frontiers | Research Topics

Traditional, complementary and integrative medicine – opportunities for managing and treating neurodegenerative diseases and ischaemic stroke

Edited by
Chun Guang Li, Bin Yu and Huazheng Liang

Published in
Frontiers in Pharmacology

FRONTIERS EBOOK COPYRIGHT STATEMENT

The copyright in the text of individual articles in this ebook is the property of their respective authors or their respective institutions or funders. The copyright in graphics and images within each article may be subject to copyright of other parties. In both cases this is subject to a license granted to Frontiers.

The compilation of articles constituting this ebook is the property of Frontiers.

Each article within this ebook, and the ebook itself, are published under the most recent version of the Creative Commons CC-BY licence. The version current at the date of publication of this ebook is CC-BY 4.0. If the CC-BY licence is updated, the licence granted by Frontiers is automatically updated to the new version.

When exercising any right under the CC-BY licence, Frontiers must be attributed as the original publisher of the article or ebook, as applicable.

Authors have the responsibility of ensuring that any graphics or other materials which are the property of others may be included in the CC-BY licence, but this should be checked before relying on the CC-BY licence to reproduce those materials. Any copyright notices relating to those materials must be complied with.

Copyright and source acknowledgement notices may not be removed and must be displayed in any copy, derivative work or partial copy which includes the elements in question.

All copyright, and all rights therein, are protected by national and international copyright laws. The above represents a summary only. For further information please read Frontiers' Conditions for Website Use and Copyright Statement, and the applicable CC-BY licence.

ISSN 1664-8714
ISBN 978-2-8325-7325-9
DOI 10.3389/978-2-8325-7325-9

Generative AI statement
Any alternative text (Alt text) provided alongside figures in the articles in this ebook has been generated by Frontiers with the support of artificial intelligence and reasonable efforts have been made to ensure accuracy, including review by the authors wherever possible. If you identify any issues, please contact us.

About Frontiers

Frontiers is more than just an open access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers journal series

The Frontiers journal series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the *Frontiers journal series* operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews. Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the *Frontiers journals series*: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area.

Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers editorial office: frontiersin.org/about/contact

Traditional, complementary and integrative medicine – opportunities for managing and treating neurodegenerative diseases and ischaemic stroke

Topic editors

Chun Guang Li — Western Sydney University, Australia

Bin Yu — Nanjing University of Chinese Medicine, China

Huazheng Liang — Monash University - Southeast University Joint Research Institute (Suzhou), China

Citation

Li, C. G., Yu, B., Liang, H., eds. (2026). *Traditional, complementary and integrative medicine – opportunities for managing and treating neurodegenerative diseases and ischaemic stroke*. Lausanne: Frontiers Media SA.

doi: 10.3389/978-2-8325-7325-9

Table of contents

05 Editorial: Traditional, complementary and integrative medicine – opportunities for managing and treating neurodegenerative diseases and ischaemic stroke
Huazheng Liang, Chunguang Li and Bin Yu

09 A review of the *Bovis Calculus*'s intervention mechanism and clinical application in ischemic stroke
Ke Xu, Bowen Deng, Tongtong Jia, Mihong Ren, Hai Chen, Jing Zhang, Jinlin Guo, Yong Li and Jian Wang

40 Kai-Xin-San ameliorates mild cognitive impairment in SAMP8 mice by inhibiting neuroinflammation and pyroptosis via NLRP3/Caspase-1 pathway modulation
Shu Liu, Xiaochen Song, Yuefeng Sun, Ailin Sun, Yang Li, Yuyu Li and Jing Chen

56 NMN reverses D-galactose-induced neurodegeneration and enhances the intestinal barrier of mice by activating the Sirt1 pathway
Yuxian Lin, Yajing Wang, Xinxin Yang, Ziwei Ding, Mingye Hu, Xianfeng Huang, Qichun Zhang and Yingcong Yu

72 Polydatin attenuates Alzheimer's disease induced by aluminum chloride in rats: evidence for its antioxidant and anti-inflammatory effects
Seyede Nazanin Zarneshan, Sajad Fakhri, Amir Kiani, Fatemeh Abbaszadeh, Seyede Zahra Hosseini, Ehsan Mohammadi-Noori and Javier Echeverría

84 The active metabolite of Epimedii Folium promotes hippocampal neurogenesis in APP/PS1 mice by alleviating mitochondrial dysfunction
Jia-Ming Bai, Tong Li, Xue Di, Jing-Xian Yang, Zhao-Qi Cui, Dong-Yu Min, Yu-Feng Shen, Si-Yu Shan, Ye-Xin Zhang, Yi-Jun Shi, Zhi-Li Xu, De-Qiang Dou and Hong-He Xiao

101 Shenmai Injection enhances short-term outcomes in ischemic stroke patients after thrombolysis via AMPKα1
Jing Wu, Zhonghao Li, Xiaoke Dong, Jinmin Liu and Le Wang

111 Neuroprotective effects of astaxanthin in a scopolamine-induced rat model of Alzheimer's disease through antioxidant/anti-inflammatory pathways and opioid/benzodiazepine receptors: attenuation of Nrf2, NF-κB, and interconnected pathways
Zeinab Rastinpour, Sajad Fakhri, Fatemeh Abbaszadeh, Mohammad Ranjbari, Amir Kiani, Mohammed Namiq Amin and Javier Echeverría

125 Targeting ferroptosis with natural products in stroke: therapeutic mechanisms and translational opportunities
Yingyi Zheng, Xi Tan, Xiaojie Wang, Rui Mao and Jianwen Guo

148 Traditional, complementary, and integrative medicine in the management of ischemic stroke: a narrative review
S. Sowmiya, Rukaiah Fatma Begum, L. S. Dhivya, Praveen Rajendran, N. Harikrishnan and Ankul Singh S

164 Efficacy of danhong injection adjuvant therapy in patients with acute ischemic stroke: a real-world, multicenter, retrospective study
Danping Pan, Haitong Wan, Yu He, Jiehong Yang, Yilei Guo, Li Yu, Feihu Zhang, Guoqing Zheng, Bin Xu, Yaohong Song, Mingjun Zhao, Xiangzhe Liu, Jianhe Liu, Gang Sun and Yaming Lin

174 Integrative approaches in Alzheimer's disease: evaluating the potential of traditional, complementary, and integrative medicine (TCIM)
R. Sneha Sri, T. Pavithra, T. Vinciya, V. Santhosh Kumar, N. Harikrishnan, Rukaiah Fatma Begum and S. Ankul Singh

190 Multi-omics reveal the neuroprotective mechanisms of Xinshubao tablet against scopolamine-induced cognitive dysfunction in mice
Zhe Yang, Feng-Rong Zhang, Lu Ren, Jia-Ming Bai, Shi-Cong Wang, Xian-Yu Li, Hong-Jun Yang and Hong-He Xiao

212 Pharmacokinetics and tissue distribution analysis of ginsenoside Rh_3 in rats using a novel LC-MS/MS quantification strategy
Cong Hu, Yuheng Wang, Yu Liu, Xiaojing Wang, Peifang Song, Hong Ma and Ling Yang

224 Modulation of neuroinflammation by natural plant compounds: a promising approach for ischemic stroke
Pingping Niu, Yonggang Zhang, Binghao Wang, Sheng Qiu, Quanming Dong, Liqin Li and Yuntao Li

239 Efficacy and safety of Tongxinluo in the treatment of stroke: a systematic review and meta-analysis of randomized controlled trials
Zhixin Wu, Jiamei Fu, Yumeng Zhou and Yabin Zhou

260 Research progress on the mechanisms of polysaccharides derived from traditional Chinese medicine in the treatment of Parkinson's disease
Xiangling Zhou and Qi Pan

frontiers | Frontiers in Pharmacology

TYPE Editorial
PUBLISHED 15 December 2025
DOI 10.3389/fphar.2025.1749624

Check for updates

OPEN ACCESS

EDITED BY
Javier Echeverria,
University of Santiago, Chile

REVIEWED BY
Dâmaris Silveira,
University of Brasilia, Brazil

*CORRESPONDENCE
Huazheng Liang,
andy.liang@monash.edu

RECEIVED 19 November 2025
REVISED 05 December 2025
ACCEPTED 09 December 2025
PUBLISHED 15 December 2025

CITATION
Liang H, Li C and Yu B (2025) Editorial: Traditional, complementary and integrative medicine – opportunities for managing and treating neurodegenerative diseases and ischaemic stroke.
Front. Pharmacol. 16:1749624.
doi: 10.3389/fphar.2025.1749624

COPYRIGHT
© 2025 Liang, Li and Yu. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

Editorial: Traditional, complementary and integrative medicine – opportunities for managing and treating neurodegenerative diseases and ischaemic stroke

Huazheng Liang[1,2,3,4,5,6,7]*, Chunguang Li[8] and Bin Yu[9]

[1]Translational Research Institute of Brain and Brain-Like Intelligence, Shanghai Fourth People's Hospital, School of Medicine, Tongji University, Shanghai, China, [2]Shanghai Key Laboratory of Anesthesiology and Brain Functional Modulation, Shanghai, China, [3]Clinical Research Center for Anesthesiology and Perioperative Medicine, Shanghai Fourth People's Hospital, School of Medicine, Tongji University, Shanghai, China, [4]Suzhou Industrial Park Monash Research Institute of Science and Technology, Suzhou, Jiangsu, China, [5]Southeast University-Monash University Joint Graduate School, Suzhou, Jiangsu, China, [6]Monash University- Southeast University Joint Research Institute, Suzhou, Jiangsu, China, [7]Australian Regenerative Medicine Institute, Clayton, VIC, Australia, [8]NICM Health Research Institute, Western Sydney University, Westmead, NSW, Australia, [9]Jiangsu Key Laboratory for Pharmacology and Safety Evaluation of Chinese Materia Medica, Nanjing University of Chinese Medicine, Nanjing, Jiangsu, China

KEYWORDS

clinical trial, gut microbiota, ischemic stroke, natural products, neurodegenerative diseases, pharmacology, therapeutic targets

Editorial on the Research Topic
Traditional, complementary and integrative medicine – opportunities for managing and treating neurodegenerative diseases and ischaemic stroke

Ischemic stroke and Alzheimer's disease (AD) are leading neurological causes of death and disability (Avan and Hachinski, 2023; An et al., 2025), yet both face profound research and therapeutic challenges. Stroke treatment is constrained by a narrow therapeutic window, thrombolysis and endovascular therapy must be administered within hours (Ho and Powers, 2025), limiting accessibility, especially in rural or underserved regions. Post-stroke care is complicated by heterogeneous patient presentations, variable reperfusion outcomes, and a lack of neuroprotective agents that translate from animal models to humans. Chronic inflammation, blood-brain barrier disruption, and comorbidities like diabetes or atrial fibrillation further obscure treatment efficacy and recovery trajectories (Pandian et al., 2023; Zeng et al., 2025).

Alzheimer's disease research grapples with its long preclinical phase, during which pathology silently accumulates decades before symptoms emerge. This complicates early diagnosis and intervention (Marvi et al., 2025). The field has been dominated by the amyloid cascade hypothesis, yet anti-amyloid therapies have shown only modest cognitive benefits, raising questions about causality and timing (Reiss et al., 2021). Tau pathology, neuroinflammation, vascular dysfunction, and synaptic loss are increasingly recognized as multifactorial drivers (Rajendran and Krishnan, 2024; Cai et al., 2025) but integrating

these into cohesive treatment strategies remains elusive. Biomarker development is advancing, with CSF and PET imaging improving diagnostic accuracy, but high cost, limited availability, and invasiveness hinder widespread use (Krishnamurthy et al., 2025).

This research topic comprises 16 published papers tackling the abovementioned challenges. Apart from conventional secondary prevention medications, there are few drugs available to treat ischemic stroke patients. In a study by Wu et al., Shenmai Injection given to ischemic stroke patients for 10 days after rt-PA thrombolysis significantly lowered 30-day mRS scores compared with placebo, indicating better functional recovery in ischemic stroke patients without added adverse events. In H_2O_2-stressed PC12 cell culture model, Shenmai Injection significantly enhanced cell viability, reduced apoptosis, diminished the levels of reactive oxygen species and malondialdehyde. In the meantime, it increased the level of ATP and mitochondrial potential. These protective effects are abolished by AMPKα1 knockdown, indicating that these protective effects are partially mediated by AMPKα1 (Wu et al.).

In a real-world, multi-center retrospective study, Pan et al. found that Danhong Injection as an adjunct therapy significantly improved neurological outcomes in patients with acute ischemic stroke, evidenced by lower National Institutes of Health Stroke Scale (NIHSS) at discharge and higher proportions of patients achieving NIHSS ≤4 and ≤1 compared with the control group. The drug group also showed better functional recovery, with more patients achieving modified Rankin Score (mRS) ≤1. There was no significant difference in in-hospital complications (Pan et al.). These findings suggest Danhong Injection may enhance short-term recovery in acute ischemic stroke patients and further randomized trials are needed.

In a systematic review and meta-analysis by Wu et al., results from 51 RCTs (9,577 patients) found that Tongxinluo significantly improves stroke outcomes including enhancing the functional recovery through decreasing the NIHSS, improving lipid profiles, and inflammation markers without increasing adverse events. Benefits were greater in patients ≤65 years and with ≤4 weeks treatment. However, heterogeneity and publication bias limits the fidelity (Wu et al.).

Xu et al. summarized the progress on *Bovis Calculus's* mechanism and application in ischemic stroke. This traditional medicine shows strong potential in treating ischemic stroke by mitigating oxidative stress, inflammation, and apoptosis while promoting angiogenesis and neurogenesis. Its active components, including bile acids and taurine, act through multiple pathways to protect brain tissue. Clinical studies support its safety and efficacy, especially in improving neurological function and recovery (Xu et al., 2025).

A review by Sowmiya et al. demonstrates that Traditional, Complementary and Integrative Medicine (TCIM) offers multi-targeted, clinically relevant support for ischemic-stroke patients. Evidence from controlled trials and mechanistic studies indicates that standardized herbal formulas, such as *Salvia miltiorrhiza Bunge [*Lamiaceae*; Salviae miltiorrhizae radix et rhizoma], Ginkgo biloba L. [*Ginkgoaceae*; Ginkgo folium], and Panax ginseng C.A. Mey. [*Araliaceae*; Ginseng radix]*, acupuncture, yoga, polyphenol-rich diets, aromatherapy and music therapy can significantly reduce oxidative stress, neuroinflammation, mitochondrial dysfunction, ferroptosis and autophagy, while enhancing antioxidant defenses, cerebral blood flow, neurogenesis and angiogenesis. These interventions improve neurological scores, functional independence and quality of life when used adjunctively with thrombolysis or rehabilitation. However, benefits are constrained by heterogeneous formulations, small sample sizes, short follow-ups and scarce herb-drug interaction data. The review, therefore, calls for large, multi-center, double-blind randomized trials with defined phytochemical profiles, biomarker endpoints and systems-biology analyses to validate efficacy, optimize dosing and facilitate safe, evidence-based integration of TCIM into mainstream stroke management protocols worldwide (Sowmiya et al.).

Another review by Niu et al. further highlighted the therapeutic potential of natural plant compounds in modulating neuroinflammation following ischemic stroke. It systematically summarizes how polyphenols, flavonoids, saponins, diterpenoids, and alkaloids exert anti-inflammatory effects by targeting key signalling pathways, such as NF-κB, NLRP3, TLR4, and JAK/STAT. These compounds inhibit microglial activation, reduce pro-inflammatory cytokines, and suppress immune cell infiltration, thereby attenuating post-stroke brain injury. Despite promising preclinical results, challenges like poor bioavailability, limited BBB penetration, unclear molecular targets, and translational gaps remain. This review emphasizes the need for advanced delivery systems, mechanistic clarification, and rigorous clinical trials to advance these natural products as viable neuroprotective therapies for ischemic stroke (Niu et al.).

A review by Zheng et al. identified ferroptosis, an iron-dependent form of regulated cell death, as a key therapeutic target in both ischemic and hemorrhagic stroke. It systematically summarized mechanisms driving ferroptosis, including iron overload, lipid peroxidation, and glutathione depletion. Authors analyzed 55 preclinical studies and highlighted that natural products, such as ginkgolide B, quercetin, berberine, and salvianolic acid A, effectively inhibited ferroptosis *via* the Nrf2/HO-1, GPX4, and SLC7A11 pathways. Despite promising animal data, clinical translation remains limited due to issues like poor bioavailability, unclear targets, and lack of standardized formulations. This review emphasizes the need for pharmacokinetic profiling, target validation, and robust clinical trials to advance these natural compounds into stroke therapeutics (Zheng et al.).

Alzheimer's disease (AD) remains a challenging medical condition with no effective treatments. However, traditional medicine offers some empirical recipes to halt the progression of this condition. A study by Liu et al. demonstrated that Kai-Xin-San (KXS), a traditional Chinese herbal formula, improved cognitive functions in SAMP8 mice, a model of mild cognitive impairment, by reducing amyloid-beta deposition, suppressing neuroinflammation, and inhibiting pyroptosis. These protective effects are mediated through modulating the NLRP3/Caspase-1 signalling pathway (Liu et al., 2025). These findings suggest that KXS is a promising multi-target therapeutic strategy for early-stage cognitive decline.

In another study by Lin et al., NMN was reported to rescue D-galactose-induced aging in mice by restoring NAD^+, activating Sirt1/AMPK/PGC-1α, alleviating oxidative stress, neuroinflammation and apoptosis, while enhancing neurotransmission and rebuilding the intestinal barrier. All these benefits vanished when a Sirt1 inhibitor Ex527 was applied, proving that these benefits are mediated through Sirt1 (Lin et al., 2025).

In an APP/PS1 mouse model of AD, Bai et al. reported that Icariside II, the most potent *Epimedii Folium* metabolite, attenuated cognitive deficits of these mice through restoring hippocampal neurogenesis. This benefit required intact mitochondria, as it normalized fusion/fission proteins, ATP and ROS, yet vanished when rotenone disrupted mitochondrial functions. Other metabolites of *Epimedii Folium* including Icarlin and Icaritin also showed similar effects but they were weaker compared to Icariside II (Bai et al., 2025).

In a scopolamine-induced rat model of AD, Rastinpour et al. showed that Astaxanthin significantly mitigated cognitive deficits by enhancing antioxidant defenses (catalase, glutathione), reducing oxidative stress (nitrite), and modulating inflammatory markers (MMP-2↑, MMP-9↓). It also regulated key transcription factors like upregulating Nrf2 and downregulating NF-κB while preserving hippocampal neurons. Notably, these neuroprotective effects were partially reversed by opioid (naloxone) and GABA-A (flumazenil) receptor antagonists, indicating that astaxanthin's benefits involve modulation of these neurotransmitter systems (Rastinpour et al.).

In a similar model of AD, Yang et al. demonstrated that Xinshubao tablet (XSB), a traditional Chinese medicine, significantly improved cognitive functions. Using a multi-omics approach, the study revealed that XSB mitigated neuroinflammation, oxidative stress, and cholinergic dysfunction while enhancing synaptic integrity. XSB modulated gut microbiota by increasing beneficial bacteria (e.g., *Enterococcus*, *Actinobacteriota*) and reducing harmful species (*Helicobacter rodentium*). It also restored metabolic balance by regulating pathways, such as glycerophospholipid and unsaturated fatty acid metabolism. Fecal microbiota transplantation from XSB-treated mice replicated these cognitive and intestinal barrier benefits, confirming that the gut-brain axis is a key pathway for mediating the protective effect. These findings highlight XSB's potential as a multifactorial therapeutic strategy for AD (Yang et al.).

In an aluminum chloride-induced rat AD model, Zarneshan et al. reported that polydatin dose-dependently reversed memory deficits and anxiety-like behaviors, performing comparably to donepezil. Polydatin elevated antioxidant defenses (glutathione, catalase), reduced oxidative nitrite, shifted matrix metalloproteinase balance (downregulated pro-inflammatory MMP-9, upregulated protective MMP-2), and preserved hippocampal CA2, CA4 and dentate gyrus neurons. These antioxidant, anti-inflammatory and neuroprotective mechanisms underlie polydatin's therapeutic potential against AD (Zarneshan et al., 2025).

An LC-MS/MS study by Hu et al. revealed that oral ginsenoside Rh3 (GRh_3) exhibited sustained-release pharmacokinetics in rats: delayed Tmax (8 h), long half-life (14.7 h), low clearance (13 L/h/kg) and large volume of distribution (280 L/kg). After being administered at 100 mg/kg, the compound accumulated most in intestine > stomach > liver, but notably penetrated brain tissue, reaching 520 ng/g in the hippocampus, which is contradicting the rule that only <500 Da molecules can cross the blood brain barrier. This brain enrichment, coupled with prolonged systemic exposure, supports GRh3's potential for chronic neuroprotective therapies and warrants investigation of transporter-mediated BBB mechanisms (Hu et al.).

In another study, Sneha et al. reported the therapeutic potential of Traditional, Complementary, and Integrative Medicine (TCIM) as an adjunct to conventional treatments for AD. Unlike standard pharmaceuticals that offer only symptomatic relief, TCIM employs a holistic, multi-targeted approach, addressing key pathological mechanisms, such as amyloid-beta aggregation, tau hyperphosphorylation, oxidative stress, neuroinflammation, and mitochondrial dysfunction. Promising herbal agents including *Withania somnifera (L.) Dunal*, *Curcuma longa L*, *Bacopa monnieri (L.) Wettst*, *Centella asiatica (L.) Urb*, and *G. biloba L* demonstrate neuroprotective, anti-inflammatory, and antioxidant properties in preclinical and limited clinical studies. Mind-body practices like meditation also contribute to the therapeutic effect by reducing stress, a known modifiable risk factor. However, the review emphasized that despite encouraging results, most TCIM studies suffer from methodological limitations, including small sample sizes, lack of standardization, poor bioavailability, and insufficient randomized controlled trials. To advance TCIM's role in AD management, future research must prioritize rigorous, large-scale, multi-center randomized controlled trials, pharmacokinetic profiling, herb-drug interaction studies, and personalized, integrative therapeutic strategies combining traditional and modern medicine (Sneha et al.).

Parkinson's disease (PD) is the second most common neurodegenerative disease. No cure is available for this condition. In the review by Zhou and Pan, the therapeutic potential of polysaccharides derived from Traditional Chinese Medicine in treating PD through multiple mechanisms has been summarized. These polysaccharides, extracted from sources like *Astragalus mongholicus Bunge [*Fabaceae*, Gastrodia elata Blume [*Orchidaceae*], Lycium barbarum L. [*Solanaceae*]*, and *Cistanche deserticola Ma [*Orobanchaceae*]*, exhibit neuroprotective effects by inhibiting apoptosis, reducing oxidative stress, enhancing mitochondrial function, promoting autophagy, and alleviating neuroinflammation. They also modulate key signalling pathways, such as PI3K/AKT/mTOR and AMPK/mTOR, and may influence the gut-brain axis by restoring microbiota balance. Preclinical studies showed improvements in motor and non-motor symptoms in PD models, with some evidence of dopaminergic neuron protection and α-synuclein aggregation inhibition. Despite promising results, clinical translation is limited by lack of standardized formulations, bioavailability issues, and absence of large-scale human trials. Future research should focus on clinical validation, mechanism elucidation, and formulation optimization to advance these polysaccharides as viable PD therapeutics (Zhou and Pan).

Taking these studies together, this Research Topic has provided pharmaceutical and clinical evidence on naturally derived products in managing ischemic stroke and neurodegenerative diseases like AD and PD. They take therapeutic effects through multiple mechanisms. However, robust clinical evidence is still needed to overcome limitations of current studies, such as small sample sizes, patient heterogeneity, and poor translational potential. Nevertheless, these natural products might become drug candidates against ischemic stroke and neurodegenerative diseases and open a new era of drug development.

Author contributions

HL: Conceptualization, Data curation, Investigation, Resources, Supervision, Writing – original draft, Writing – review and editing.

CL: Conceptualization, Data curation, Investigation, Resources, Supervision, Validation, Writing – original draft, Writing – review and editing. BY: Conceptualization, Data curation, Investigation, Resources, Supervision, Validation, Writing – original draft, Writing – review and editing.

Funding

The author(s) declared that financial support was not received for this work and/or its publication.

Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declared that generative AI was not used in the creation of this manuscript.

Any alternative text (alt text) provided alongside figures in this article has been generated by Frontiers with the support of artificial intelligence and reasonable efforts have been made to ensure accuracy, including review by the authors wherever possible. If you identify any issues, please contact us.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

An, X., Liu, Z., Zhang, L., Zhao, J., Gu, Q., Han, W., et al. (2025). Co-occurrence patterns and related risk factors of ischaemic heart disease and ischaemic stroke across 203 countries and territories: a spatial correspondence and systematic analysis. *Lancet Glob. Health* 13 (5), e808–e819. doi:10.1016/S2214-109X(25)00013-0

Avan, A., and Hachinski, V. (2023). Global, regional, and national trends of dementia incidence and risk factors, 1990-2019: a global burden of disease study. *Alzheimers Dement.* 19 (4), 1281–1291. doi:10.1002/alz.12764

Bai, J. M., Li, T., Di, X., Yang, J. X., Cui, Z. Q., Min, D. Y., et al. (2025). The active metabolite of epimedii folium promotes hippocampal neurogenesis in APP/PS1 mice by alleviating mitochondrial dysfunction. *Front. Pharmacol.* 16, 1546256. doi:10.3389/fphar.2025.1546256

Cai, J., Liu, Y., and Fan, H. (2025). Review on pathogenesis and treatment of alzheimer's disease. *Dev. Dyn.* 254 (4), 296–309. doi:10.1002/dvdy.762

Ho, J. P., and Powers, W. J. (2025). Contemporary management of acute ischemic stroke. *Annu. Rev. Med.* 76 (1), 417–429. doi:10.1146/annurev-med-050823-094312

Krishnamurthy, H. K., Jayaraman, V., Krishna, K., Wang, T., Bei, K., Changalath, C., et al. (2025). An overview of the genes and biomarkers in alzheimer's disease. *Ageing Res. Rev.* 104, 102599. doi:10.1016/j.arr.2024.102599

Lin, Y., Wang, Y., Yang, X., Ding, Z., Hu, M., Huang, X., et al. (2025). NMN reverses D-galactose-induced neurodegeneration and enhances the intestinal barrier of mice by activating the Sirt1 pathway. *Front. Pharmacol.* 16, 1545585. doi:10.3389/fphar.2025.1545585

Liu, S., Song, X., Sun, Y., Sun, A., Li, Y., Li, Y., et al. (2025). Kai-xin-san ameliorates mild cognitive impairment in SAMP8 mice by inhibiting neuroinflammation and pyroptosis *via* NLRP3/Caspase-1 pathway modulation. *Front. Pharmacol.* 16, 1528011. doi:10.3389/fphar.2025.1528011

Marvi, F., Chen, Y. H., and Sawan, M. (2025). Alzheimer's disease diagnosis in the preclinical stage: normal aging or dementia. *IEEE Rev. Biomed. Eng.* 18, 74–92. doi:10.1109/RBME.2024.3376835

Pandian, J. D., Padma, S. M. V., Aaron, S., Ranawaka, U. K., Venketasubramanian, N., Sebastian, I. A., et al. (2023). The burden, risk factors and unique etiologies of stroke in south-east Asia region (SEAR). *Lancet Reg. Health Southeast Asia* 17, 100290. doi:10.1016/j.lansea.2023.100290

Rajendran, K., and Krishnan, U. M. (2024). Mechanistic insights and emerging therapeutic stratagems for alzheimer's disease. *Ageing Res. Rev.* 97, 102309. doi:10.1016/j.arr.2024.102309

Reiss, A. B., Montufar, N., DeLeon, J., Pinkhasov, A., Gomolin, I. H., Glass, A. D., et al. (2021). Alzheimer disease clinical trials targeting amyloid: lessons learned from success in mice and failure in humans. *Neurologist* 26 (2), 52–61. doi:10.1097/NRL.0000000000000320

Xu, K., Deng, B., Jia, T., Ren, M., Chen, H., Zhang, J., et al. (2025). A review of the bovis Calculus's intervention mechanism and clinical application in ischemic stroke. *Front. Pharmacol.* 15, 1510779. doi:10.3389/fphar.2024.1510779

Zarneshan, S. N., Fakhri, S., Kiani, A., Abbaszadeh, F., Hosseini, S. Z., Mohammadi-Noori, E., et al. (2025). Polydatin attenuates alzheimer's disease induced by aluminum chloride in rats: evidence for its antioxidant and anti-inflammatory effects. *Front. Pharmacol.* 16, 1574323. doi:10.3389/fphar.2025.1574323

Zeng, J., Zhou, H., Wan, H., and Yang, J. (2025). Single-cell omics: moving towards a new era in ischemic stroke research. *Eur. J. Pharmacol.* 1000, 177725. doi:10.1016/j.ejphar.2025.177725

frontiers | Frontiers in Pharmacology

TYPE Review
PUBLISHED 15 January 2025
DOI 10.3389/fphar.2024.1510779

Check for updates

OPEN ACCESS

EDITED BY
Huazheng Liang,
Monash University - Southeast University Joint Research Institute (Suzhou), China

REVIEWED BY
Corina Bubueanu,
National Institute For Chemical - Pharmaceutical Research and Development – ICCF Bucharest, Romania
Hee Geun Jo,
Gachon University, Republic of Korea

*CORRESPONDENCE
Jian Wang,
lczyx712@163.com
Yong Li,
leeyoung@stu.cdutcm.edu.cn

†These authors have contributed equally to this work and share first authorship

RECEIVED 13 October 2024
ACCEPTED 20 December 2024
PUBLISHED 15 January 2025

CITATION
Xu K, Deng B, Jia T, Ren M, Chen H, Zhang J, Guo J, Li Y and Wang J (2025) A review of the *Bovis Calculus*'s intervention mechanism and clinical application in ischemic stroke. *Front. Pharmacol.* 15:1510779. doi: 10.3389/fphar.2024.1510779

COPYRIGHT
© 2025 Xu, Deng, Jia, Ren, Chen, Zhang, Guo, Li and Wang. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

A review of the *Bovis Calculus*'s intervention mechanism and clinical application in ischemic stroke

Ke Xu†, Bowen Deng†, Tongtong Jia, Mihong Ren, Hai Chen, Jing Zhang, Jinlin Guo, Yong Li* and Jian Wang*

College of Pharmacy, Chengdu University of Traditional Chinese Medicine, Chengdu, China

Background: *Bovis Calculus* (BC), also named Niuhuang in Chinese, is utilized as a resuscitation drug in Traditional Chinese Medicine (TCM) for the treatment of neurological disorders. Ischemic stroke (IS) is a significant global public health issue that currently lacks safe and effective therapeutic drugs. Ongoing efforts are focused on identifying effective treatment strategies from Traditional, Complementary, and Integrative Medicine. Noticeably, BC has been used in TCM for thousands of years to prevent or treat IS-related diseases.

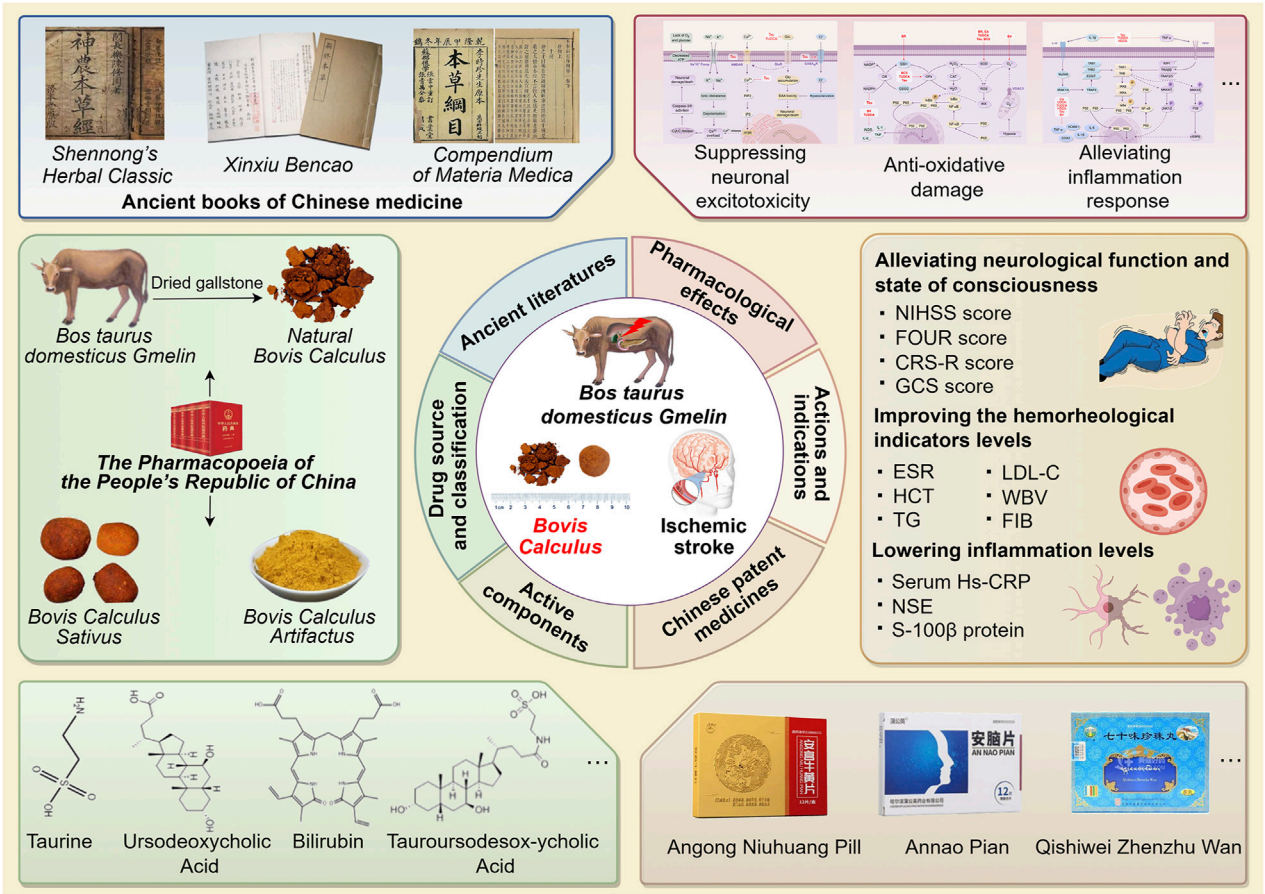
Methods: The historical origins of BC in the treatment of IS were investigated through the examination of ancient Chinese medical texts. Furthermore, the chemical components of BC were analyzed, and its mechanisms of action against IS were summarized using literature sourced from databases such as Web of Science, PubMed, and China National Knowledge Infrastructure. Information on Chinese medicine preparations and clinical reports was also integrated to provide an overview of modern applications and safety considerations.

Results: BC mainly includes chemical components such as bile pigments, bile acids, cholesterol, proteins amino acids, and trace elements. Additionally, the efficacy of BC in treating cerebral ischemia/reperfusion injury (CI/RI) is certain, particularly due to the components of bile pigments, bile acids, and amino acids that can interfere with the enzymatic cascade reaction of CI/RI through multiple components, targets, and pathways. The active components of BC exert neuroprotective effects by reducing microcirculation disturbance, excitatory amino acid toxicity, and oxidative stress injury in the acute stage; inhibiting inflammatory injury, apoptosis, and blood-brain barrier (BBB) disruption in the subacute stage; and promoting angiogenesis and neurogenesis in the restoration stage. Furthermore, as a crude drug, BC appears in many Chinese patent medicine (CPM) preparations for the treatment of IS, and clinical and preclinical studies have proved its safety.

Conclusion: The use of BC in the treatment of IS has a long history, proven efficacy, and widespread application. Future efforts should focus on elucidating its mechanisms of action and exploring its applications.

KEYWORDS

***Bovis Calculus*, chemical composition, ischemia stroke, clinical application, drug safety, pharmacokinetics, traditional application, pharmacological effect**



GRAPHICAL ABSTRACT

1 Introduction

Bovis Calculus (BC) is the desiccated gallstone of *Bos taurus domesticus* Gmelin. It has been employed for cardio-cerebrovascular ailment treatment for over two millennia in China. According to the *Chinese Pharmacopoeia*, this substance is described as having a cooling and sweet nature and is associated with the heart and liver meridians. It is known for its ability to cleanse the heart, eliminate phlegm, induce resuscitation, cool the liver, relieve wind-related symptoms, and detoxify the body. Thus, people use BC to treat stroke and phlegm confusion, fever and dizziness, epileptic convulsions, etc. (Chinese Pharmacopoeia Committee of People's Republic of China, 2020). Due to the scarce source and high price of natural BC, nowadays, *Bovis Calculus Artifactus* and *Bovis Calculus Sativus* have been studied as alternatives to natural BC.

Stroke is a severe disease of cerebral blood circulation disturbance and a major public health event that results in death and disability on a global scale. It is caused by cerebral vascular obstruction or rupture, leading to damage to tissue function and structure. With its high mortality and disability rates, stroke has brought a heavy burden to society and is now the second most deadly disease in the world (Owolabi et al., 2015; Johnson et al., 2016; Wu et al., 2019). It is noteworthy that CI is the primary type of stroke, accounting for about 85% of all strokes (Sarvari et al., 2020). Studies found that CI/RI is the result of the interaction of multiple pathological components such as microcirculation disturbance, energy metabolism disorder, oxidative stress, inflammatory responses, apoptosis, and necrosis (Sekerdag et al., 2018; Przykaza, 2021). Nowadays, thrombolysis with recombinant tissue-type plasminogen activator (rt-PA) is the main approach to restoring cerebral blood flow (CBF). However, thrombolysis is subject to a strict time window. The use of rt-PA also increases the risk of intracranial hemorrhage, potentially converting CI to cerebral hemorrhage (Cheng et al., 2014; Emberson et al., 2014). Therefore, it is imperative to determine the ideal treatment drug.

As studies showed, the active components of BC that reduce the acute stage of CI/RI are taurine (Tau), ursodeoxycholic acid (UDCA), bilirubin (BR), biliverdin (BV), cholic acid (CA), tauroursodeoxycholic acid (TUDCA) and hyodeoxycholic acid (HDCA). Besides, the active components of BC that inhibit the subacute stage of CI/RI are CA, UDCA, TUDCA, HDCA, Tau, BV, BR and glycoursodeoxycholic acid (GUDCA). Otherwise, the active components of BC that promote the restoration stage of cerebral ischemic recovery are Tau, CA and HDCA.

Up to now, there have been numerous pharmacological studies on the treatment of IS by BC and its active components. The effectiveness and mechanism of BC has also been extensively validated in animal studies (Wang et al., 2014; Zhang et al.,

2021). However, there is a lack of a review to systematically sort out the relevant content. This study not only seeks to combine three fundamental sources of BC and its chemical constituents to investigate the molecular mechanism through which BC influences CI/RI, but also discussed its widely clinical application.

2 Materials and methods

A comprehensive search was conducted on the medical databases, including Web of Science, PubMed, China National Knowledge Infrastructure (CNKI), Wanfang Data, and VIP Database, using terms such as "*Bovis Calculus*" "natural *Bovis Calculus*" "*Bovis Calculus* artifactus" "*Bovis Calculus* sativus" "cerebral ischemia" "ischemic stroke" "traditional applications" "chemical components" "clinical applications" "clinical safety" "pharmacokinetic characteristics" "safety evaluation". The languages of retrieval include English and non-English. The types of literature include journals, papers, books, and patents. A total of 376 articles were retrieved. Then the retrieved literature was categorized and summarized. Exclusion criteria included literature with poor quality, old data and lack of innovation. And the search time frame is from 1 January 1955, to 31 August 2024. Finally, 196 relevant papers were obtained. There were 112 English papers and 84 non-English papers, and the ratio of English to non-English papers was approximately 1.33:1. All the papers included in this manuscript can fully support the conclusions of this manuscript.

3 Traditional applications of *Bovis Calculus*

The traditional applications of BC can be traced back to *Shennong's Herbal Classic* (Wang and Xue, 2008). For over two thousand years, its effectiveness and recommended uses have evolved, from *Shennong's Herbal Classic* to the latest edition of the *Chinese Pharmacopoeia* (Chen and Wang, 2023). Earlier studies have indicated that BC can be used as follows, clear the heart, sweep phlegm, open the neorifices, cool the liver, extinguish wind, and remove toxin, indications loss of consciousness in febrile disease, wind-stroke and phlegm clouding the heart, seizures and convulsions, epilepsy, manic psychosis, swollen sore throat, mouth and tongue sores, swelling abscess, deep-rooted boil and sore.

In the periods of the *Qin* and *Han* Dynasties (221 B.C. to 220 A.D.), *Shennong's Herbal Classic* recorded that BC was mainly used for convulsive epilepsy, aversion to cold first and then fever, hyperpyrexia, syncope, and spasm. It was the first time to record the application of BC in infantile convulsions, febrile convulsions, and mental and emotional diseases. After that, the efficacy and indications of BC in the *Collection of herbal classics* (Tao, 1955) in the *Liang* Dynasty (502 A.D. to 557 A.D.) and the *Xinxiu Bencao* (Su et al., 2004) in the *Tang* Dynasty (618 A.D. to 907 A.D.) were consistent. Such as treatment for children with various diseases including epilepsy, excessive internal heat leading to mouth failure to open, and adult mania. Later, the *Five* Dynasties (907 A.D. to 979 A.D.) *Rihuazi herbal medicine* (Ri and Shang, 2005): treatment of apoplectic aphasia, lockjaw, palpitations, infectious disease, forgetfulness, and debility, increased the records of BC in stroke and infectious diseases treatment. Hundred years later, in the *Yuan* Dynasty (1271 A.D. to 1368 A.D.), the record of diuretic phlegm and anticonvulsants was added to the *Daily Materia medica* (Wu, 2019). Specifically, in the *Ming* Dynasty (1368 A.D. to 1644 A.D.), Li Shizhen's *Compendium of Materia Medica* (Li et al., 2014) recorded that BC could be used for children with febrile convulsions, chewing tongue, and delirium.

4 Chemical components of *Bovis Calculus*

Modern research has elucidated the chemical composition of NBC more clearly. However, due to the scarcity and high price of NBC, in recent years, *Bovis Calculus Artifactus* (BCA), *Bovis Calculus Sativus* (BCS), cultured *Bovis Calculus* (CBC), and other substitutes for NBC have been successfully developed to address urgent clinical needs (Yang et al., 1996). It is noteworthy that the BC discussed in this paper does not pertain to the CBC and is consistent with medical ethics. Besides, the chemical composition and content of BC from different base sources differ (Kong et al., 2010; 2012; Yu et al., 2020). The chemical composition of NBC and its common clinical substitutes along with their structures, are sorted out as follows (Table 1; Figure 1).

4.1 Natural *Bovis Calculus*

Natural *Bovis Calculus* (NBC) mainly contains bile pigments, bile acids, cholesterol, peptides, and inorganic elements (Kong et al., 2012). Among them, Bile pigments mainly exist in the form of conjugated BR and also contain a small amount of free BR and BV. Conjugated BR has been found to be one of the important active components of BC (Kong et al., 2010). Besides, Bile acid is one of the main medicinal components in NBC. Additionally, Bile acids in NBC are categorized as free bile acids and conjugated bile acids. Researches found that the free bile acids are mainly CA, deoxycholic acid (DCA), chenodeoxycholic acid (CDCA), UDCA, HDCA, and lithocholic acid. Another studies showed that the conjugated bile acids are mainly taurocholic acid (TCA), glycocholic acid (GCA), taurodeoxycholic acid (TDCA), glycodeoxycholic acid (GDCA), taurochenodeoxycholic acid (TCDCA), glycochenodeoxycholic acid (GCDCA), TUDCA, GUDCA, taurolithocholic acid and glycolithocholic acid (Kong et al., 2012; Yu et al., 2020). Tau is also one of the important active components in NBC (Kong et al., 2012). Furthermore, NBC contains components such as vitamin D and carotenoids (Kong et al., 2010). According to the *Pharmacopoeia of the People's Republic of China,* NBC must contain not only a minimum of 4.0% bile acid but also at least 25% BR in dried form (Chinese Pharmacopoeia Committee of People's Republic of China, 2020).

4.2 *Bovis Calculus* artifactus

The *Bovis Calculus Artifactus* (BCA) process is based on the known composition of NBC. Earlier studies have indicated that BCA consists of bovine bile powder, CA, DCA, Tau, BR, cholesterol,

TABLE 1 The main chemical composition of BC.

Classification	Chemical component	Molecular formula	PubChem CID	Reference
Bile pigments	Bilirubin	$C_{33}H_{36}N_4O_6$	5280352	Jia et al. (2013), Miao et al. (2013), Feng et al. (2017), Huang et al. (2018a), Liu et al. (2019b), Yu et al. (2020)
	Bilirubin calcium	$C_{32}H_{42}CaN_4O_6$	101620642	
	Bilirubin glucuronic acid conjugates	$C_{39}H_{44}N_4O_{12}$	6438344	
	Biliverdin	$C_{33}H_{34}N_4O_6$	5280353	
Bile acids	Cholic acid	$C_{24}H_{40}O_5$	221493	
	Deoxycholic acid	$C_{24}H_{40}O_4$	222528	
	Chenodeoxycholic acid	$C_{24}H_{40}O_4$	10133	
	Ursodeoxycholic acid	$C_{24}H_{40}O_5$	31401	
	Hydeoxycholic acid	$C_{24}H_{40}O_4$	5283820	
	Lithocholic acid	$C_{24}H_{40}O_3$	9903	
	Taurocholic acid	$C_{26}H_{45}NO_7S$	6675	
	Glycocholic acid	$C_{26}H_{43}NO_6$	10140	
	Taurodeoxycholic acid	$C_{26}H_{45}NO_6S$	2733768	
	Glycodeoxycholic acid	$C_{26}H_{43}NO_5$	3035026	
	Taurochenodeoxycholic acid	$C_{26}H_{45}NO_6S$	387316	
	Glycochenodeoxycholic acid	$C_{26}H_{43}NO_5$	12544	
	Tauroursodeoxycholic acid	$C_{26}H_{45}NO_6S$	9848818	
	Glycoursodeoxycholic acid	$C_{26}H_{43}NO_5$	12310288	
	Taurolithocholic acid	$C_{26}H_{45}NO_5S$	439763	
	Glycolithocholic acid	$C_{26}H_{43}NO_4$	115245	
Proteins and amino acids	Taurine	$C_2H_7NO_3S$	1123	
	Glycine	$C_2H_5NO_2$	750	
	Alanine	$C_3H_7NO_2$	5950	
	Valine	$C_5H_{11}NO_2$	6287	
	Leucine	$C_6H_{13}NO_2$	6106	
	Isoleucine	$C_6H_{13}NO_2$	6306	
	Methionine	$C_5H_{11}NO_2S$	6137	
	Proline	$C_5H_9NO_2$	145742	
	Tryptophan	$C_{11}H_{12}N_2O_2$	6305	
	Serine	$C_3H_7NO_3$	5951	
	Tyrosine	$C_9H_{11}NO_3$	6057	
	Cysteine	$C_3H_7NO_2S$	5862	
	Phenylalanine	$C_9H_{11}NO_2$	6140	
	Threonine	$C_4H_9NO_3$	6288	
	Aspartic acid	$C_4H_7NO_4$	5940	
	Glutamic acid	$C_5H_9NO_4$	33032	
	Lysine	$C_6H_{14}N_2O_2$	5962	

(Continued on following page)

TABLE 1 (*Continued*) The main chemical composition of BC.

Classification	Chemical component	Molecular formula	PubChem CID	Reference
	Arginine	$C_6H_{14}N_4O_2$	6322	
	Histidine	$C_6H_9N_3O_2$	6274	
	Asparagine	$C_4H_8N_2O_3$	6267	
	Glutamine	$C_5H_{10}N_2O_3$	5961	
Cholestenes	Ergosterol	$C_{28}H_{44}O$	444679	
	Cholesteryl ester	$C_{45}H_{76}O_3$	90471597	
	Cholesterol	$C_{27}H_{46}O$	5997	
Others	Vitamin D3	$C_{27}H_{44}O$	5280795	
	Phosphatidylcholine	$C_{44}H_{84}NO_8P$	6441487	
	Oleanic acid	$C_{30}H_{48}O_3$	10494	
	Ursolic acid	$C_{30}H_{48}O_3$	64945	
	Methyl cholate	$C_{25}H_{42}O_5$	10960835	
	Carotenoid B	$C_{40}H_{58}O$	14730337	
Microelement	K, Na, Ca, Mg, Fe, P, Mn, Cu, Cl, Co, Ti, Cr, Ni, Pb, Zn, Ba, La, Li, Mo, Al, V, Sr and Y			

calcium phosphate, magnesium sulfate, and other trace elements (Kong et al., 2010; 2012). Compared to NBC, BCA has a higher concentration of total bile acids, but a lower level of BR (Yan et al., 2007). Noticeably, BCA and NBC share similar physical and chemical properties, but their chemical components' content and ratio differ. As a result, there may be some differences in clinical efficacy compared to NBC. The *Pharmacopoeia of the People's Republic of China* therefore specifies that BCA must contain a minimum of 13.0% bile acid and at least 0.63% BR in dried form (Chinese Pharmacopoeia Committee of People's Republic of China, 2020).

4.3 Bovis Calculus sativus

Bovis Calculus Sativus (BCS) is artificially cultivated using fresh bile from cattle as the mother liquor. It involves the addition of CA, DCA, and other compounds to mimic the process of stone formation in cattle *in vitro* through physical and biochemical pathways. According to researches, it has similar physical and chemical properties to NBC and has similar clinical efficacy. However, the BR and BV content of BCS is lower than that of NBC. And the total bile acid content is higher than that of NBC (Cai et al., 2004; Wan et al., 2009). *The Pharmacopoeia of the People's Republic of China* stipulates that BCS must contain not only a minimum of 6.0% bile acid but also at least 35% BR in dried form (Chinese Pharmacopoeia Committee of People's Republic of China, 2020).

In addition, it has been demonstrated that the bound component of BC drugs has better efficacy compared to the free component. For instance, the NBC contains more bound bile acids and bound bile pigments, which may be one of the reasons for its better efficacy (Xu et al., 2017).

4.4 Comparison of chemical components of three types BC

The experiments demonstrated that Tau and conjugated bile acids content in BCS and NBC was significantly higher compared to the content in BCA (Feng et al., 2015). There was a significant difference in bile acids content between NBC and its two alternatives, BCS and BCA (Kong et al., 2012). Researchers utilized the Hierarchical Cluster Analysis (HCA) to categorize NBC into three groups, A, B, and C, based on quality. They also assessed conjugated bile acids and free bile acids levels in BCS and the various NBC groups. The results indicated higher levels of conjugated bile acids in BCS and group A (High-quality NBC), and higher levels of free bile acids in group B (Common NBC). High-quality NBC is similar to BCS in bile acids (Li et al., 2022). Additionally, study revealed that the total content of six bile acids in 33 batches of NBC samples ranged from 0 to 245.89 mg/g, while the total bile acids content in 2 batches of BCA samples ranged from 178.48 to 194.22 mg/g, and 3 batches of BCS samples ranged from 41.01 to 107.3 mg/g (Kong et al., 2012).

Furthermore, TCA content in NBC is significantly higher compared to the content of TCA in BCA. The content of GCA in BCA is notably higher than in BCS, while the content of GCA in NBC is relatively lower (Shimada et al., 2013). Utilizing liquid chromatography coupled with triple quadrupole mass spectrometry, researchers measured the levels of various bile acids in NBC, with GCA being the highest at 46%, followed by TCA at 25%, GDCA at 17%, TDCA at 10%, and GCDCA, GHDCA, and TCDCA each below 1%. In addition, in BCA, the content of CA reached 35%, TCA at 19%, DCA at 11%, HDCA at 10%, TDCA at 9%, GCA at 9%, and GDCA and CDCA both at 3% (Qiao et al., 2011). Chromatographic analysis also revealed that in BCA, the content of CA, CDCA, DCA, UDCA, TCDCA, and TDCA

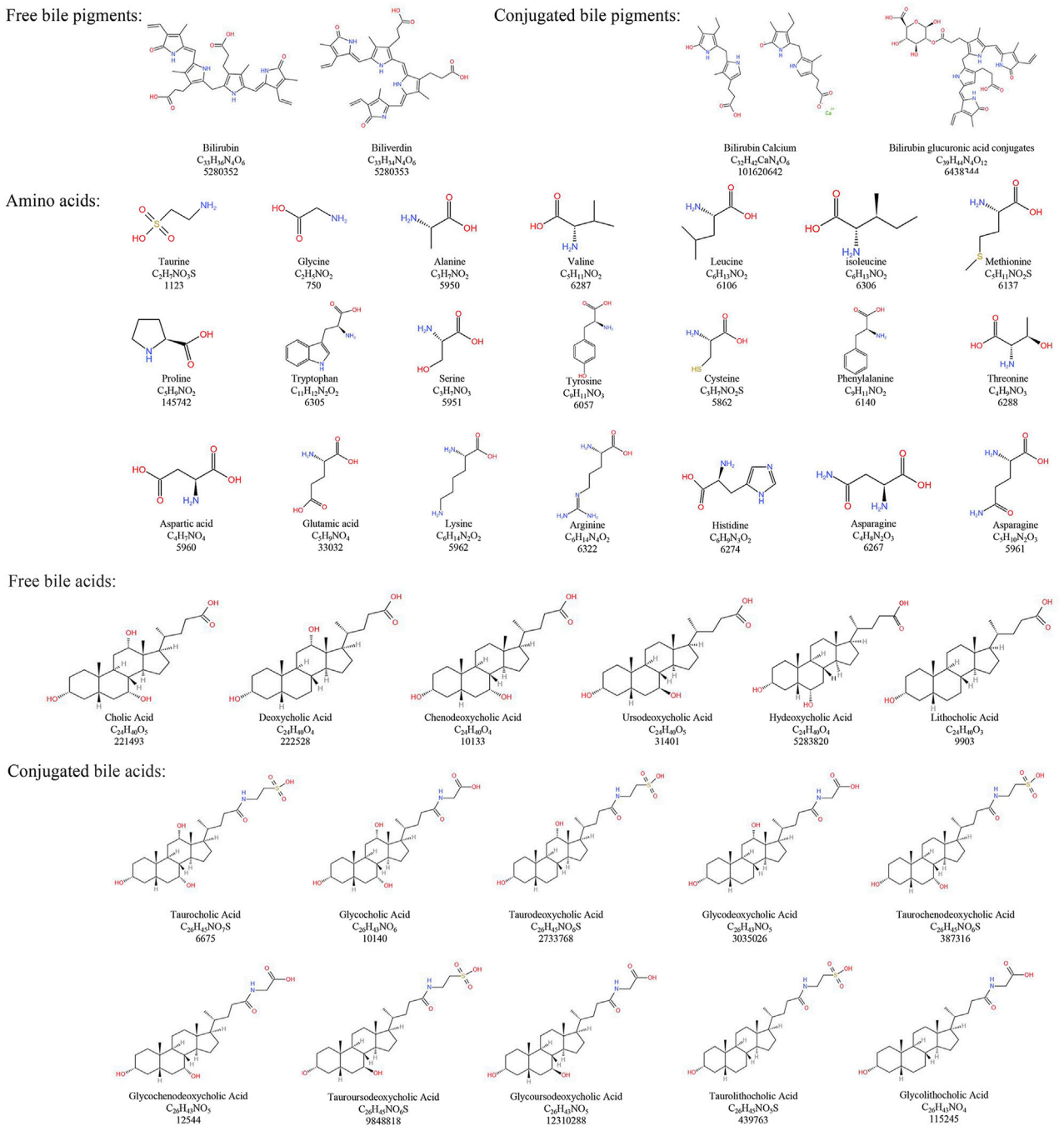


FIGURE 1
Chemical structure of bile pigments, amino acids, and bile acids in *Bovis Calculus*.

exceeded 9.88 μg/mg, while the bile acids content in NBC was lower (Ye et al., 2016). Due to commercial factors, there were significant variations in the three types of BC bile acids content, with bile acids content generally lower in NBC compared to BCS and BCA; it is possible that BCA may have higher bile acids content than NBC.

Additionally, the two alternatives to NBC, BCA and BCS, contain higher amounts of Tau, CA, Fe, Mg, and Ca. These components can serve as markers to differentiate NBC from its alternatives (Shimada et al., 2013). Researchers identified a new chemical compound in BCS named 3α, 12α-dihydroxy-7-oxo-5α-cholanic acid, which was scarcely detected in NBC and BCA. Furthermore, glycocholic acid, glycodeoxycholic acid, and taurocholic acid (TCA) were detected as biomarkers in NBC and BCA (Liu et al., 2015).

In conclusion, BCS is uniformly produced by pharmaceutical companies, with stable chemical composition. BCA is synthesized from various raw materials in specific proportions, resulting in higher purity and uniform chemical composition. On the other hand, NBC has a complex origin closely linked to the cattle's

TABLE 2 Summary of anti-cerebral ischemia injury experiment of *Bovis Calculus*.

Drugs	Animal models and methods	Results	Reference
Taurine	1. Animals: SD rats (♂), 280–340 g 2. Model: MCAO 3. Ischemia time: Unclear 4. Route of administration: iv 5. Drug treating times: 4 h after MCAO 6. Administration dosage: 50 mg/kg	1. Tau treatment reduced cerebral infarction volume in rats 2. Tau treatment reduced the permeability of BBB in rats 3. Tau treatment almost completely abolished gelatinolytic activity in ischemic brain microvessels 4. Tau treatment significantly inhibited the increase of MMP-9 activity induced by CI 5. Tau treatment significantly inhibited the expression of CD147 protein induced by CI 6. Tau blocks tPA-related bleeding by inhibiting CD147 - dependent MMP-9 pathway in ischemic brain endothelial cells after IS	Jin et al. (2018)
Taurine	1. Animals: SD rats (♂), 250–280 g 2. Model: tMCAO 3. Ischemia time: 2 h 4. Route of administration: ip 5. Drug treating times: 48 h, 24 h and 2 h before MCAO, 0.5 h and 12 h after MCAO 6. Administration dosage: 200 mg/kg	1. Tau treatment reduced neurological deficit score in rats 2. Tau treatment reduced the volume of cerebral infarction in rats 3. Tau pretreatment reduced brain water content in rats 4. Tau (600 mg/kg ig) treatment reduces mean blood pressure and heart rate in conscious and anesthetized rats 5. Tau treatment can significantly restore the number of neurons in hippocampus and cortex after MCAO 6. Tau inhibits the expression of caspase-3 in cerebral infarction area	Wang et al. (2007a)
TUDCA	1. Animals: SD rats (♂), 180–200 g 2. Model: tMCAO 3. Ischemia time: 2 h 4. Route of administration: iv 5. Drug treating times: 1 h after modeling 6. Administration dosage: 400 mg/kg	1. TUDCA treatment decreased the level of Glu 2. TUDCA treatment remarkably improved the degree of cerebral infarction in ACI rats 3. TUDCA treatment improved the blood lipid level in ACI rats 4. TUDCA treatment inhibited mRNA and protein expressions of TNF-α, IL-8 and hs-CRP and alleviated inflammatory response 5. TUDCA treatment inhibited the expression of MDA and ox-LDL, increased the expression of SOD and GSH-Px, and alleviated the ACI-induced oxidative stress injury 6. TUDCA reduced the expression of VLDLR and NF-κB proteins and downregulated the expression of Bax and caspase-3 through negative regulation of Nrf2 signaling pathway	Bian et al. (2019)
Taurine	1. Animals: SD rats 2. Model: MCAO 3. Ischemia time: Unclear 4. Route of administration: iv 5. Drug treating times: 1 h after CI 6. Administration dosage: 50 mg mL^{-1}·kg^{-1}	1. Tau treatment significantly reduced the number of microglia containing MHC I antigen in rat CI area 2. Tau treatment significantly reduced the number of MHC II antigen positive microglia in cerebral ischemic area of rats 3. Tau inhibited NF-κB activity in ischemic penumbra of rats 4. Tau significantly inhibited TNF-α activity in ischemic penumbra of rats	Wang and Xu (2017)
CA + HDCA	1. Animals: SD rats (♂), 310–350 g 2. Model: pMCAO 3. Ischemia time: permanent 4. Route of administration: ip 5. Drug treating times: 1 h before MCAO 6. Administration dosage: 21 mg/kg	1. CA + HDCA treatment reduced infarct volume in CI rats 2. CA + HDCA treatment downregulated the expression of TNF-α in CI rats 3. CA + HDCA treatment inhibited IL-1β level in CI rats 4. CA + HDCA treatment reduced endothelial cell injury and plasma vWF concentration in CI rats 5. CA + HDCA treatment reduced neuronal damage and serum NSE level in rats with CI	Hua et al. (2009)
CA	1. Animals: Newborn SD rats 2. Model: *in vitro* NVU 3. Ischemia time: 1 h 4. Route of administration: coculture 5. Drug treating times: 24 h before OGD/R, 1 h under OGD, 24 h after OGD/R 6. Administration dosage: 93.75 μg/mL, 11.72 μg/mL	1. CA treatment improved the BBB function remarkably 2. CA has a significant protective effect on the BBB characteristics and neurons in the NVU after OGD/R 3. CA treatment inhibited the expression of IL-1β, IL-6 and TNF-α 4. CA pretreatment fully reversed OGD/R induced cell apoptosis in neurons	Li et al. (2020b)
HDCA	1. Animals: SD rat pups 2. Model: *in vitro* NVU 3. Ischemia time: 1 h 4. Route of administration: coculture 5. Drug treating times: 24 h before OGD/R, 1 h under OGD 6. Administration dosage: 10.16 μg/mL, 2.54 μg/mL	1. HDCA treatment protected the cell in a dose-dependent manner 2. HDCA treatment markedly improved the integrity of the BBB following OGD/R 3. HDCA treatment significantly inhibited the release of TNF-α, IL-1βand IL-6 4. HDCA treatment significantly lower the apoptosis cells of OGD/R 5. HDCA treatment increased GDNF and BDNF expression and secretion levels in a dose-dependent manner	Li et al. (2019a)
Taurine	1. Animals: SD rats (♂), 300–320 g 2. Model: tMCAO 3. Ischemia time: Unclear 4. Route of administration: iv 5. Drug treating times: 1 h after MCAO 6. Administration dosage: 50 mg mL^{-1}·kg^{-1}	1. Tau treatment reduced the number of MHC I antigen positive microglia in ischemic gray matter, corpus callosum and caudate putamen of rats with CI 2. Tau treatment reduced the number of MHC II antigen positive microglia in ischemic gray matter, corpus callosum and caudate putamen of rats with CI	Wang et al. (2018)

(Continued on following page)

TABLE 2 (*Continued*) Summary of anti-cerebral ischemia injury experiment of *Bovis Calculus*.

Drugs	Animal models and methods	Results	Reference
Taurine	1. Animals: SD rats (♂), 315–340 g 2. Model: tMCAO 3. Ischemia time: 2 h 4. Route of administration: iv 5. Drug treating times: 2 h after MCAO 6. Administration dosage: 50 mg/kg	1. Tau treatment decreased the infarct volume and lessened the brain swelling significantly 2. Tau treatment markedly reduced the PARP levels in the cytosolic fractions in the core and in the nuclear fractions in the penumbra and core 3. Tau treatment suppressed the induction and activation of NF-κB in the penumbra and core after experimental stroke 4. Tau treatment markedly reduced the protein levels of TNF-α, IL-1β and ICAM-1 in the penumbra and core 5. Tau treatment markedly reduced the number of neutrophils in the penumbra and core 6. Tau treatment significantly reduced the cell death scores in the penumbra and core	Sun et al. (2012)
Taurine	1. Animals: SD rats (♂), 200–250 g 2. Model: pMCAO 3. Ischemia time: permanent 4. Route of administration: ip 5. Drug treating times: 1 week before MCAO 6. Administration dosage: 250 mg/kg	1. Tau pretreatment significantly improved cerebral infarction volume in rats with CI 2. Tau pretreatment inhibited the decrease of SOD activity after ischemia for 6 h 3. Tau pretreatment inhibited ICAM-1 expression in CI rats	Luo et al. (2004)
Taurine	1. Animals: Wistar rats, 250–300 g 2. Model: tMCAO 3. Ischemia time: 1 h 4. Route of administration: iv 5. Drug treating times: 30 min before MCAO 6. Administration dosage: 200 mg/kg	1. Tau pretreatment reduced cerebral infarction volume in rats with CI 2. Tau pretreatment reduced the swelling volume of ischemic brain tissue in ischemic rats 3. Tau pretreatment inhibits the expression of NF-κB in CI rats	Yang et al. (2006)
Biliverdin	1. Animals: SD rats (♂), 200–240 g 2. Model: tMCAO 3. Ischemia time: 2 h 4. Route of administration: ip 5. Drug treating times: 15 min before reperfusion, 4 h after reperfusion 6. Administration dosage: 35 mg/kg in 2 mL	1. BV treatment markedly reduced the NSS of rats 2. BV treatment markedly reduced the infarction 3. BV treatment significantly downregulated the mRNA expression level of HO-1 4. BV treatment significantly decreased the mRNA expression of TNF-α, IL-1β, iNOS and IL-6	Li et al. (2017)
Biliverdin	1. Animals: 1). SD rats (♂), 200–240 g, 2). SD rats pups 2. Model: 1). tMCAO, 2). in vitro hippocampal neurons I/R injury 3. Ischemia time: 2 h 4. Route of administration: 1). ip, 2). coculture 5. Drug treating times: 15 min before reperfusion, 4 h、12 h and 20 h after reperfusion 6. Administration dosage: 1). 35 mg/kg, 2). 2 μg/mL	1. BV treatment remarkedly suppressed the mRNA and protein expression levels of IL-6, IL-1β and TNF-α 2. BV treatment remarkably enhanced cell viability following OGD/R injury 3. BV treatment remarkably suppress the expression of caspase-3 and Bax 4. BV treatment promoted the expression of Bcl-2	Li et al. (2021)
Bilirubin	1. Animals: SD rats (♂), 280–300 g 2. Model: tMCAO 3. Ischemia time: 2 h 4. Route of administration: ip 5. Drug treating times: 2 h after reperfusion 6. Administration dosage: 2 mmol/L	1. Apoptosis rate, MDA, 8-OHdG, Bax and caspase-3 levels in ischemic penumbra decreased significantly after BR treatment in rats with CI 2. Expression of SOD, GSH and Bcl-2 in ischemic penumbra after BR treatment in rats with CI	Zhang et al. (2016)
Biliverdin	1. Animals: Wistar rats (♂), 250–250 g 2. Model: tMCAO 3. Ischemia time: 1.5 h 4. Route of administration: ip 5. Drug treating times: Immediately after reperfusion 6. Administration dosage: 100 mg/kg	1. BV treatment rapidly increased the serum levels of BR in a dose-dependent 2. BV treatment significantly deceased the infarct area in the cerebral cortices 3. BV treatment prevented superoxide generation following tMCAO 4. BV treatment significantly decreased the number of stained cells for these oxidative injury markers in the cortex 5. BV treatment significantly reduced 4-HNE expressions	Deguchi et al. (2008)
BCS	1. Animals: SD rats (♂), 240–280 g 2. Model: tMCAO 3. Ischemia time: 1.5 h 4. Route of administration: ig 5. Drug treating times: pre-dose for 3 days, 1 h before MCAO, 6 h after MCAO 6. Administration dosage: 50 mg/kg, 100 mg/kg	1. BCS treatment has a protective effect on CI/RI 2. BCS treatment inhibited neuronal apoptosis in response to CI/RI 3. BCS treatment alleviated the mitochondrial damage by CI/RI	Lu et al. (2020)

Abbreviations: ig, intragastric administration; ip: intraperitoneal injection; iv: intravenous injection; pMCAO, permanent middle cerebral artery occlusion; SD-rats: Sprague-Dawley rats; tMCAO, transient middle cerebral artery occlusion; Tau, Taurine; BV, biliverdin; CA, cholic acid; TUDCA, tauroursodeoxycholic acid; BR, bilirubin; BCS, *bovis calculus sativus;* BCA, *bovis calculus artifactus;* NBC, natural *bovis calculus;* BBB, Blood-brain barrier; MMP-9, Matrix metalloproteinase-9; Glu, Glutamic acid; TNF-α, Tumor necrosis factor-α; hs-CRP, High-sensitivity c-reactive protein; MDA, malondialdehyde; SOD, superoxide dismutase; ROS, reactive oxygen species; GSH, glutathione; Bax, Bax, Bcl-2, associated X protein; NF-κB, Nuclear factor-κb; LDL-C, Low-density lipoprotein cholesterol; MCP-1, Monocyte chemoattractant protein-1; IL-1β, Interleukin-1β; NVU, neurovascular unit; OGD/R, Oxygen-glucose deprivation/reperfusion; GDNF, Glia cell line-derived neurotrophic factor; BDNF, Brain-derived neurotrophic factor; iNOS, inducible nitric oxide synthase; IS, Ischemic stroke.

environment, leading to significant variations in chemical composition among different batches.

5 Mechanisms of *Bovis Calculus* against CI/RI

The progression of CI/RI can be categorized into acute, subacute, and restoration stages. BC and its active constituents exhibit therapeutic effects on various aspects of CI/RI, with their mechanisms of action elucidated below (Table 2).

5.1 Mechanisms of against CI/RI in the acute stage

During the acute stage, reduced CBF leads to an insufficient supply of glucose and oxygen, resulting in unmet energy requirements for neurons, glial cells, and endothelial cells. Depolarization and reduced glutamate reuptake activity due to hypoxia leads to elevated extracellular glutamate levels. Elevated glutamate levels can cause intracellular calcium overload via N-methyl-D-aspartate ionotropic receptors (NMDAR) and metabotropic glutamate receptors.

5.1.1 Improving microcirculation disturbances

The optimal treatments for CI involve administering thrombolysis promptly within the treatment time frame, and quickly and efficiently rebuilding microvascular collateral circulation. Reinstating blood reperfusion in the ischemic region and penumbra is also a critical intervention. If CBF is not restored in time, microcirculation abnormalities in the ischemic core and penumbra will be closely associated with inflammatory cell infiltration, microthrombosis, vascular endothelial cell damage, and other variables. Cerebral edema resulting from IS exacerbates microcirculatory disruptions by compressing capillaries, underscoring the importance of minimizing microcirculatory disturbances post-ischemia. Furthermore, enhancing CBF in the ischemic penumbra is equally crucial during the acute phase of CI (Kulik et al., 2008; Przykaza, 2021).

Tau is a crucial active ingredient in BC that increases CBF in cerebral ischemic tissue (Zhou et al., 2004; Wang et al., 2016). Following CI, the interaction between extracellular matrix metalloproteinase inducer and platelet glycoprotein VI can lead to intravascular fibrin deposition and platelet adhesion, and subsequent microthrombus formation (Seizer et al., 2009; Jin et al., 2017). According to research, Tau helps reduce secondary thrombosis of microvessels post-CI, aiding in facilitating reperfusion. Furthermore, Tau can mitigate the risk of hemorrhagic transformation following recombinant tissue-type plasminogen activator (rt-PA) treatment by inhibiting the extracellular matrix protease-inducible factor-dependent matrix metalloproteinase-9 (MMP-9) pathway in cerebral tissue post-ischemia (Jin et al., 2018).

Earlier studies have indicated that platelet aggregation is a significant contributor to thrombosis. Tau can stimulate the release of endogenous arachidonic acid (AA) triggered by collagen and inhibit the synthesis of thromboxane A2 (Chen et al., 1991). Tau also demonstrates a notable inhibitory impact on platelet aggregation, which is induced by adenosine diphosphate (ADP), collagen, and AA. In addition, the combination of Tau with tetrandrine or neferine has the potential to further suppress platelet aggregation, which is induced by ADP, collagen, and AA (Huang and Rao, 1995; Qi and Rao, 1995). Hypertension, a major risk factor for CI, has a complex interplay with microcirculatory disturbances, contributing to endothelial dysfunction and hypoperfusion due to increased shear stress (Feihl et al., 2009; Cipolla et al., 2018). In patients with essential hypertension, Tau can lower blood pressure. Moreover, Tau helps to prevent the increase of serum Ca^{2+} in spontaneously hypertensive rats on a high-salt diet, leading to reduced blood pressure (Dawson Jr et al., 2000; Militante and Lombardini, 2002; El Idrissi et al., 2013).

When combined with indapamide, Tau has the ability to significantly reduce blood pressure in spontaneously hypertensive rats and also shorten the duration of the dosing cycle. The mechanism is to affect the intracellular Ca^{2+} concentration, then promote the phosphorylation of endothelium-derived nitric oxide (NO) synthase, and increase the release of NO. Thus, leading to blood vessel relaxation and a reduction in blood pressure (Zhao Y. Y. et al., 2018). Research has also revealed that Tau exerts a non-endothelium-dependent vasodilatory effect, which is through the concentration-dependent inhibition of the contraction. The contraction is that isolated porcine coronary artery rings induced by KCl, histamine, 5-hydroxytryptamine, and extracellular Ca^{2+} (Zhang et al., 2009).

In clinical settings, the combination of BCS with conventional drugs, such as antiplatelet medications, has been shown to effectively reduce intracranial pressure and improve circulation for CI. This combined approach yields significant therapeutic benefits, improving neurological deficit scores, blood lipid profiles, and hemorheological parameters (Cai et al., 2004).

Overall, the above findings indicate that BCS and Tau have the effects of alleviating microcirculation disturbance after CI and promoting complete reperfusion. The therapeutical effects are achieved by inhibiting thrombosis and dilating blood vessels during the acute stage injury of CI.

5.1.2 Suppressing neuronal excitotoxicity

Glutamate and aspartate are examples of excitatory amino acids (EAA), while gamma-aminobutyric acid (GABA) and glycine (Gly) are examples of inhibitory amino acids (IAA). All of these are neurotransmitters in the central nervous system. Following the occurrence of CI, neurons release large amounts of EAA, such as glutamate and aspartate. Due to insufficient ATP supply and diminished EAA reuptake, the accumulation of EAA triggers excitatory neurotoxicity by stimulating their respective receptors. This cascade of events is linked to inflammatory responses, oxidative stress, leading to neuronal demise (Deng et al., 2004; Korde et al., 2007; Xu et al., 2007; Li X. X. et al., 2016). Therefore, it is imperative to counteract the detrimental effects of amino acid overstimulation to alleviate the immediate damage caused by CI.

Tau serves as a prevalent inhibitory neurotransmitter in the central nervous system (Huxtable, 1992). Studies indicate that Tau in NBC comprises approximately 4.71% of the total amino acid

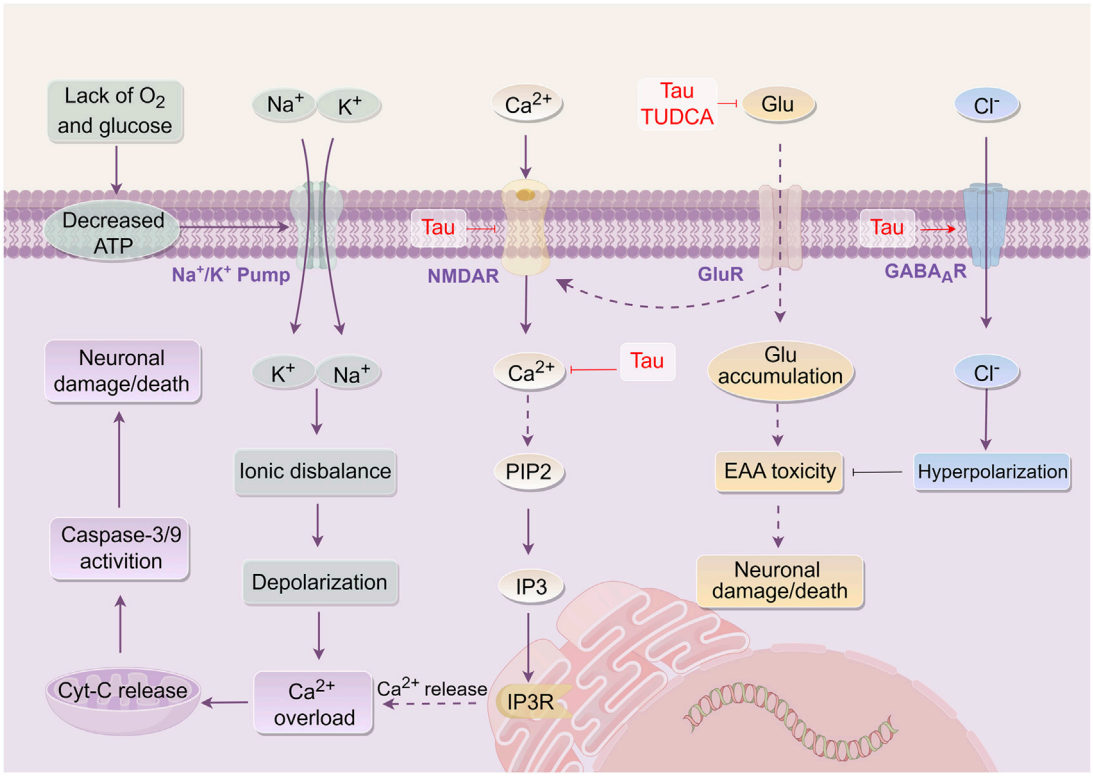


FIGURE 2
Mechanism of *Bovis Calculus* against excitatory amino acid toxicity in CI. Energy disturbances in mitochondria, abnormal ion pump function, and cell depolarization. Glutamate and other EAA are released into the intercellular space. Glutamate activates GluR, such as NMDAR, leading to the promotion of Ca^{2+} influx. This influx then produces IP3-3K, activating the binding of IP3 to endoplasmic reticulum IP3R, resulting in endoplasmic reticulum stress and the release of stored Ca^{2+} from the endoplasmic reticulum. The accumulation of Ca^{2+} in cells inhibits the function of mitochondria and worsens the disruption of energy metabolism. 1). Tau promotes the influx of Cl^- and hyperpolarizes the cell membrane by activating $GABA_AR$ and opening the chloride channel. 2). Tau promotes Ca^{2+} outflow and reduces intracellular Ca^{2+} concentration by inhibiting the activation of NMDAR. 3). Tau inhibits the binding of glutamate to its receptor, which ultimately affects endoplasmic reticulum stress and inhibits the release of Ca^{2+} from endoplasmic reticulum storage. 4). Tau and TUDCA can decrease the level of extracellular EAA and alleviate their toxicity. Abbreviations: ATP, Adenosine triphosphate; Tau, Taurine; TUDCA, Tauroursodeoxycholic acid; NMDAR, N-methyl-D-aspartate receptor; Glu, Glutamic acid; $GABA_AR$, γ-aminobutyric acid type a receptors; CytC, Cytochrome C; PIP2, phosphatidylinositol (4,5)bisphosphate; IP3, Inositol triphosphate; EAA, Excitatory amino acids.

content (Wen et al., 2020). Noticeably, Tau exhibits an anti-EAA toxicity effect (Wu and Prentice, 2010) by reducing neuronal excitability through the activation of γ-aminobutyric acid type A receptors ($GABA_AR$), increasing Cl^- influx, and hyperpolarizing the cell membrane (Wang G. H. et al., 2007). Additionally, Tau can mitigate hypoxia induced by focal CI, or the influx of Ca^{2+} caused by N-methyl-D-aspartate (NMDA). By modulating the transmembrane movement of Ca^{2+}, stabilizing the intracellular Ca^{2+} concentration, enhancing the energy barrier, and inhibiting the release of glutamate and aspartic acid (Asp), Tau aids in reducing EAA neurotoxicity (Sun et al., 2008; Zhao et al., 2012). Furthermore, TUDCA has been demonstrated to lower serum glutamate levels and inhibit EAA toxicity in rats subjected to transient CI in animal studies (Bian et al., 2019). Other studies demonstrated that NBC could elevate the levels of IAA, such as GABA and Gly in the rat brain striatum, thereby exert anti-EAA toxicity (Liu P. et al., 2010).

The aforementioned findings indicate that the active components of Tau and TUDCA exert anti-EAA toxicity by reducing EAA levels, activating $GABA_AR$, and increasing IAA content, thereby eliciting protective effects on the brain (Figure 2).

5.1.3 Anti-oxidative damage

CI/RI is closely associated with oxidative stress as a prominent pathological mechanism. Upon the occurrence of CI, an excess of reactive oxygen species (ROS) is generated. These ROS disrupt the delicate balance of the intracellular oxidation-antioxidation system. Besides, the excessive accumulation of ROS causes oxidative damage to lipids, proteins, and tissue cells. This oxidative stress triggers various signaling pathways, including mitochondrial and endoplasmic reticulum pathways, leading to the release of Cyt-C and apoptosis-inducing factors, ultimately resulting in nerve cell apoptosis (Wang and Huang, 2012; Rodrigo et al., 2013; Orellana-Urzúa et al., 2020). Thus, mitigating oxidative stress injury contributes to alleviating symptoms during the acute stage of CI.

BR, a key active component of bile components, serves as a potent antioxidant (Suh et al., 2018). It effectively reduces oxidative damage by enhancing the activity of antioxidant enzymes such as superoxide dismutase (SOD) and glutathione peroxide (GSH-Px). Furthermore, it diminishes the levels of oxidative injury markers, including malondialdehyde (MDA) and 8-hydroxy-2′-deoxyguanosine (8-OHdG) (Zhang et al., 2016). BV displays the ability to inhibit the generation of superoxide anion ($O_2^{\cdot-}$), lower

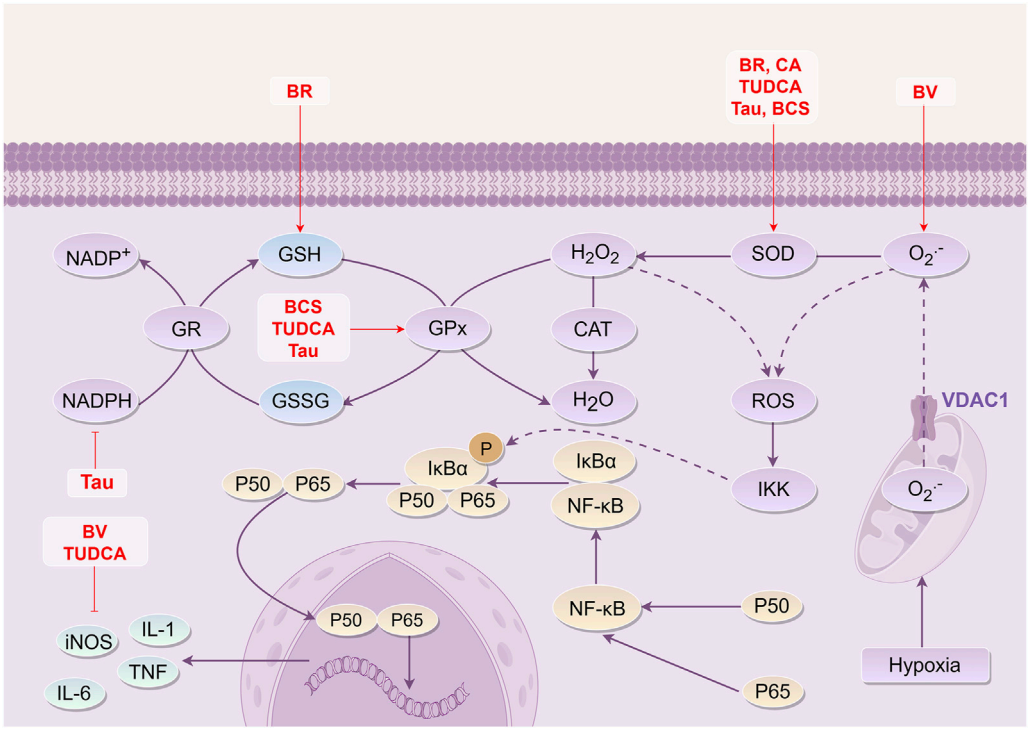


FIGURE 3
Mechanism of *Bovis Calculus* against oxidative stress injury in CI. Mitochondrial hypoxia disrupts ATP synthesis, causing a significant release of oxygen ions through the voltage-dependent anion channel 1 (VDAC1) on the mitochondrial membrane, which in turn disrupts the intracellular oxidation-antioxidation balance system. ROS reacts with intracellular organelles and phospholipid membranes, leading to cell damage. 1). BR can increase the content of SOD and GSH. 2). BV can inhibit the production of $O_2^{\cdot-}$ and the expression level of iNOS. 3). CA can increase the content of SOD. 4). TUDCA can inhibit the expression of iNOS and increase the content of SOD and GSH-Px. 5). Tau can inhibit the transformation between NADPH and $NADP^+$, increase the content of SOD and GSH-Px, and reduce the production of ROS. 6). BCS can increase the content of SOD and GSH-Px to play a role in anti-oxidative stress injury. Abbreviations: NADPH, Nicotinamide adenine dinucleotide phosphate; GR, gluathione reductase; GSH, Glutathione; GSSG, Glutathione (Oxidized); Gpx, Glutathione peroxidase; CAT, Catalase From *Micrococcus* Lysodeikticus; SOD, Superoxide dismutase; ROS, Reactive oxygen species; VDAC1, Voltage Dependent Anion Channel 1; IκBα, I-kappa-B-alpha; NF-κB, Nuclear factor-κb; IKK, IκB kinase; iNOS, Inducible nitric oxide synthase; IL-1, Interleukin-1; IL-6, Interleukin-6; TNF, Tumor necrosis factor; BR, Bilirubin; BCS, *Bovis Calculus sativus;* TUDCA, Tauroursodeoxycholic acid; Tau, Taurine; BV, Biliverdin; CA, Cholic acid.

lipid peroxidation levels, and reduce DNA oxidative damage. Additionally, it decreases MDA and 8-OHdG content while downregulating the expression of inducible nitric oxide synthase (iNOS) (Deguchi et al., 2008; Li et al., 2017).

CA, contained in BC, has been shown to elevate SOD levels and reduce MDA content following oxygen-glucose deprivation/reperfusion (OGD/R) in the neurovascular unit (NVU), exerting antioxidant effects (Li C. X. et al., 2020). TUDCA is one of the essential bile acid components of BC. It has the ability to reduce serum MDA and oxidized low-density lipoprotein levels, enhance antioxidant SOD and GSH-Px levels, and modulates oxidative stress injury through the Nrf2-VLDLR pathway in rats with transient middle cerebral artery occlusion (Bian et al., 2019). HDCA, present in BC, plays a significant role in regulating oxidative stress post-OGD/R in the NVU and exhibits an antioxidative stress injury effect (Li C. X. et al., 2019).

Although Tau within BC does not directly scavenge $O_2^{\cdot-}$, it can eliminate other oxygen radicals, such as hydroxyl radicals, thereby reducing tissue damage caused by oxygen radicals. This action enhances SOD, total anti-oxidant capacity (T-AOC), and GSH-Px levels, reduces MDA content, and inhibits the activity of nicotinamide adenine dinucleotide phosphate (NADPH) to confer antioxidant effects (Raschke et al., 1995; Hanna et al., 2004; Sun et al., 2008; Fang and Peng, 2013; Han et al., 2016; Zhu et al., 2016; Cai et al., 2018). Furthermore, BCS has been shown to enhance cellular tolerance to hypoxia, suppress free radical generation, and modulate neurotransmitters. These effects are linked to the augmentation of SOD and GSH-Px activity as well as T-AOC by BCS (Cai et al., 2007). Collectively, the findings suggest that BR, BV, TUDCA, HDCA, and Tau, as the active components of BCS or NBC, have antioxidant effects against stress injuries. These mechanisms involve scavenging oxygen free radicals or enhancing antioxidant activity (Figure 3).

5.2 Mechanisms of against CI/RI in the subacute stage

During the subacute stage, alterations in BBB permeability and the release of signaling molecules,such as cytokines, by astrocytes, microglia, and oligodendrocytes contribute to inflammatory injuries. In turn, reperfusion initiates a cascade of free radicals that result in apoptosis by inducing mitochondrial damage, cytochrome-C (Cyt-C) release, and activation of multiple

pathways. These interconnected factors create a "waterfall" cascade reaction of IS, ultimately leading to various forms of neuronal cell death, including necrosis and apoptosis, through continuous accumulation (Campbell et al., 2019).

5.2.1 Alleviating inflammation response

Excessive local inflammatory response tends to worsen brain damage during CI, leading to a poor prognosis (Lawrence, 2009; Cheng et al., 2020). Following CI, the build-up of ROS triggers the activation of complement, platelets, and endothelial cells, resulting in inflammatory substances generation such as interleukin-6 (IL-6), interleukin-1β (IL-1β), and tumor necrosis factor-α (TNF-α). Moreover, necrotic neurons release nucleosides that stimulate purine receptors on microglia and circulating macrophages. The process leads to the aggregation of inflammatory cells and the infiltration of neutrophils (Iadecola and Anrather, 2011; Fluri et al., 2015). According to research, Nuclear factor-κB (NF-κB) is activated by the phosphorylation of inflammatory transcription factors during inflammation. This activation allows NF-κB to enter the nucleus and regulate cytokine expression, leading to the production of numerous adhesion molecules. Simultaneously, inflammatory factors and adhesion molecules further induce NF-κB phosphorylation, intensifying the inflammatory response (Sun et al., 2014; Zhao H. et al., 2018). Therefore, anti-inflammatory measures are crucial for alleviating adverse symptoms following CI-reperfusion.

Different researches have revealed that CA, UDCA, TUDCA, HDCA, and Tau present in BC, possess anti-inflammatory properties that can reduce levels of TNF-α, IL-6, and IL-1β in CI. Additionally, these compounds demonstrate the capacity to inhibit the activation of NF-κB and mitigate the inflammatory response (Joo et al., 2003; Zhu et al., 2004; Hua et al., 2009; Wang and Xu, 2017; Bian et al., 2019; Li C. X. et al., 2020).

Among these constituents, CA has been shown to suppress TNF-α and IL-1β expression post-CI, reducing monocyte chemoattractant protein-1 (MCP-1) expression at the site of CI. Moreover, CA has the ability to impede microglia activation and the aggregation of monocyte macrophages to the injury site (Zhu et al., 2004; Hua et al., 2009; Li C. X. et al., 2020). Furthermore, UDCA has shown the ability to lower NO levels, decrease IL-1β and TNF-α expression, and inhibit microglia activation (Joo et al., 2003).

Specifically, TUDCA has the ability to stimulate adenylate cyclase and enhance cyclic adenosine monophosphate (cAMP) expression. TUDCA activates Takeda G-protein receptor 5 (TGR5) in microglia and triggers cyclic adenosine-dependent protein kinase A and cyclic adenosine-independent protein kinase A pathways to regulate the transcription of pro- and anti-inflammatory proteins. TUDCA also reduces the levels of high-sensitivity c-reactive protein (hs-CRP), TNF-α, and interleukin-8 (IL-8) through transcription and translation inhibition. Additionally, it negatively regulates the Nrf2 inflammatory pathway, contributing to its anti-inflammatory effects (Yanguas-Casás et al., 2017; Bian et al., 2019). Additionally, HDCA significantly reduces inflammatory response in NVU following OGD/R, and may exert anti-inflammatory effects through its conversion to bile acids like CA and TUDCA (Li C. X. et al., 2019).

Furthermore, Tau acts as a suppressive amino acid in the brain, suppressing NF-κB activation, and preventing neutrophil infiltration and neuron apoptosis by hindering microglia activation in ischemic tissues. Tau also hinders NO production, suppresses pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6, as well as adhesion molecules like intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1), and prostaglandin E2 (PGE2) (Luo et al., 2004; Yang et al., 2006; Sun et al., 2011; Wang and Xu, 2017; Wang et al., 2018). BV, another essential bile pigment in BC, exerts anti-inflammatory effects by down-regulating inflammatory mediators expression, including TNF-α, IL-6, IL-1β, and iNOS mRNA, and negatively regulating heme oxygenase-1 mRNA expression (Li et al., 2017; 2021).

Clinical studies have indicated that BCS is effective in reducing the inflammatory response in patients with CI, thereby decreasing neurological damage and providing neuroprotective effects (Wang et al., 2021).

In summary, the active constituents of BC, CA, UDCA, TUDCA, HDCA, and Tau exhibit anti-inflammatory effects. They achieve that by suppressing the production of inflammatory factors IL-1β, IL-6, and TNF-α. Additionally, these active constituents reduce the expression of MCP-1 at ischemic sites, lower NO levels, increase cAMP and activate TGR5. Furthermore, they regulate the transcription of pro- and anti-inflammatory proteins, reduce hs-CRP, inhibit microglia activation, suppress ICAM-1, VCAM-1, and PGE2, decrease iNOS mRNA, and regulate heme oxygenase-1 mRNA expression (Figure 4).

5.2.2 Protecting the blood-brain barrier

The BBB is a complex structure consisting of cerebral microvascular endothelial cells, endothelial cell occluding, and claudin, intercellular zonula occludens-1 (ZO-1), astrocyte terminal foot, pericyte, and basement membrane (Mark and Davis, 2002; Lampugnani and Dejana, 2007). More importantly, the BBB is responsible for maintaining the overall stability of the central nervous system and safeguard the brain from the infiltration of detrimental substances.

When CI occurs, the BBB function is compromised, leading to increased permeability. This allows harmful substances and cytokines to enter the brain to penetrate the brain tissue, causing damage (Marı´n-Teva et al., 2004; Choi et al., 2008; 2008; Hjort et al., 2008). Noticeably, when large amounts of matrix metalloproteinases are released, the tight junctions of the BBB are impaired, and permeability is increased (Vecsernyés et al., 2014). Additionally, increased expression of water channel proteins like aquaporin-4 (AQP-4) can result in hemorrhagic brain edema (Zhang H. et al., 2020; Shi et al., 2021). Additionally, endothelial cells express amounts of inflammatory substances such as TNF-α, IL-6, IL-1β, and ICAM. These substances enhance the permeability of vascular endothelial cells and contribute to the accumulation, adhesion, and infiltration of leukocytes in the area affected by infarction. Furthermore, ICAM-1-mediated neutrophil adhesion after reperfusion blocks microcirculatory channels and exacerbates tissue damage in CI (Amantea et al., 2007; Tuttolomondo et al., 2008; Vakili et al., 2011; Treda et al., 2016). Therefore, protecting the BBB's integrity is crucial in enhancing the therapeutic effect of cerebral ischemia-reperfusion and alleviate patients' symptoms.

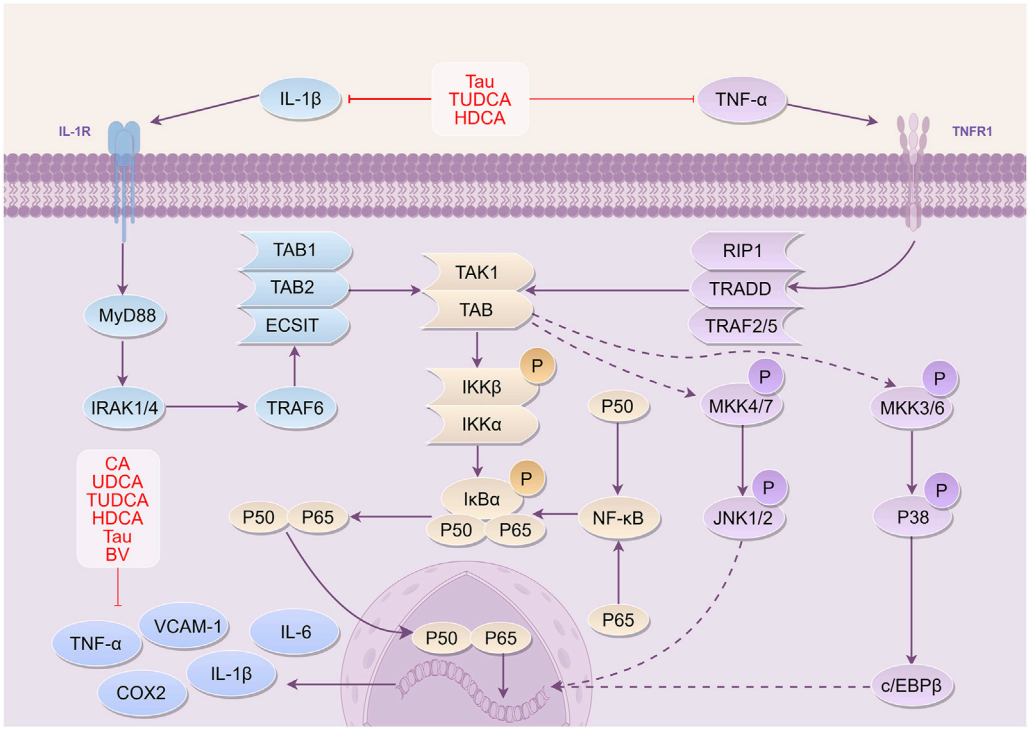


FIGURE 4
Mechanism of *Bovis Calculus* against inflammation in CI IL-1β binds to its receptor IL-1R and ultimately activates IκBα phosphorylation. NF-κB enters the nucleus to participate in the transcriptional expression of DNA and produce inflammatory cytokines. Binding of TNF-α to its receptor TNFR1 similarly phosphorylates and activates IκBα, allowing NF-κB to enter the nucleus. 1). CA inhibits the expression of inflammatory factors and adhesion molecules, reducing the accumulation of monocytes at the site of injury. 2). UDCA inhibits the expression of IL-1β and TNF-α and suppresses the activation of microglia. 3). TUDCA reduces the production of inflammatory factors and inhibits their binding to receptors. 4). Tau reduces TNF-α and IL-1β levels and inhibits their binding to receptors. 5). BV can downregulate the expression of inflammatory mediators such as TNF-α, IL-6, and IL-1β, thereby reducing inflammatory damage. 6). HDCA can inhibit the expression of inflammatory factors and can be converted into components such as CA and TUDCA, which exert neuroprotective effects. Abbreviations: IL-1β, Interleukin-1β; TNF-α, Tumor necrosis factor-α; TNFR1, tumor necrosis factor receptor 1; IRAK1, IL-1 receptor associated kinase 1; IRAK4, IL-1 receptor associated kinase 4; TRAF6, Tumor necrosis factor receptor-associated factor 6; ECSIT, Evolutionarily conserved signaling intermediate in Toll pathway; TAK1, TGF beta-Activated Kinase 1; TAB1, TGF-beta-activated kinase 1-binding protein 1; IKKβ, inhibitor of kappa B kinase β; IKKα, inhibitor of kappa B kinase α; IκBα, I-kappa-B-alpha; NF-κB, Nuclear factor-κb; RIP1, receptor-interacting protein 1; TRADD, tumor necrosis factor receptor-associated death domain protein; TRAF2, tumor necrosis factor receptor-associated factors 2; TRAF5, tumor necrosis factor receptor-associated factors 5; MKK4, Mitogen-activated protein kinase kinase 4; MKK7, Mitogen-activated protein kinase kinase 7; JNK1, c-Jun N-terminal kinase 1; JNK2, c-Jun N-terminal kinase 2; c/EBPβ, CCAAT/enhancer binding protein beta; TNF-α, Tumor necrosis factor-α; VCAM-1, Vascular cell adhesion molecule-1; IL-6, Interleukin-6; COX2, Cyclooxygenase-2; TUDCA, Tauroursodeoxycholic acid; Tau, Taurine; BV, Biliverdin; CA, Cholic acid; UDCA, Ursodeoxycholic acid.

Tau has been shown to reduce secondary brain injury after CI by down-regulating the expression of matrix metalloproteinases-3 (MMP-3) in brain-injured rats, thereby protecting the BBB (Li et al., 2010). Moreover, GUDCA inhibits the activation of MMP-9 and caspase-9 in a model of superoxide dismutase-1-induced neuronal degeneration, thereby exerting a protective effect on the BBB (Vaz et al., 2015). Additionally, the CA can protect the BBB function following NVU OGD/R. The mechanism is associated with the reduction of serum factor permeability coefficient and the increase in transepithelial electrical resistance (TEER) value and Gamma-glutamyl transferase activity (γ-GT) (Li C. X. et al., 2020).

Furthermore, investigation revealed that BC and its active constituents, including Tau, TUDCA, and UDCA, can decrease lactate dehydrogenase levels, increase γ-GT content, raise TEER values, and decrease fluorescein sodium permeability coefficient. These medications alone can lower the expression levels of inflammatory factors TNF-α, IL-6, and IL-1β, and reduce the protein expression of MMP-2 and MMP-9. Among them, Tau alone can stimulate the protein expression of tight junction protein ZO-1, occludin, and claudin-5 (Zhang et al., 2019). Combining Tau with UDCA can inhibit the MyD88/NF-κB pathway, while Tau and TUDCA combination can suppress the P38MAPK pathway, thereby exerting cerebral protective effects (Xu et al., 2017).

In conclusion, BC and its bioactive constituents, such as Tau, GUDCA, CA, TUDCA, and UDCA, collectively inhibit caspase-9 activation, reduce serum factors permeability coefficient, improve TEER and γ-GT activity, decrease inflammatory factors expression, block the MyD88/NF-κB and P38MAPK pathways, and promote the expression of tight junction proteins such as ZO-1, occludin, and claudin-5. These mechanisms play a crucial role in protecting the BBB's integrity.

5.2.3 Anti-apoptosis

Apoptosis plays a crucial role in neuronal death following CI. After CI, the brain has two important regions that are independent

of ischemia: the ischemic core and the ischemic penumbra (Kulik et al., 2008). Insufficient ATP supply renders Ca^{2+}-related transporter proteins ineffective. Conversely, when EAA is released and binds to EAA receptors on the postsynaptic membrane, it induces cell depolarization. This leads to a significant influx of Ca^{2+}, triggering the activation of Ca^{2+} proteases, the hydrolysis of cellular proteins, and ultimately resulting in neuronal death (Radak et al., 2017; Sekerdag et al., 2018).

The accumulation of calcium ions within cells can potentially induce the release of Cytochrome C (CytC) in mitochondria, which activates caspase-9 and the final executor protein caspase-3. This process leads to the breakdown of functional proteins within cells, causing DNA damage, and ultimately initiating apoptosis (He et al., 2020; Tuo et al., 2022). In addition, cell injury induces endoplasmic reticulum stress, which is an important site for intracellular protein translation, synthesis, modification, folding, and storage of Ca^{2+}. This stress will lead to the accumulation of unfolded proteins, disrupting Ca^{2+} homeostasis, and ultimately resulting in a further increase in intracellular Ca^{2+} concentration (Xu et al., 2017). Thus, the mitigation of symptoms during the subacute phase of CI and the enhancement of recovery outcomes are significantly influenced by preventing neuronal cell death.

Additionally, CA has been shown to inhibit the expression of caspase-9, caspase-3, and Bax after NVU OGD/R while increasing the expression of Bcl-2, exerting an anti-apoptotic effect (Li C. X. et al., 2020). Different researches have shown that Tau effectively prevents cell death and tissue damage by suppressing Bax activity, thus preventing the release of Cyt-C from mitochondria to the cytoplasm. Tau safeguards mitochondrial function, inhibits caspase-3 and calpain activity, and enhances the production of the anti-apoptotic protein Bcl-2. These actions help regulate the Bax/Bcl-2 ratio, ultimately resulting in anti-apoptotic effects (Schaffer et al., 2002; Zhang, 2006; Sun et al., 2011; Zhu et al., 2016).

Furthermore, Tau has been found to suppress endoplasmic reticulum stress by inhibiting the activation of transcriptional activator 6 and inositol-requiring enzyme-1 molecular pathways (Gharibani et al., 2013). TUDCA, as an important component of BC, inhibits the phosphorylation activation of protein kinase R-like endoplasmic reticulum kinase, eukaryotic initiation factor 2α, and transcriptional activator 4 expression in cortical and hippocampal regions. TUDCA also blocks caspase-12-dependent endoplasmic reticulum stress-mediated apoptosis (Rodrigues et al., 2002; Xu et al., 2017; Chen et al., 2020).

The active constituents of BC, BR, and BV, play a crucial role in inhibiting apoptosis. This is achieved by reducing the levels of caspase-3, Bax, and other proteins associated with apoptosis, while simultaneously elevating the concentration of Bcl-2 (Zhang et al., 2016; Li et al., 2021). A study demonstrated that pretreatment with BCS reduced brain swelling, damaged tissue size, neuronal cell death, and neurological impairments in rats. BCS also decreased the number of dying neurons, possibly by suppressing caspase-9 and caspase-3 activity, and Cyt-C release. This inhibition of mitochondria-mediated apoptotic signaling contributed to CI/RI reduction (Lu et al., 2020).

Overall, these findings highlight that the active components of BC, CA, Tau, TUDCA, BV, and BR, can protect mitochondria, inhibit endoplasmic reticulum stress, impede apoptosis, and exert neuroprotective effects through various mechanisms of action (Figure 5).

5.3 Mechanisms of against CI/RI in the restoration stage

In the late phase of CI, cell proliferation and differentiation lead to predominant repair mechanisms, which are characterized by extensive reconstruction of cell populations, angiogenesis, and regeneration of myelin. Among them, neovascularization helps to relieve local microcirculation disturbance after CI and reduces CI/RI. Specifically, neurogenesis plays a reparative role by producing neurotrophic factors at the injury site, thereby promoting neuronal regeneration (Steliga et al., 2020).

5.3.1 Promoting angiogenesis

The process of angiogenesis plays a crucial role in restoring blood oxygen supply to injured brain tissue and mitigating damage from IS. Additionally, within the central nervous system, there exists a significant interplay between blood vessels and axons. Angiogenesis also plays a crucial role in the growth of axons and the generation of new neurons, ultimately facilitating the development and differentiation of neurons. These contribute to restoring typical brain function (Millikan, 1970; Hatakeyama et al., 2020). Hence, the promotion of angiogenesis is crucial for brain function repair during the recovery phase following an IS.

Vascular endothelial growth factor (VEGF) has mitogenic and anti-apoptotic effects on endothelial cells and plays a pivotal role in angiogenesis and remodeling in the restoration stage of IS (Melincovici et al., 2018). According to research, Hypoxia-inducible factor (HIF-1α) plays a key role in promoting angiogenesis after IS by regulating the expression of downstream genes (Shi, 2009). Moreover, Angiotensin-1 (Ang-1) has been found to promote cerebral angiogenesis after IS through the Mas/eNOS-dependent pathway, reduce ischemic tissue injury, and enhance its tolerance to subsequent ischemia (Jiang et al., 2014).

Studies demonstrated that Tau presence in ischemic brain tissue enhances the production of HIF-1α mRNA, thereby promoting angiogenesis, microcirculation reconstruction, and reducing ischemic brain tissue damage following IS (Wang H. R. et al., 2007). Additionally, BCS activates 3-carboxyphosphatidylinositol kinase (p-PI3K) and serine/threonine protein kinase (p-Akt) proteins, leading to an upregulation in the expression of p-PI3K, p-Akt protein, and PI3K mRNA. This activation results in increased levels of serum VEGF and Ang-1 (Ren et al., 2023). Furthermore, BCS modulates the HIF-1α/VEGF and PI3K/AKT pathways, thus aiding in the restoration of normal brain function post-IS (Zhang et al., 2019).

In conclusion, BCS and its active component Tau promote angiogenesis following IS by modulating HIF-1α/VEGF and PI3K/Akt pathways, thereby contributing significantly to the restoration of normal brain function subsequent to an IS.

5.3.2 Facilitating nerve regeneration

CI can lead to limited endogenous neurogenesis within the body, failing to adequately repair nerve damage following ischemia. The rehabilitation and reconstruction of neurological function play a vital role in the treatment of IS. Studies have indicated that stem cell-based therapy, which includes cell transplantation and stimulating endogenous neurogenesis, holds promise as a potential strategy for repairing and regenerating damaged brains. This innovative approach may offer a secondary treatment window for IS patients (Zhu et al., 2018).

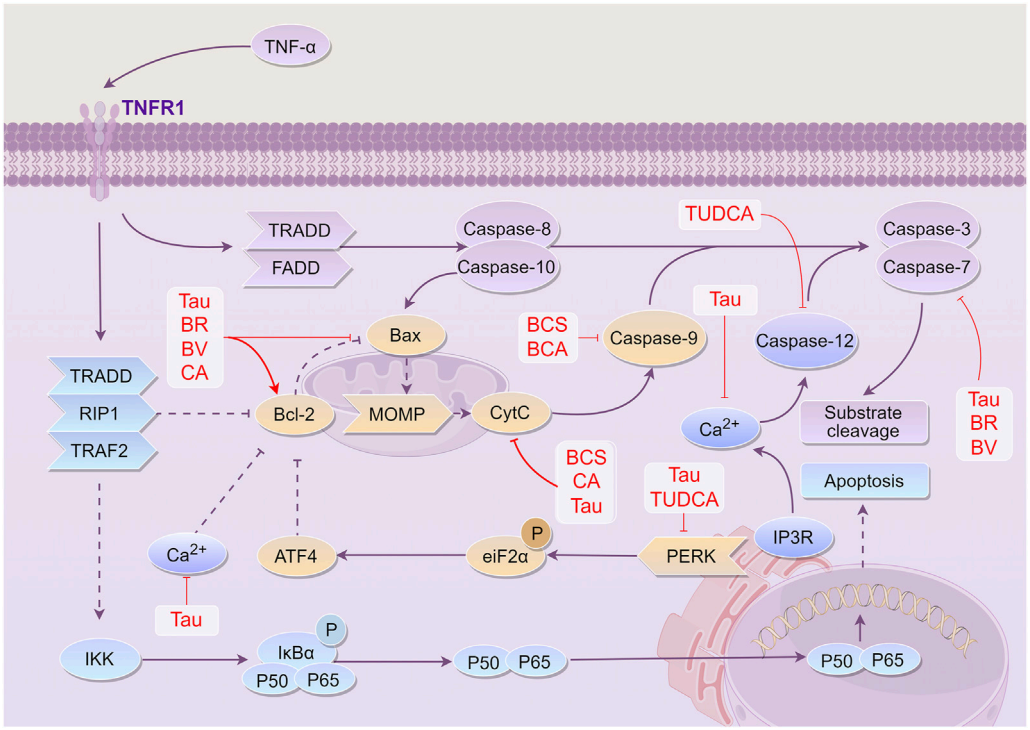


FIGURE 5
Mechanism of *Bovis Calculus* against cell apoptosis in CI. The ratio of Bcl-2 to Bax, a group of proteins involved in apoptosis and anti-apoptosis, determines whether cells are more likely to survive or die. TNF-α promotes the expression of Bax and inhibits the expression of Bcl-2 by activating the TNFR1 receptor. This activation eventually leads to the production of the apoptotic end protein caspase-3, which breaks down most of the functional proteins in the cell, resulting in apoptosis. 1). Tau inhibits the expression of caspase-3, the release of Ca^{2+}, activation of the PERK pathway, the expression of Bax, release of Cyt-C, and expression of caspase-9, and promotes the expression of Bcl-2. 2). BV inhibits the expression of caspase-3 and Bax and promotes the expression of Bcl-2. 3). BR promotes the expression of Bcl-2 and inhibits the expression of Bax and caspase-3. 4). CA can inhibit the expression of Bax, the release of Cyt-C, and the expression of caspase-9, and promote the expression of Bcl-2. 5). BCS can inhibit the release of Cyt-C and the expression of caspase-9. 6). TUDCA can inhibit the expression of the PERK pathway and CASP12 activated by endoplasmic reticulum stress, thus playing a role in cell protection and preventing apoptosis. Abbreviations: TNF-α, Tumor necrosis factor-α; TNFR1, tumor necrosis factor receptor 1; TRADD, tumor necrosis factor receptor-associated death domain protein; FADD, Fas-associating protein with a novel death domain; TRAF2, tumor necrosis factor receptor-associated factors 2; Bcl-2, B-cell lymphoma-2; Bax, Bcl-2 associated X protein; MOMP, mitochondrial outer membrane permeabilization; CytC, Cytochrome C; IP3, Inositol triphosphate; ATF4, Activating transcription factor 4; eiF2α, Eukaryotic translation initiation factor 2α; PERK, protein kinase RNA-like endoplasmic reticulum kinase; IKK, Inhibitor of kappa B kinase; IκBα, I-kappa-B-alpha; Tau, Taurine; BV, Biliverdin; CA, Cholic acid; TUDCA, Tauroursodeoxycholic acid; BR, Bilirubin; BCS, *Bovis Calculus sativus;* BCA, *Bovis Calculus artifactus*

Tau, a natural amino acid found in the brain, has been demonstrated to exert positive effects on brain growth and development (Huxtable, 1992; Tochitani, 2017). In cases of CI/RI, Tau has been shown to enhance the expression of neural stem cell factor (SCF) mRNA in the cortex, striatum, and paraventricular zone. Moreover, it promotes nestin expression and augments the population of neural stem cells during the ischemic recovery process (Zhang et al., 2008; Zang et al., 2011).

Tau facilitates the proliferation and differentiation of hippocampal neural stem cells induced by glutamic acid (Glu). This was achieved by activating the brain-derived neurotrophic factor (BDNF)/ERK/CREB signaling pathway and repairing brain injury after CI (Zhou, 2019). BDNF plays a crucial role in neuronal growth and development. Upon binding to the tyrosine kinase B receptor (TrkB), BDNF triggers downstream signaling pathways such as mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (Erk) and PI3K/Akt, governing neuronal proliferation and differentiation (Choo et al., 2017; Li C. X. et al., 2020).

As one of the important free bile acids present in BC, HDCA can effectively protect NVU in NVU ODG/R. HDCA might achieve this by promoting the expression and release of BDNF and glia cell line-derived neurotrophic factor (GDNF), thereby fostering neural recovery (Li C. X. et al., 2019). Moreover, Nerve growth factor (NGF) contributes to nerve growth. After an IS, the content of NGF in brain tissue decreases, weakening the body's protective effect. One study demonstrated that BCA can increase the content of NGF and aid in nerve recovery in the recovery stage of IS (Li and Li, 2004).

In conclusion, BC and its active constituents have a significant impact on restoring nerve function after IS. This is achieved by stimulating SCF mRNA production, activating the BDNF/ErK/CREB signaling pathway, boosting BDNF and GDNF expression, and facilitating neuronal repair.

6 Clinical applications

6.1 Clinical actions

In *Chinese Pharmacopoeia*, the actions of NBC is to clear the heart, sweep phlegm, open the orifices, cool the liver, extinguish

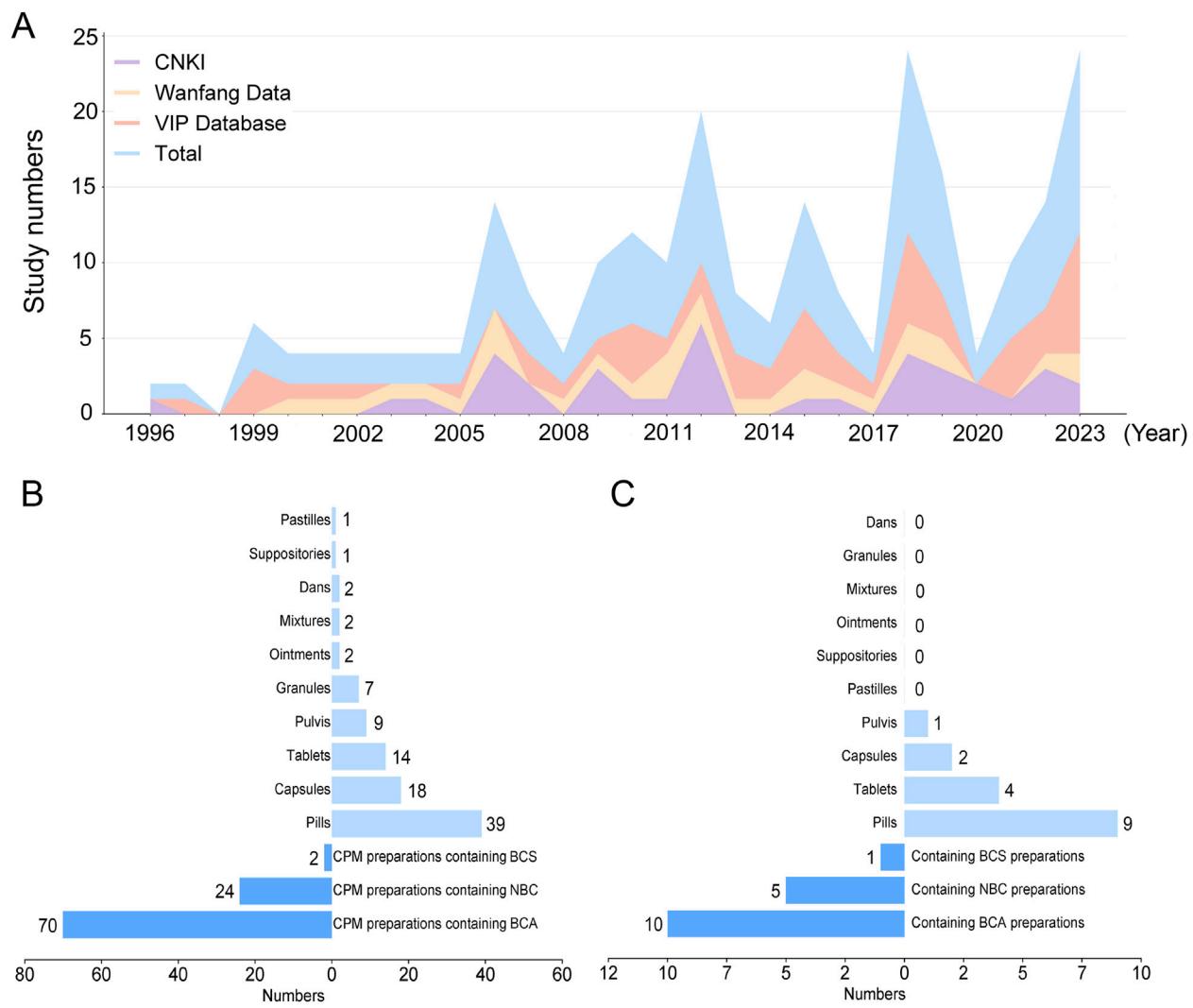


FIGURE 6
(A) A stacked area chart of the annual publication count and cumulative publication count for three databases each year (From 1996 to 2023) **(B)** The respective proportions of three types BC preparations in 95 preparations and the proportions of different dosage forms **(C)** The respective proportions of three types BC preparations in 16 preparations for the IS treatment and the proportions of different dosage forms Abbreviations: BCS, *Bovis Calculus sativus;* BCA, *Bovis Calculus artifactus;* NBC, Natural *Bovis Calculus.*

wind, and remove toxin. In addition, the actions of BCA is to clear the heart, remove toxin, resolve phlegm and relieve fright. Furthermore, the actions of BCS is to clear the heart, sweep phlegm, open the orifices, cool the liver, extinguish wind, and remove toxin (Chinese Pharmacopoeia Committee of People's Republic of China, 2020). Therefore, the actions of the three is similar, and NBC and BCS have the same effect. But because NBC is very expensive, its substitute BCS is widely used.

6.2 Clinical effects of BC

Currently, an increasing number of researchers are focusing on the clinical and preclinical studies of BC for IS (Figure 6A). Notably, there are 95 formulations of CPMs containing BC listed in the *Chinese Pharmacopoeia*. Among these, 16 formulations are explicitly indicated for the treatment of IS, accounting for 16.8% of the total. Therefore, from a formulations perspective, it is evident that BC is frequently used in the treatment of IS. In addition, in clinical practice, BC is also being used in combination with other drugs for IS treatment. Compared to the conventional treatment group, BC (particularly BCS and NBC) has been shown to enhance the therapeutic effects of conventional treatment. Furthermore, it has demonstrated the ability to promote the recovery of patients' neurological function and consciousness, improve patients' hemorheological indexes and inflammatory levels, as well as enhance the medium-to-long-term prognosis of patients (Table 3).

6.2.1 Alleviating neurological function and state of consciousness

Quantitative indicators play a crucial role in evaluating the neurological function and consciousness status of stroke patients. For instance, a higher National Institute of Healthstroke scale (NIHSS) score indicates that a stroke may lead to more severe

TABLE 3 Clinical application of BC in IS.

Study	Drug	Randomized controlled trial	Experimental subject		Treatment method		Course of treatment	Result	Clinical safety
			Treatment group	Control group	Treatment group	Control group			
Cai et al. (2004)	BCS	Yes	95 cases	32 cases	BCS (Three times a day, two tablets per time, 50 mg per tablet, oral application or nasal feeding)	NBC (Three times a day, two tablets per time, 50 mg per tablet, oral application or nasal feeding)	5 days	The effective rate was 86.3% in the treatment group and 80.6% in the control group	Routine blood test, urine test, stool test, liver function, and kidney function all showed no obvious abnormalities
Chen (2022)	BCS and Zhongfeng Xingnao liquid	Yes	23 cases	25 cases	BCS (Twice a day, 0.15 g per time, oral application) + Zhongfeng Xingnao liquid + Conventional treatment	Zhongfeng Xingnao liquid + Conventional treatment	14 days	1) The effective rate was 91.3% in the treatment group and 76.00% in the control group 2) The treatment group showed some improvement in NIHSS score, FOUR score, CRS-R score, and GCS score	1) The medications in the treatment regimen have no significant impact on liver function (Alanine Transaminase, Aspartate Aminotransferase), kidney function (serum creatinine), and electrolytes (K+, Na+, Ca2+); 2) The medications in the treatment regimen have no significant impact on coagulation function (APTT, INR); 3) The treatment regimen has no significant impact on the blood routine (RBC, WBC, PLT); 4) The adverse reactions related to the experimental drug were mild diarrhea, with 5 cases in the treatment group and 4 cases in the control group. Symptoms improved after taking the medication; 5) No signs of active gastrointestinal bleeding were observed
Liao (2020)	BCS and Gastrodin Injection	Yes	32 cases	32 cases	BCS (Twice a day, 0.15 g per time, nasal feeding) + Gastrodin Injection + Conventional treatment	Conventional treatment	14 days	1) The effective rate was 96.87% in the treatment group and 93.75% in the control group 2) Treatment group patients showed better improvement in ESR, HCT, TG, and LDL-C levels compared to the control group	The incidence of adverse reactions was 6.25% in the treatment group and 15.63% in the control group, this indicates that the treatment group can reduce adverse reactions
Liu et al. (2010b)	BCS	Yes	45 cases	45 cases	BCS (Twice a day, oral application or nasal feeding) + Conventional treatment	Conventional treatment	14 days	1) The effective rate was 95.6% in the treatment group and 84.4% in the control group 2) The treatment group showed a significant decrease in WBV, HCT, and FIB levels 3) The treatment group showed a significant decrease in cholesterol, TG, and LDL-C levels	There were no obvious adverse reactions in all cases, no significant changes in liver, kidney function and blood routine, and no bleeding tendency
Peng et al. (2019)	BCS	Yes	40 cases	40 cases	BCS (Twice a day, 0.3 g per time) + Conventional treatment	Vinpocetine Injection + Conventional treatment	14 days	1) The effective rate was 95.00% in the treatment group and 80.00% in the control group 2) The NIHSS score of the treatment group was significantly lower than that of the control group	The incidence of adverse reactions was 2.50% in the treatment group and 17.50% in the control group
Wang et al. (2021)	BCS	Yes	54 cases	54 cases	BCS (0.3 g per day, nasal feeding) + Conventional treatment	Conventional treatment	7 days	1) The GCS score of the treatment group was higher than that of the control group	There were no liver and kidney function damage and abnormal coagulation function

(Continued on following page)

10.3389/fphar.2024.1510779

TABLE 3 (*Continued*) Clinical application of BC in IS.

Study	Drug	Randomized controlled trial	Experimental subject		Treatment method		Course of treatment	Result	Clinical safety
			Treatment group	Control group	Treatment group	Control group			
								2) The NIHSS score and mRS score in treatment group were lower than that of the control group 3) Serum Hs-CRP, NSE and S-100β protein levels in the treatment group was lower than that in the control group	
Wu et al. (2023)	BCS	Yes	49 cases	49 cases	BCS (0.3 g per day, oral application) + rt-PA thrombolytic treatment	rt-PA thrombolytic treatment	7 days	1) The treatment group has a higher GCS score than the control group 2) The mRS score in treatment group were higher than that of the control group, BCS can significantly improve the prognosis of stroke patients	_
Xiang and Xiong (2023)	BCS	Yes	50 cases	50 cases	BCS (Twice a day, 0.15 g per time) + Conventional treatment	Conventional treatment	30 days	1) The treatment group has lower ESR, HCT, TG and LDL-C levels than the control group 2) The treatment group showed a significant decrease in NSE level and NIHSS score	The treatment group had two cases of adverse reactions, while the control group had nine cases
Zeng (2024)	BCS and Huatan Tongluo Decoction	Yes	30 cases	30 cases	BCS (Twice a day, 0.15 g per time, nasal feeding or oral application) + Huatan Tongluo Decoction + Conventional treatment	BCS (Twice a day, 0.15 g per time, nasal feeding or oral application) + Conventional treatment	14 days	1) The effective rate was 93.3% in the treatment group and 76.7% in the control group 2) The NIHSS score of the treatment group was significantly lower than that of the control group 3) The TCMSSS of the treatment group was significantly better than that of the control group	_
Zhang et al. (2015)	BCS	Yes	40 cases	38 cases	BCS (Once a day, 0.15 g per time) + Conventional treatment	Conventional treatment	14 days	The average G-Pcs scores in the treatment group at each time point after treatment were higher than those in the control group	_

Abbreviations: NIHSS, national institute of healthstroke scale; FOUR, Full Outline of UnResponsiveness; CRS-R, ComaRecoveryScale-Revised; GCS, glasgow coma scale; mRS, modified rankin scale; ESR, erythrocyte sedimentation rate; HCT, red blood cell hematocrit; TG, triglycerides; LDL-C, low density lipoprotein cholesterin; APTT, activated partial thromboplastin time; INR, international normalized ratio; RBC, red blood count; WBC, white blood cell; PLT, blood platelet; WBV, whole blood viscosity; FIB, fibrinogen; NSE, neuron specific enolase; Hs-CRP, hypersensitive C-reactive protein; TCMSSS, traditional chinese medicine syndrome score scale; G-Pcs, Glasgow-Pittsburgh coma scale.

10.3389/fphar.2024.1510779

neurological dysfunction. Building upon the NIHSS, the Full Outline of UnResponsiveness (FOUR) score considers indicators such as breathing pattern, eye movements, and swallowing reflex in patients. In clinical practice, the use of BCS in the treatment of IS with impaired consciousness can significantly reduce the NIHSS score, improve the FOUR score, indicating a marked enhancement in the neurological function and consciousness status of patients. Additionally, BCS is safe and stable, with a low incidence of adverse reactions (Chen, 2022).

Furthermore, a lower Glasgow Coma Scale (GCS) score indicates more severe neurological damage. Newly clinical research found that BCS can increase the GCS score and improve the prognosis of patients with IS accompanied by impaired consciousness (Wu et al., 2023). It is noteworthy that Coma Recovery Scale-Revised (CRS-R) is used to assess patients' cognitive function and neurological function. One study demonstrated that BCS can increase the CRS-R score, improving the cognitive function of patients with IS and impaired consciousness (Chen, 2022). Otherwise, a lower Modified Rankin Scale (mRS) score indicates a better consciousness state in patients. In a new clinical study, treatment with BCS for IS patients can lower the mRS score, leading to a favorable prognosis (Wu et al., 2023).

6.2.2 Improving the hemorheological indicators levels

Clinical studies have shown that elevated levels of cholesterol, triglycerides (TG), and low-density lipoprotein cholesterol (LDL-C) are closely associated with the occurrence of stroke. For example, abnormal levels of erythrocyte sedimentation rate (ESR), hematocrit (HCT), whole blood viscosity (WBV), and fibrinogen (FIB) can lead to abnormal blood viscosity, resulting in reduced blood rheology. Otherwise, clinical trials found that BCS has been shown to significantly improve cholesterol, TG, and LDL-C levels, alleviate symptoms of acute IS, promote the recovery of neurological function, improve CBF, and enhance clinical treatment outcomes (Liao, 2020). Additionally, BCS can lower HCT, WBV, and FIB levels, improve blood viscosity, and enhance blood circulation (Liu X. Y. et al., 2010).

6.2.3 Lowering inflammation levels

Serum levels of hs-CRP, neuron specific enolase (NSE), and S-100β protein are commonly used to evaluate neurological damage. Hs-CRP serves as an inflammatory marker, and elevated levels indicate the presence of an inflammatory response in patients. S-100β protein levels can reflect the severity of central nervous system inflammatory responses. The serum levels of hs-CRP and NSE are associated with the severity and prognosis of IS. Noticeably, NSE can serve as a quantitative index of central nervous system damage (Pandey et al., 2014; Selçuk et al., 2014). Clinically, BCS can reduce serum levels of hs-CRP, NSE, and S-100β protein, effectively reducing central nervous system inflammatory responses, alleviating neurological damage, improving medium-to-long-term prognosis, and possessing high clinical utility (Wang et al., 2021).

6.3 Combination drug therapy

The positive therapeutic impact of BC and its active constituents on IS injury has been demonstrated. When combined with other medications, BC can enhance therapeutic outcomes and minimize adverse reactions. According to research, NBC combined with *Gardenia jasminoides* have the ability to increase SOD activity and MDA content in brain tissue after 72 h of ischemia-reperfusion, reduce the injury from lipid peroxidation, and enhance brain tissue antioxidant capacity (Li et al., 2004). Additionally, CA combined with geniposide has been shown to reduce the levels of inflammatory factors such as TNF-α, IL-1β, and ICAM-1 in ischemic tissue, thereby decreasing the inflammatory response (Zhu et al., 2004). Moreover, Tau combined with *Panax notoginseng* saponins improved SOD and MDA levels in ischemic tissue and enhanced the antioxidant capacity in ischemic brain tissue (Fang and Peng, 2013). Noticeably, the simultaneous utilization of BC and gardenia has the potential to enhance NGF expression levels and facilitate nerve regeneration (Li and Li, 2004). In rats, the administration *Bovis Calculus*-Fel Uris-Moschus contribute to increase rCBF, lower mean arterial blood pressure, and dilate guanylate cyclase-related cerebral vessels (Park et al., 2013). Guanylate cyclase (GC) is an enzyme that converts guanosine triphosphate (GTP) into cyclic guanosine monophosphate (cGMP), a second messenger that plays a critical role in various physiological processes, including vasodilation. In the context of cerebral vessels, guanylate cyclase is involved in mediating the effects of nitric oxide (NO), which is produced by endothelial cells in response to various stimuli. Activation of guanylate cyclase by nitric oxide leads to an increase in cGMP levels, resulting in smooth muscle relaxation and subsequent vasodilation of cerebral blood vessels. This mechanism is crucial for regulating cerebral blood flow and maintaining adequate oxygen supply to the brain (Xiong et al., 2023) Otherwise, Gastrodia elata contains various active compounds, such as gastrodin, which have been shown to have antioxidant effects and promote neuronal survival following ischemic injury. Research indicates that these compounds can enhance cerebral blood flow and improve neurological outcomes by mitigating oxidative stress and inflammation in the brain. Thus, when combined with BCS, they may play a crucial role in improving not only the peripheral venous blood sedimentation rate, HCT, TG, and LDL-C in patients with acute CI, but also in enhancing the overall rehabilitation of neurological functions (Liao, 2020).

In the laboratory, Tau combined with diazepam effectively activate both GABA and Gly receptors, hyperpolarize the cell membrane, reduce neuronal excitability, antagonize the EAA toxicity after CI, and regulate the synthesis and release of EAA and ROS (Wang et al., 2010). In addition, Tau combined with strychnine could promote nerve repair in the CA1 area of the hippocampus of ischemic rats. Result in the density of surviving neurons was significantly higher than that in the control group (Li, 2006). One study demonstrated that the combination of Tau and tPA had the ability to reduce the risk of stroke hemorrhagic transformation and secondary thrombosis of microvessels. This is achieved through inhibiting the CD147 protein-dependent MMP-9 pathway in endothelial cells of rats with CI (Jin et al., 2018). Furthermore, Tau combined with urokinase has been shown to significantly improve the infarct volume, prolong the treatment time window, enhance the neurological prognosis, and reduce the risk of hemorrhagic transformation after IS in rats with CI (Guan et al., 2011).

6.4 Analysis of preparations containing BC

We analyzed the proportions of three types of BC in 95 preparations and the proportions of various dosage forms. Among these CPM preparations, BCA is the most widely used with 70 preparations (73.7%), 24 preparations (25.3%) containing NBC, and two preparations (2.1%) containing BCS. Specifically, the type of BC used in Xihuang pill is either NBC or BCS. Besides, our findings indicate that the clinical applications of BC mainly include 10 dosage forms, including 39 pills (41.1%), 18 capsules (18.9%), 14 tablets (14.7%), 9 pulvis (9.5%), 7 granules (7.4%), 2 ointments (2.1%), 2 mixtures (2.1%), 2 dans (2.1%), one suppository and one pastille. It is noteworthy that among the 39 pills, Angong Niuhuang Pill is the most widely used (Figure 6B).

Furthermore, we analyzed the proportions of three types of BC in IS treatment (16 preparations) and the proportions of various dosage forms. Among these CPM preparations, BCA is the most widely used with 10 preparations (62.50%), 5 preparations (31.25%) containing NBC, and one preparation (6.25%) containing BCS. According to our statistics and analysis, the clinical applications of BC in IS treatment mainly include 4 dosage forms, including 9 pills (56.25%), 4 tablets (25.00%), 2 capsules (12.50%), and one pulvis (6.25%) (Figure 6C). Overall, these insights provide valuable information about the prevalence of different types of BC in CPM preparations and their applications in the treatment of IS, along with the diverse dosage forms utilized in clinical practice.

6.5 Representative CPM preparations containing BC

Notably, three specific BC preparations targeting anti-IS are detailed in (Table 4), each providing essential information such as the preparation name, type, SFDA approval number, actions, and indications. Among these formulations, the noteworthy prescriptions incorporating NBC, BCA, and BCS are Ershiwuwei Zhenzhu Wan, Angong Niuhuang Pill, and Annao Pian, respectively (Chinese Pharmacopoeia Committee of People's Republic of China, 2020; Peng et al., 2022). These meticulously crafted preparations hold significance in the field of traditional Chinese medicine, underscoring their role in combating IS as highlighted in the *Chinese Pharmacopoeia*.

Ershiwuwei Zhenzhu Wan functions to decrease TNF-α, IL-1β, and IL-6 expression in the serum of pMCAO rats, thereby exerting anti-inflammation and antioxidation. Additionally, it enhances the activity of SOD and CAT while decreasing MDA levels. It participates in neuronal repair and regeneration by up-regulating Notch1, Jagged1, and Hes1. Moreover, Ershiwuwei Zhenzhu Wan inhibits neuronal apoptosis by up-regulating Bcl-2 mRNA and reducing the expression of caspase-3 protein. Specifically, in rats with CI, this treatment shows promise in increasing VEGF levels, promoting angiogenesis, and decreasing cerebral capillary permeability, brain water content, and brain edema. This may effectively prevent brain edema resulting from experimental CI in rats (Zhang et al., 2006; Liu et al., 2022). Different clinical research has demonstrated that Ershiwuwei Zhenzhu Wan may enhance the average velocity of blood flow in the brain, as well as improve blood circulation in the left and right vertebral arteries and the basilar artery. These improvements in hemodynamics help prevent blood stagnation, lower blood lipid levels, and reduce platelet aggregation and adhesion. Furthermore, it has the potential to enhance blood flow in microcirculation and exhibits a positive therapeutic impact on memory impairment resulting from cerebrovascular disease (Wang et al., 2013; Wang, 2021).

Studies demonstrated that Annao tablet has the ability to facilitate the transformation of astrocyte phenotype from A1 to A2 and microglia phenotype from M1 to M2 in the rat cortex, contributing to the maintenance of brain homeostasis and the enhancement of brain function after injury (Zhang, 2019). Annao tablet also improves mitochondrial fusion and mitotic disorders by increasing fusion and cleavage proteins Opa1 and Drp1, activating the PINK1/Parkin pathway, promoting mitochondrial autophagy in neurons, and timely removing damaged mitochondria. Additionally, Annao tablet has the potential to enhance the transcription level of Bcl-2 mRNA, augment the ratio of Bcl-2/Bax to impede apoptosis, diminish the count and protein expression of caspase-3 positive cells, decrease the number of neuronal apoptosis at the periphery of cerebral infarction, ameliorate the neurological deficit following ischemia, and elevate the count of positive neurons (Zhang Y. et al., 2020). Moreover, Annao tablet significantly enhances the cognitive abilities of rats with bilateral common carotid artery ischemia. It effectively elevates the concentration of acetylcholine in the hippocampus and mitigates neuronal apoptosis (Xu and Luan, 2016). Clinical trials in this century found Annao tablet is effective in improving acquired cognitive dysfunction syndrome and headache symptoms in patients with cerebral apoplexy (Yang and Luo, 2015).

Angong Niuhuang Pill exhibits anti-inflammatory properties by suppressing TNF-α, IL-1β, and iNOS mRNA expressing in the cerebral tissue of ischemic rats (Liu Y. X. et al., 2011; Yan et al., 2017). Angong Niuhuang Pill also diminishes MDA levels, enhances SOD activity, triggers the gsk-3β/HO-1 pathway activation, boosts the brain tissue's resistance against oxidative damage (Feng and Sun, 2007; Zhang et al., 2021), and mitigates apoptosis by inhibiting the expression of Bax, Bcl-2, and caspase-3 (Wang et al., 2014). By reducing the water content of the tissue surrounding the cerebral hematoma, enhancing the deformability of red blood cells, improving brain edema, and inhibiting the levels of MMP9 mRNA and AQP4 mRNA in brain tissue, the integrity of the BBB is protected (Fang et al., 2007; Zheng et al., 2014; Li J. Z. et al., 2019; Chen et al., 2022). Furthermore, Angong Niuhuang Pill reduces overall blood thickness, plasma thickness, platelet clumping rate, red blood cell clumping and stiffness index, and red blood cell deformation index in the brain tissue of rats with CI (Liu Z. T. et al., 2011). Clinical studies have shown that Angong Niuhuang Pill can aid in managing high fever, promoting consciousness recovery, and reducing convulsions in patients with cerebral apoplexy. When combined with standard comprehensive treatment, it is effective in patients with cerebral apoplexy. Overall, Angong Niuhuang Pill has a significant advantage in the recovery of central nervous system function (Cui, 2012; Guo et al., 2014; Huang P. et al., 2018).

7 Pharmacokinetic characteristics of effective components

CA, CDCA, TDCA, and GDCA exhibit numerous therapeutic effects in IS. A comprehensive knowledge of their metabolic

pathways is crucial in order to optimize the therapeutic effectiveness of BC. In the human intestinal Caco-2 cell model, a comparison was conducted to examine the uptake and transport of three types of BC. Furthermore, the bile acids in NBC were analyzed qualitatively and quantitatively using high-performance liquid chromatography-mass spectrometry (HPLC-MS). The results showed that GCA, CA, DCA, and TCA4 bile acids could be detected in brain tissue at different time points after intragastric administration, and the normalized area under curve (AUC) (0–60 min) was as follows: CA < GCA < DCA < TCA (Zhao et al., 2009). Moreover, ten blood entry components were found in the serum of rats after intragastric administration of NBC, and two known blood entry components were DCA and CDCA. Another study found that the pharmacokinetic characteristics of DCA and CDCA in rats were consistent with the two-compartment model (Han, 2012). After BCS and NBC intragastric administration in mice, CA, DCA, TCA, TDCA, CDCA, and Tau were detected in the blood. Additionally, BCS also detected GCA in both blood and tissues. Among them, the AUC (0–120 min) of CA, DCA, TCA, CDCA, and Tau was higher in the BCS group than in the NBC group (Feng, 2017).

The examination of the movement of various active substances in BC revealed the pharmacokinetic properties of its components. For instance, TCDCA in rats followed a first-order absorption one-compartment model. Additionally, it exhibited rapid absorption, gradual elimination, and prolonged duration in the body after being administered through the stomach (He, 2006). Furthermore, the concentrations of CA and HDCA in tissues were: kidney > lung > heart > brain. Among them, the target organs with a greater distribution were the lungs. The distribution of CDCA and DCA in the AUC and the heart, lungs, kidneys, and brain of mice was considerably higher compared to CA and HDCA. Noticeably, BR was observed to enhance bile acid absorption in BCA and reduce its elimination rate in animals, although it cannot elevate bile acid distribution in tissues. Study also foung that the oral bioavailability of monomeric bile acid is low, and the absorption and tissue distribution of bile acid in NBC is better than that in BCA (Zhao, 2007). Otherwise, after intraperitoneal injection of Qingkailing containing CA and HDCA, the peak time of CA in both normal and model groups (pMCAO model) was 4.8 min. The peak time of CA in the plasma of normal and model groups was 10.8 min and 12 min, respectively. This indicates that these two components entered the rat body quickly, and CI had no effect on its pharmacokinetic parameters (Liu X. et al., 2019).

Earlier studies have indicated that TCA concentration in serum, liver, kidney, and brain tissue of rats was determined following a single-dose intragastric administration of TCA. Additionally, it was found that the concentration-time curve of Tau in serum and liver conformed to the first-order absorption two-compartment model, while the concentration-time curve in kidney and brain tissue conformed to the first-order absorption one-compartment model. After oral administration, TCA exhibits rapid absorption, wide distribution, and slow elimination in rats (Hou, 2007). Besides, UDCA is a hydrophilic bile acid, which in consistent with the two-compartment model in rabbits. The drug is rapidly absorbed after entering the rabbit, and the peak concentration is reached in 5 min. Noticeably, the findings revealed that the majority of bile acids exhibited significantly reduced bi-directional permeability in BCA and BCS compared to NBC. Additionally, significant variations were observed in the excretion rates of CA, CDCA, TDCA, and GDCA among NBC and BCA groups, while no notable distinction was found between BCS and NBC groups (Chen et al., 2018).

Based on these findings, it can be inferred that the active constituents of BC have the ability to rapidly penetrate the bloodstream and reach the brain following absorption through the digestive system. This suggest that BC has the potential for the prompt management of stroke when taken orally.

8 Drug safety evaluation

8.1 Heavy metal problem

In recent years, the presence of heavy metals in BC and its preparations has been a concern for people. It is noteworthy that trace amounts of heavy metals, including Pb, Hg, and As, are often detected in BC (Takahashi et al., 2010). These heavy metals are typically absorbed from forage or the environment. Furthermore, BC from different sources may contain varying levels of heavy metals. Research indicates that NBC from Australia contains less As and Hg (Takahashi et al., 2010).

Importantly, the results indicating that heavy metal-containing BC preparations are not entirely absorbed after digestion by gastric fluid, with most being excreted from the body. Therefore, under proper medication guidance, the presence of heavy metals in BC and its preparations does not pose a significant risk to human health. To systematically evaluate the heavy metal problems, further research combining the absorption, metabolism, and excretion processes of heavy metals in the human body is necessary.

8.2 Drug allergy

Nowadays, BC and its related CPM preparations are widely used for medical purposes, indeed leading to some adverse events resembling allergic reactions. Studies indicated that adverse reactions caused by BC preparations (such as Niuhuang Jiedu Pill, Niuhuang Jiedu Tablet, Niuhuang Shangqing Pill, and Qingkailing Injection) mainly include allergic reactions, gastrointestinal disturbances (primarily diarrhea), and dysfunction of systems such as the nervous system (Huang et al., 2019). Additionally, allergic reactions to BC preparations are mainly characterized by rash, skin itching, and accompanied by systemic allergic reactions. Therefore, a more objective study is needed to evaluate the biosafety of BC, and it is recommended to use under the supervision of a healthcare professional.

8.2.1 Acute toxicity

To date, BC and BC-related preparations have been widely used in IS treatment. According to a research, mice were divided into three groups in the laboratory, and after an 8-h fast, they were orally administered 12.5% BC at doses of 10 g/kg, 15 g/kg, and 20 g/kg, respectively. In a 7-day acute toxicity experiment, none of the groups exhibited signs of toxicity, with normal eating and bowel movements observed (Yuan et al., 1992). Currently, data on *in vivo* and clinical toxicity experiments of BC are still lacking. Therefore, it is necessary to conduct *in vivo* and clinical research on the potential toxicity of BC, which will be crucial to determine its safe dosage.

TABLE 4 CPM preparations containing *Bovis Calculus* included in the *Chinese Pharmacopoeia* and commonly used to treat IS.

No.	CPM name	Type	SFDA approval number	Actions	Indications
1	Shixiang Fansheng Pill	NBC	Z12020754	To open orifices, resolve phlegm, tranquilize the mind with heavy medicinals	Apoplexy due to phlegm clouding the pericardium, manifested as delirious speech, coma, exuberant and congesting sputum, and trismus
2	Qishiwei Zhenzhu Pill	NBC	Z54020081	To tranquilize mind, unblock meridians, harmonize collaterals, harmonize qi and blood and open the orifices	Heimai and Baimai disease (Tibetan Medicine), Longxue disorders (Tibetan Medicine), apoplexy with paralysis or hemiplegia, epilepsy, cerebral haemorrhage, cerebral concussion, heart disease, hypertension and neurotic disorders
3	Renshen zaizao Pill	NBC	Z12020350	To tonify qi, nourish blood, dispel wind, resolve phlegm, activate blood and unblock the collaterals	Apoplexy with the pattern of qi deficiency with blood stasis and obstruction of collaterals by wind-phlegm, manifested as deviated eyes and mouth, hemiplegia, numbness of the limbs, pain, spasm, and sluggish speech
4	Angong Niuhuang Pill	NBC	Z21021326	Actions to clear heat, remove toxin, settle fright, and open the orifices	Febrile disease due to pathogenic factors entering the pericardium, manifested as high fever with seizure, loss of consciousness, and delirious speech; apoplex with coma, encephalitis, meningitis, toxic encephalopathy, cerebral haemorrhage, and septicemia with the symptoms described above
5	Angong Niuhuang Pill	NBC	Z11021084	To clear heat, remove toxin, settle fright and open the orifices	Febrile disease due to pathogenic factors entering the pericardium, manifested as high fever with seizure, loss of consciousness, and delirious speech; Apoplexy with coma, encephalitis, meningitis, toxic encephalopathy, cerebral hemorrhage, and septicemia with the symptoms described above
6	Tiandan Tongluo Tablet	BCA	Z20090152	To activate blood, unblock the collaterals, extinguish wind, and resolve phlegm	Apoplexy striking the meridians and collaterals with the pattern of wind-phlegm and blood stasis obstructing the meridians and collaterals, manifested as hemiplegia, hemianesthesia, deviated eyes and mouth, and sluggish speech; Acute stage and early stage of convalescence of cerebral infarction with the symptoms described above
7	Tiandan Tongluo Capsule	BCA	Z20010029	To activate blood, unblock the collaterals, extinguish wind, and resolve phlegm	Indications Apoplexy striking the meridians and collaterals with the pattern of wind-phlegm and blood stasis obstructing the meridians and collaterals, manifested as hemiplegia, hemianesthesia, deviated eyes and mouth, and sluggish speech; Acute stage and early stage of convalescence of cerebral infarction with the symptoms described above
8	Xinnaojing Tablet	BCA	Z21021359	To pacify the liver, subdue yang, clear the heart and tranquilize the mind	Dizziness and stroke (apoplexy) due to ascendant hyperactivity of liver yang, manifested as dizziness, blurred vision, vexation, restlessness, slurred speech, and paralyzed limbs; Hypertension with the symptoms described above
9	Annao Pill	BCA	Z23020126	To clear heat, remove toxin, tranquilize the mind and induce resuscitation, sweep phlegm, open the orifices, settle fright and extinguish wind	High fever, loss of consciousness, vexation, restlessness, delirious speech, convulsions, syncope, wind stroke with blocked orifices, headache and dizziness; Hypertension and cerebral apoplexy with the symptoms described above
10	Jiannao Anshen tablet	BCA	Z23020125	To clear heat, remove toxin, tranquilize the mind and induce resuscitation, sweep phlegm, open the orifices, settle fright and extinguish wind	High fever, loss of consciousness, vexation, restlessness, delirious speech, convulsions, syncope, wind stroke with blocked orifices, headache and dizziness; Hypertension and cerebral apoplexy with the symptoms described above
11	Qingxuan Zhitan Pill	BCA	Z11021187	To pacify the liver, extinguish wind, resolve phlegm and unblock the collaterals	Dizziness, vertigo, stiffness of the neck, distension in the head, heat in the chest, fear, deficiency vexation, exuberant and congesting phlegm, slobber, sluggish speech, numbness of the limbs, deviated eyes and mouth and hemiplegia due to ascendant hyperactivity of liver yang, and internal stirring of liver wind

(Continued on following page)

TABLE 4 (*Continued*) CPM preparations containing *Bovis Calculus* included in the *Chinese Pharmacopoeia* and commonly used to treat IS.

No.	CPM name	Type	SFDA approval number	Actions	Indications
12	Zaizao Wan	BCA	Z14021477	To dispel wind, resolve phlegm, activate blood and unblock the collaterals	Apoplexy due to wind phlegm obstructing the collaterals, manifested as hemiplegia, deviated tongue and mouth, numbness of the extremities, pain, spasm, and sluggish speech
13	Xueshuan Xinmaining Pill	BCA	Z20030145	To tonify qi, activate blood, open the orifices and relieve pain	Stroke and chest bi disorder due to qi deficiency with blood stasis, manifested as dizziness, vertigo, hemiplegia, oppression in the chest, heart pain, palpitations, and shortness of breath; Recovery phase of IS, coronary heart disease and angina pectoris with the symptoms described above
14	Xueshuan Xinmaining Capsule	BCA	Z22021474	To tonify qi, activate blood, open the orifices and relieve pain	Stroke and chest bi disorder due to qi deficiency with blood stasis, manifested as dizziness, vertigo, hemiplegia, oppression in the chest, heart pain, palpitations, and shortness of breath; Recovery phase of IS, coronary heart disease and angina pectoris with the symptoms described above
15	Kangshuan Zaizao Pill	BCA	Z21021307	To activate blood, resolve stasis, relax sinews, unblock the collaterals, extinguish wind and arrest convulsions	Wind stroke due to static blood obstructing orifices and failing to nourish meridians and collaterals, manifested as numbness of the limbs, difficulty in walking, paralysis, deviated eyes and mouth, slurred speech; Convalescence stage and sequela of apoplexy with the symptoms described above
16	Ershiwuwei Zhenzhu Pill	BCS	Z54020085	To tranquilize the mind and open the orifices	Apoplexy manifested as hemiplegia, deviated eyes and mouth, coma, disordered consciousness, delirious speech, and mania, etc

Abbreviations: BCS, *bovis calculus sativus;* BCA, *bovis calculus artifactus;* NBC, natural *bovis calculus;* CPM, chinese patent medicine; SFDA, state food and drug administration.

8.3 Potential side effects of drug interactions

When BC or its alternative preparations are used concomitantly with other Chinese herbal medicines or chemical substances, they generally do not induce or exacerbate side effects. Furthermore, studies have indicated that BC may reduce the incidence of adverse reactions to other drugs. For instance, BCS can reduce the incidence of Fecal occult blood in patients with acute IS and disturbance of consciousness treated with Zhongfeng Xingnao Liquid (Chen, 2022). Other research has reported that the co-administration of BCS with Gastrodin Injection reduced the incidence of adverse reactions in acute IS patients from 15.63% to 6.25% (Liao, 2020). Noticeably, novel drug delivery system has demonstrated good therapeutic efficacy, with researchers developing a new mucoadhesive film combining BCS with Ornidazole (OD) for oral ulcers treatment. This CBS-OD mucoadhesive film can reduce the adverse effects associated with conventional OD membranes, including significantly alleviating mucosal damage (Li W. et al., 2016).

9 Ethics of *Bovis Calculus*

The naturally occurring BC was first discovered by ancient Chinese ancestors while slaughtering sick cattle. Through clinical research and practice, they gradually realized the medicinal value of BC and began using it in clinical settings. From the perspective of sources, NBC forms naturally in cattle during the course of digestive or hepatobiliary diseases. Butchers intentionally search for NBC when inspecting the liver and gallbladder of cattle. This method is fundamentally different from the controversial practice of extracting bile from live bears, ensuring that NBC fully aligns with humanitarian and medical ethics.

Considering the scarcity and high price of NBC, it is important to develop its substitutes (Li C. et al., 2020). In the 1970s, Chinese researchers successfully developed the CBC, and the Ministry of Drug Administration approved three substitutes for NBC, namely, BCS, BCA, and CBC (Cai, 1978; Qiao et al., 2011; Shimada et al., 2013). However, due to the high cost, long production cycle, and ethical concerns in the medical field, CBC has limited circulation in the market and has not been included in the *Chinese Pharmacopoeia* (Liu et al., 2019a). The analysis and discussion presented in this study fall under the categories of NBC, BCA, and BCS, all of which adhere to the medical ethics of BC and the collection standards of the *Chinese Pharmacopoeia*.

10 Discussions and outlooks

10.1 Active ingredients

Clinical trials in the past found that IS injury involves complex pathophysiological processes. Reviewing the literature reveals that BC and its active components offer advantages in improving microcirculation disturbance, reducing excitatory amino acid

toxicity, inhibiting oxidative stress, protecting the BBB, preventing apoptosis, and promoting nerve and vascular regeneration. Further analysis revealed that the primary anti-excitatory amino acid toxic components of BC were TUDCA and Tau. The main components that counteract oxidative stress injury are BV, BR, CA, TUDCA, HDCA, and Tau. Moreover, the main anti-inflammatory components were CA, UDCA, TUDCA, HDCA, and Tau. Besides, the main components protecting the BBB function were GUDCA, CA, TUDCA, and UDCA. Furthermore, the primary anti-apoptosis components included CA, Tau, TUDCA, BV, and BR.

10.2 The correlation analysis of composition and therapeutic effect

BCA and BCS are substitutes for NBC. According to the existing literature, it is found that the main active components of the three types of BC are bile acids, bile pigments, Tau, amino acids, and a variety of trace elements. Among them, Tau, BR, BV, HDCA, CA, HDCA, and TUDCA have been widely studied in the research and application of IS. While NBC and its substitutes have been extensively utilized in clinical settings, NBC is acknowledged to possess superior effectiveness in comparison to its surrogates. This is possibly attributed to the varying proportions of the constituents found in NBC and its substitutes (Yan et al., 2007).

BCA is one of the main substitutes for NBC, and there are some differences and similarities between them in composition. For example, the content and proportion of bile acids in BCA are prepared based on the proportion of NBC. The bile acid content in BCA is higher than that in NBC, but other non-bile acid components are lower in BCA compared to NBC. These components include BR, inorganic ions, amino acids, and so on. It is noteworthy that BR is the most abundant component in NBC, constituting 35%–60% of its content. NBC contains substances that are absent in BCA, which can enhance the absorption and distribution of bile acids in BCA. In addition, BR can enhance the absorption of bile acid and reduce its elimination rate in mice (Zhao, 2007). Moreover, as a substitute for NBC, BCS has been included in the *Chinese Pharmacopoeia* and can replace NBC in clinical practice. This suggests that in a wider range of treatment scenarios, such as remote areas where medical personnel and equipment are less developed, the lower price of BCS can replace NBC to some extent. The analysis of the content properties of Tau and 12 bile acids in BCS and NBC revealed that the concentration of CA/DCA in BCS was approximately double that in NBC. Furthermore, the ratio of unbound bile acids to bound bile acids was higher in NBC compared to BCS. However, there was no notable disparity in the Tau/(TCA + TDCA + TCDCA) content between BCS and NBC (Feng, 2017). The aforementioned findings indicate that there are certain resemblances and disparities in the chemical makeup and substance of the three types of BC, which may be associated with their differences in pharmacodynamics.

10.3 Current research challenges and future research directions

The current research on BC and its pharmacological effects in IS faces several limitations and challenges. Many studies have concentrated on the mechanism of BC intervention in CI/RI. However, the following issues persist:

1) There is confusion around the terminology "*Bovis Calculus*". *Bovis Calculus* is clearly defined in the *Chinese Pharmacopoeia*, but in commercial trade or scientific research, its Chinese pronunciation is very similar to that of another TCM, "*Sulfur*". However, the pharmacological actions of these two substances are completely different, as *Sulfur* is a mineral extracted from natural sulfur-containing ores.
2) Lack of comprehensive and well-controlled clinical trials to evaluate the efficacy and safety of BC in the treatment of IS. Many existing studies have small sample sizes, use limited evaluation methods, and lack quality, making the reliability of their conclusions questionable.
3) Inconsistencies in the doses of BC and its preparations used by different research institutions and researchers, pose new challenges in deriving meaningful comparative results and conclusions.
4) Most of the existing literature focuses on the anti-IS damage effects of some active components in BC, but there are few comparative studies on the anti-CI effects of single BC and different sources of BC. The mechanisms of other active components, such as cholesterol components, in BC against IS injury and their relationship with the main active components of BC are not clear.
5) NBC substitutes are widely used in clinics, and it is believed that there are variations in therapeutic effects among these substitutes as well as compared to NBC, but the underlying reasons for these variances have not been elucidated. Whether the combined components have better therapeutic effects than the individual ones still requires further study.
6) The exact mechanisms of action of BC in treating IS are not fully elucidated. Although some studies have shown that BC can improve hemorheological parameters, alleviate inflammatory responses, and enhance antioxidant capacity, the precise molecular mechanisms remain unclear.
7) According to the location of CI, IS can be categorized into focal CI and global CI. It can also be classified as permanent CI or ischemia-reperfusion based on whether reperfusion occurs after the ischemic event. However, most researchers mainly focus on the focal IS model. Due to the treatment time window and other factors, more patients experience permanent CI (Dalli et al., 2019). It is advocated to verify the neuroprotective effect of BC using a variety of CI models. It is suggested that the timing and method of administration in the model should align with clinical practice.
8) The restoration stage of CI has a significant effect on the quality of life of patients. At present, research on the anti-IS effects of BC and its active components is primarily concentrated on alleviating IS injury in the acute stage, with insufficient focus on the restoration stage post-IS. Future research should pay attention to the follow-up period.
9) Patients often develop a fever after undergoing IS. Long-term fever has adverse effects on the prognosis of patients. Tau and bile acids in BC also play an anticoagulant and antipyretic role in the process of CI (Zhang et al., 2013; Xu, 2015). The mechanisms of these actions need to be further explored.

10) There is a lack of research on the potential chronic toxicity and hypersensitivity reactions of BC, as well as a lack of studies on the interchangeability of NBC with other substances like BCA and BCS in clinical applications.

In response to the limitations and challenges in the pharmacological treatment of IS using BC, we propose the following research directions:

1) The bioactive compounds, potential targets, and potential mechanisms of BC were studied using the method of network pharmacology. It was found that the PI3K/AKT and MAPK signal pathways were the key targets of BC against IS (Liu et al., 2021). BC in the treatment of IS is associated with steroid hormone biosynthesis, metabolic pathways, and neuroactive ligand-receptor interactions (Chen et al., 2021). Furthermore, network pharmacology combined with cell experiments further confirmed that BCS can protect against OGD/R damage through anti-apoptotic effects, maintaining BBB tight junction proteins, anti-inflammatory actions, and inhibiting oxidative stress. BCS also protects NVU by regulating the HIF-1/VEGF and PI3K/Akt signaling pathways (Du et al., 2022). The conclusion of network pharmacology is only a conjecture based on big data. It is also limited by the selected database. Therefore, further verification is needed to determine if BC interferes with IS through other pathways.
2) Conduct larger-scale and more standardized pharmacological experiments and clinical trials to actively evaluate the efficacy and safety of BC in IS treatment. Establish standard specifications for BC and its preparations to ensure the reliability of the research.
3) Further in-depth research is needed to elucidate the molecular mechanisms of how BC exerts its therapeutic effects on IS. The research aims should focus on clarifying how BC improves blood flow, reduces inflammation, and enhances antioxidant activity, providing a solid scientific foundation for its clinical applications.
4) Efforts should be made to address the issue of the confusing nomenclature of BC products, ensuring consistency between commercial circulation and scientific research. Emphasis should be placed on using "*Bovis Calculus*" as the specific identifier that complies with the *Chinese Pharmacopoeia*.
5) Investigations should be conducted to explore the potential causes of adverse reactions associated with the use of BC, and to study the interchangeability of artificial substitutes in clinical applications. Through the discussion of these research directions, we can further deepen the understanding and utilization of BC in IS treatment.

11 Conclusion

This research highlights the tremendous potential of BC in the prevention and treatment of IS through a multi-component, multi-target, multi-pathway approach. Comprehensive studies have shown that BC can treat CI/RI through mechanisms such as improving microcirculation disturbances, inhibiting neurotoxicity, reducing ROS damage, alleviating inflammatory responses, suppressing cell apoptosis, and reducing ischemic brain tissue damage. Furthermore, BC has the ability to mitigate BBB injury and prevent calcium overload caused by CI. It can also promote angiogenesis and functional recovery of the brain during the post-stroke rehabilitation period. Additionally, BC exhibits anti-thrombotic effects by inhibiting platelet aggregation and fibrinolysis, and exerts anti-CI/RI effects by improving lipid metabolism and regulating the PI3K/AKT and MAPK signaling pathways. Therefore, BC not only can alleviate the damage caused by ischemia to the brain, but also has significant interventional effects on related complications and sequelae. Besides, Chinese patent medicine formulations containing BC are clinically widely used in the treatment of IS, which offers a new and promising avenue for IS. In summary, BC has notable advantages and tremendous development potential in the prevention and treatment of IS. Future research should delve deeper into exploring its broader biological activities and clinical application value.

Author contributions

KX: Writing–original draft, Writing–review and editing. BD: Data curation, Formal Analysis, Investigation, Writing–original draft. TJ: Formal Analysis, Investigation, Writing–review and editing. MR: Formal Analysis, Investigation, Writing–review and editing. HC: Formal Analysis, Investigation, Writing–review and editing. JZ: Formal Analysis, Investigation, Writing–review and editing. JG: Formal Analysis, Investigation, Writing–review and editing. YL: Conceptualization, Data curation, Formal Analysis, Investigation, Writing–review and editing. JW: Formal Analysis, Funding acquisition, Investigation, Supervision, Writing–review and editing.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. Thanks for the support of the National Natural Science Foundation of China (81873023 and 81473371).

Acknowledgments

Specifically, thanks to Professor Wang for providing writing assistance. Thanks to all those who have helped with this article.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declare that Generative AI was used in the creation of this manuscript. The authors thank Figdraw for providing drawing support.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

Amantea, D., Russo, R., Gliozzi, M., Fratto, V., Berliocchi, L., Bagetta, G., et al. (2007). Early upregulation of matrix metalloproteinases following reperfusion triggers neuroinflammatory mediators in brain ischemia in rat. *Int. Rev. Neurobiol.* 82, 149–169. doi:10.1016/S0074-7742(07)82008-3

Bian, K. Y., Jin, H. F., Sun, W., and Sun, Y. J. (2019). DCA can improve the ACI-induced neurological impairment through negative regulation of Nrf2 signaling pathway. *Eur. Rev. Med. Pharmacol. Sci.* 23, 343–351. doi:10.26355/eurrev_201901_16782

Cai, H., Guang, Y., Liu, L., Yao, P., and Qiu, F. (2007). The protective effects of *in vitro* cultivated calculus bovis on the cerebral and myocardial cells in hypoxic mice. *J. Huazhong Univ. Sci. Technol. Med. Sci.* 27, 635–638. doi:10.1007/s11596-007-0603-2

Cai, H. J., Zhang, X. Q., Li, C. Y., Haung, C. Y., Wang, Q., and Lai, S. L. (2004). Clinical studies on in-vitro-cultured calculus bovis in the treatment of apoplexy. Tradit. *Chin. Drug Res. Clin. Pharmacol.* 15, 287–289. doi:10.3321/j.issn:1003-9783.2004.04.025

Cai, J. G., Luo, L. M., Tang, H., and Zhou, L. (2018). Cytotoxicity of malondialdehyde and cytoprotective effects of taurine via oxidative stress and PGC-1α signal pathway in C2C12 cells. *Mol. Biol. (Mosk.)* 52, 616–627. doi:10.1134/S0026898418040043

Cai, Z. C. (1978). A preliminary report on the study of artificial cultivation of natural calculus bovis. *Chin. Herb. Med. Commun.* 36-39, 49.

Campbell, B. C. V., De Silva, D. A., Macleod, M. R., Coutts, S. B., Schwamm, L. H., Davis, S. M., et al. (2019). Ischaemic stroke. *Nat. Rev. Dis. Primer* 5, 70. doi:10.1038/s41572-019-0118-8

Chen, C. X., Jin, R. M., Xu, P. K., Wang, Q., Li, Y. K., and Yang, S. Y. (1991). Effects of taurine on platelet aggregation and experimental thrombosis. *Chin. Pharm.* J., 207–209+251.

Chen, H. (2022). *Clinical observation of Zhongfeng Xingnao liquid combined with calculus bovis cultured* in vitro *in the treatment of acute ischemic stroke with disturbance of consciousness*. Chengdu University of TCM. doi:10.26988/d.cnki.gcdzu.2021.000530

Chen, H., Li, H. Y., Wang, J., Xie, Q., and Fu, Y. (2021). Mechanism of calculus bovis in the treatment of ischemic stroke based on network pharmacology. *Her. Med.* 40, 768–772. doi:10.3870/j.issn.1004-0781.2021.06.011

Chen, H., Luo, Y., Tsoi, B., Gu, B., Qi, S., and Shen, J. (2022). Angong Niuhuang Wan reduces hemorrhagic transformation and mortality in ischemic stroke rats with delayed thrombolysis: involvement of peroxynitrite-mediated MMP-9 activation. *Chin. Med.* 17, 51. doi:10.1186/s13020-022-00595-7

Chen, H., and Wang, J. (2023). Exploration of the property effect and usage of Niuhuang. *J. Shanxi Univ. Tradit. Chin. Med.* 24, 509–512. doi:10.19763/j.cnki.2096-7403.2023.05.08

Chen, X., Wang, J. H., Gao, X. L., Wu, Y., Gu, G., Shi, M. M., et al. (2020). Tauroursodeoxycholic acid prevents ER stress-induced apoptosis and improves cerebral and vascular function in mice subjected to subarachnoid hemorrhage. *Brain Res.* 1727, 146566. doi:10.1016/j.brainres.2019.146566

Chen, Y., Hu, X. R., Cheng, X. L., Ma, S. C., Ran, Q. S., Yang, Q., et al. (2018). Quantitative comparison of bile acid distribution and intestinal transport from native cow-bezoar and artificial and *in vitro* cultured substitutes using caco-2 cell monolayer model. *Digit. Chin. Med.* 1, 261–271. doi:10.1016/s2589-3777(19)30051-5

Cheng, X., Yang, Y. L., Li, W. H., Liu, M., Wang, Y. H., and Du, G. H. (2020). Cerebral ischemia-reperfusion aggravated cerebral infarction injury and possible differential genes identified by RNA-Seq in rats. *Brain Res. Bull.* 156, 33–42. doi:10.1016/j.brainresbull.2019.12.014

Cheng, Y., Xi, G., Jin, H., Keep, R. F., Feng, J., and Hua, Y. (2014). Thrombin-induced cerebral hemorrhage: role of protease-activated receptor-1. *Transl. Stroke Res.* 5, 472–475. doi:10.1007/s12975-013-0288-8

Chinese Pharmacopoeia Committee of People's Republic of China (2020). Pharmacopoeia of the People's Republic of China.

Choi, S. H., Veeraraghavalu, K., Lazarov, O., Marler, S., Ransohoff, R. M., Ramirez, J. M., et al. (2008). Non-cell-autonomous effects of presenilin 1 variants on enrichment-mediated hippocampal progenitor cell proliferation and differentiation. *Neuron* 59, 568–580. doi:10.1016/j.neuron.2008.07.033

Choo, M., Miyazaki, T., Yamazaki, M., Kawamura, M., Nakazawa, T., Zhang, J., et al. (2017). Retrograde BDNF to TrkB signaling promotes synapse elimination in the developing cerebellum. *Nat. Commun.* 8, 195. doi:10.1038/s41467-017-00260-w

Cipolla, M. J., Liebeskind, D. S., and Chan, S. L. (2018). The importance of comorbidities in ischemic stroke: impact of hypertension on the cerebral circulation. *J. Cereb. Blood Flow. Metab.* 38, 2129–2149. doi:10.1177/0271678X18800589

Cui, A. Y. (2012). Recent pharmacologic actions and clinical applications of Angong niuhuang wan. *Chin. J. Exp. Tradit. Med. Formulae* 18, 341–344. doi:10.13422/j.cnki.syfjx.2012.20.087

Dalli, L. L., Kim, J., Thrift, A. G., Andrew, N. E., Lannin, N. A., Anderson, C. S., et al. (2019). Disparities in antihypertensive prescribing after stroke: linked data from the Australian stroke clinical registry. *Stroke* 50, 3592–3599. doi:10.1161/STROKEAHA.119.026823

Dawson, J. R., Liu, S., Jung, B., Messina, S., and Eppler, B. (2000). Effects of high salt diets and taurine on the development of hypertension in the stroke-prone spontaneously hypertensive rat. *Amino Acids* 19, 643–665. doi:10.1007/s007260070014

Deguchi, K., Hayashi, T., Nagotani, S., Sehara, Y., Zhang, H., Tsuchiya, A., et al. (2008). Reduction of cerebral infarction in rats by biliverdin associated with amelioration of oxidative stress. *Brain Res.* 1188, 1–8. doi:10.1016/j.brainres.2007.07.104

Deng, X., Li, J., Liu, S., and Zhu, C. (2004). Changes in amino acid neurotransmitters during cerebral ischemia and reperfusion in awake rats. *Chin. J. Pathophysiol.* 20, 660–663. doi:10.3321/j.issn:1000-4718.2004.04.037

Du, X., Li, C. X., Zhang, S., Sun, C. Y., Zhang, X. L., Chen, C. A., et al. (2022). Exploring the pharmacological mechanism of calculus bovis in cerebral ischaemic stroke using a network pharmacology approach. *J. Ethnopharmacol.* 284, 114507. doi:10.1016/j.jep.2021.114507

El Idrissi, A., Okeke, E., Yan, X., Sidime, F., and Neuwirth, L. S. (2013). Taurine regulation of blood pressure and vasoactivity. *Adv. Exp. Med. Biol.* 775, 407–425. doi:10.1007/978-1-4614-6130-2_31

Emberson, J., Lees, K. R., Lyden, P., Blackwell, L., Albers, G., Bluhmki, E., et al. (2014). Effect of treatment delay, age, and stroke severity on the effects of intravenous thrombolysis with alteplase for acute ischaemic stroke: a meta-analysis of individual patient data from randomised trials. *Lancet lond. Engl.* 384, 1929–1935. doi:10.1016/S0140-6736(14)60584-5

Fang, D. S., and Peng, M. Y. (2013). Protective effects of Panax notoginseng total saponins combined with sulfuric acid against cerebral ischemia-reperfusion injury model rats. *China Pharm.* 24, 4436–4438. doi:10.6039/j.issn.1001-0408.2013.47.06

Fang, F., Sun, J. N., Yang, L., and Yang, Z. Y. (2007). Effects of Angongniuhuang Pills and its simplified prescription on intracerebral hemorrhage injury in rats. *J. Beijing Univ. Tradit. Chin. Med.* 30, 611–614. doi:10.3321/j.issn:1006-2157.2007.09.011

Feihl, F., Liaudet, L., and Waeber, B. (2009). The macrocirculation and microcirculation of hypertension. *Curr. Hypertens. Rep.* 11, 182–189. doi:10.1007/s11906-009-0033-6

Feng, C. Y. (2017). *Study on quality standard and pharmacokinetics of calculus bovis sativus based on bile acids*. Huazhong University of Science and Technology. doi:10.7666/d.D01309529

Feng, C. Y., Li, X. P., Zhang, C. L., He, G. Z., Xu, Y. J., Li, W., et al. (2015). Development of a rapid and simple LC-MS/MS method for identification and quality control of natural Calculus bovis and Calculus bovis sativus. *Anal. METHODS* 7, 7606–7617. doi:10.1039/c5ay01288k

Feng, C. Y., Zhang, C. L., and Liu, D. (2017). Modern research of calculus bovis (second): the quality control. *Her. Med.* 36, 117–122. doi:10.3870/j.issn.1004-0781.2017.02.001

Feng, S. Y., and Sun, J. N. (2007). Research of protective effect on intracranial hemorrhage rat model of Angong niuhuang pill and its simplified prescription. *Prog. Mod. Biomed.* 7, 571–572. doi:10.3969/j.issn.1673-6273.2007.04.022

Fluri, F., Grünstein, D., Cam, E., Ungethuem, U., Hatz, F., Schäfer, J., et al. (2015). Fullerenols and glucosamine fullerenes reduce infarct volume and cerebral inflammation after ischemic stroke in normotensive and hypertensive rats. *Exp. Neurol.* 265, 142–151. doi:10.1016/j.expneurol.2015.01.005

Gharibani, P. M., Modi, J., Pan, C., Menzie, J., Ma, Z., Chen, P.-C., et al. (2013). "The mechanism of taurine protection against endoplasmic reticulum stress in an animal stroke model of cerebral artery occlusion and stroke-related conditions in primary neuronal cell culture," in *Taurine 8*. Editors A. El Idrissi and W. J. L'Amoreaux (New York, NY: Springer), 241–258. doi:10.1007/978-1-4614-6093-0_23

Guan, W. H., Zhao, Y. M., and Xu, C. (2011). A combined treatment with taurine and intra-arterial thrombolysis in an embolic model of stroke in rats: increased neuroprotective efficacy and extended therapeutic time window. *Transl. Stroke Res.* 2, 80–91. doi:10.1007/s12975-010-0050-4

Guo, Y., Yan, S. H., Xu, L. P., Zhu, G. X., Yu, X. T., and Tong, X. L. (2014). Use of Angong niuhuang in treating central nervous system diseases and related research. *Evid. Based Complement. Altern. Med.* 2014, 346918–346919. doi:10.1155/2014/346918

Han, Y. Y. (2012). *Research on the serum pharmaceutical chemistry of calculus bovis cultivated by glucuronidase and natural calculus bovis*. Taishan Medical University. doi:10.7666/d.Y2280331

Han, Z., Gao, L. Y., Lin, Y. H., Chang, L., Wu, H. Y., Luo, C. X., et al. (2016). Neuroprotection of taurine against reactive oxygen species is associated with inhibiting NADPH oxidases. *Eur. J. Pharmacol.* 777, 129–135. doi:10.1016/j.ejphar.2016.03.006

Hanna, J., Chahine, R., Aftimos, G., Nader, M., Mounayar, A., Esseily, F., et al. (2004). Protective effect of taurine against free radicals damage in the rat myocardium. *Exp. Toxicol. Pathol. Off. J. Ges. Toxikol. Pathol.* 56, 189–194. doi:10.1016/j.etp.2004.08.004

Hatakeyama, M., Ninomiya, I., and Kanazawa, M. (2020). Angiogenesis and neuronal remodeling after ischemic stroke. *Neural Regen. Res.* 15, 16–19. doi:10.4103/1673-5374.264442

He, X. L. (2006). *Studies on antiinflammatory immunopharmacology and pharmacokinetics of TCDCA*. Inner Mongolia Agricultural University. doi:10.7666/d.y919372

He, Z., Ning, N., Zhou, Q., Khoshnam, S. E., and Farzaneh, M. (2020). Mitochondria as a therapeutic target for ischemic stroke. *Free Radic. Biol. Med.* 146, 45–58. doi:10.1016/j.freeradbiomed.2019.11.005

Hjort, N., Wu, O., Ashkanian, M., Sølling, C., Mouridsen, K., Christensen, S., et al. (2008). MRI detection of early blood-brain barrier disruption: parenchymal enhancement predicts focal hemorrhagic transformation after thrombolysis. *Stroke* 39, 1025–1028. doi:10.1161/STROKEAHA.107.497719

Hou, X. J. (2007) "Studies on pharmacokinetics of taurocholic acid in rats," in *Inner Mongolia agricultural university*. doi:10.7666/d.y1138113

Hua, Q., Zhu, X., Li, P., Liu, Y., Zhang, N., Xu, Y., et al. (2009). The inhibitory effects of cholalic acid and hyodeoxycholalic acid on the expression of TNFalpha and IL-1beta after cerebral ischemia in rats. *Arch. Pharm. Res.* 32, 65–73. doi:10.1007/s12272-009-1119-z

Huang, H. L., and Rao, M. R. (1995). Effects of neferine and its combination with taurine on platelet aggregation and experimental thrombosis in rats. *Acta Pharm. Sin.* 30, 486–490. doi:10.16438/j.0513-4870.1995.07.002

Huang, M. R., Zhao, W. J., Li, J. S., Zhang, J. Y., and Wang, Z. B. (2018a). Research advance of chemical constituents,analytical methods and pharmacological effects of cow-bezoar and its substitutes. *Chin. J. Pharm. Anal.*

Huang, P., Guo, Y. H., Zhao, J. X., and Liu, Q. Q. (2018b). Clinical research progress of Angong niuhuang wan. J. Emerg. Tradit. *Chin. Med.* 27, 361–364+376. doi:10.3969/j.issn.1004-745X.2018.02.054

Huang, Q. Q., Sun, J., Gan, G., and Li, M. (2019). Analysis of 646 cases of adverse drug reactions induced by niuhuangjiedu preparation. *Chin. J. Pharmacoepidemiol.* 28, 591–594. doi:10.19960/j.cnki.issn1005-0698.2019.09.008

Huxtable, R. J. (1992). Physiological actions of taurine. *Physiol. Rev.* 72, 101–163. doi:10.1152/physrev.1992.72.1.101

Iadecola, C., and Anrather, J. (2011). The immunology of stroke: from mechanisms to translation. *Nat. Med.* 17, 796–808. doi:10.1038/nm.2399

Jia, J., Sun, J. M., Zang, H., and Zhang, H. (2013). Research progress on chemical constituents and pharmacological activities of natural bezoar. *Jilin J. Chin. Med.* 33, 271–274. doi:10.3969/j.issn.1003-5699.2013.03.026

Jiang, T., Yu, J. T., Zhu, X. C., Zhang, Q. Q., Tan, M. S., Cao, L., et al. (2014). Angiotensin-(1-7) induces cerebral ischaemic tolerance by promoting brain angiogenesis in a Mas/eNOS-dependent pathway. *Br. J. Pharmacol.* 171, 4222–4232. doi:10.1111/bph.12770

Jin, R., Xiao, A. Y., Chen, R., Granger, D. N., and Li, G. (2017). Inhibition of CD147 (cluster of differentiation 147) ameliorates acute ischemic stroke in mice by reducing thromboinflammation. *Stroke* 48, 3356–3365. doi:10.1161/STROKEAHA.117.018839

Jin, R., Xiao, A. Y., Liu, S., Wang, M., and Li, G. H. (2018). Taurine reduces tPA (Tissue-Type plasminogen activator)-induced hemorrhage and microvascular thrombosis after embolic stroke in rat. *Stroke* 49, 1708–1718. doi:10.1161/STROKEAHA.118.020747

Johnson, W., Onuma, O., Owolabi, M., and Sachdev, S. (2016). Stroke: a global response is needed. *Bull. World Health Organ.* 94, 634–634A. doi:10.2471/BLT.16.181636

Joo, S. S., Kang, H. C., Won, T. J., and Lee, D. I. (2003). Ursodeoxycholic acid inhibits pro-inflammatory repertoires, IL-1 beta and nitric oxide in rat microglia. *Arch. Pharm. Res.* 26, 1067–1073. doi:10.1007/BF02994760

Kong, W. J., Jin, C., Xiao, X. H., Zhao, Y. L., Liu, W., Li, Z. L., et al. (2010). Determination of multicomponent contents in Calculus bovis by ultra-performance liquid chromatography-evaporative light scattering detection and its application for quality control. *J. Sep. Sci.* 33, 1518–1527. doi:10.1002/jssc.200900751

Kong, W. J., Xing, X. Y., Xiao, X. H., Wang, J. B., Zhao, Y. L., and Yang, M. H. (2012). Multi-component analysis of bile acids in natural Calculus bovis and its substitutes by ultrasound-assisted solid-liquid extraction and UPLC-ELSD. *Analyst* 137, 5845–5853. doi:10.1039/c2an36084e

Korde, A. S., Pettigrew, L. C., Craddock, S. D., Pocernich, C. B., Waldmeier, P. C., and Maragos, W. F. (2007). Protective effects of NIM811 in transient focal cerebral ischemia suggest involvement of the mitochondrial permeability transition. *J. Neurotrauma* 24, 895–908. doi:10.1089/neu.2006.0122

Kulik, T., Kusano, Y., Aronhime, S., Sandler, A. L., and Winn, H. R. (2008). Regulation of cerebral vasculature in normal and ischemic brain. *Neuropharmacology* 55, 281–288. doi:10.1016/j.neuropharm.2008.04.017

Lampugnani, M. G., and Dejana, E. (2007). Adherens junctions in endothelial cells regulate vessel maintenance and angiogenesis. *Thromb. Res.* 120 (Suppl. 2), S1–S6. doi:10.1016/S0049-3848(07)70124-X

Lawrence, T. (2009). The nuclear factor NF-kappaB pathway in inflammation. *Cold Spring Harb. Perspect. Biol.* 1, a001651. doi:10.1101/cshperspect.a001651

Li, C., Li, L. M., Cao, S., Li, L., Li, J. H., Hu, Q., et al. (2020a). Herbal textual research of Calculus Bovis varieties of past dynasties. *Chin. Tradit. Pat. Med.* 42, 1865–1871. doi:10.3969/j.issn.1001-1528.2020.07.036

Li, C. X., Wang, X. Q., Cheng, F. F., Yan, X., Luo, J., and Wang, Q. G. (2019a). Hyodeoxycholic acid protects the neurovascular unit against oxygen-glucose deprivation and reoxygenation-induced injury *in vitro*. *Neural Regen. Res.* 14, 1941–1949. doi:10.4103/1673-5374.259617

Li, C. X., Wang, X. Q., Yan, J. T., Cheng, F. F., Ma, X. N., Chen, C. A., et al. (2020b). Cholic acid protects *in vitro* neurovascular units against oxygen and glucose deprivation-induced injury through the BDNF-TrkB signaling pathway. *Oxid. Med. Cell. Longev.* 2020, 1–14. doi:10.1155/2020/1201624

Li, C. Y., Li, P. T., Pan, Y. S., Jia, X., and Li, K. L. (2004). Effects of combination of calculus bezoar and fructus gardeniae on lipid peroxidation in different phases of focal cerebral IR injuries in rat. *China J. Tradit. Chin. Med. Pharm.* 19, 528–530. doi:10.3969/j.issn.1673-1727.2004.09.006

Li, J. J., Peng, L. J., Bai, W. Y., Peng, P. H., Chen, W. D., Yang, W., et al. (2021). Biliverdin protects against cerebral ischemia/reperfusion injury by regulating the miR-27a-3p/rgs1 Axis. *Neuropsychiatr. Dis. Treat.* 17, 1165–1181. doi:10.2147/NDT.S300773

Li, J. J., Zou, Z. Y., Liu, J., Xiong, L. L., Jiang, H. Y., Wang, T. H., et al. (2017). Biliverdin administration ameliorates cerebral ischemia reperfusion injury in rats and is associated with proinflammatory factor downregulation. *Exp. Ther. Med.* 14, 671–679. doi:10.3892/etm.2017.4549

Li, J. Z., Jiang, Y., Zeng, Y., Yan, X. G., and Yan, J. W. (2019b). Effect of An-gong Niu-huang Wan on the expression of MMP9 and AQP4 in rats with acute cerebral ischemia-reperfusion. *J. Zunyi Med. Univ.* 42, 412–415. doi:10.3969/j.issn.1000-2715.2019.04.011

Li, O. (2006). *The successive administation of taurine and strythnine improves the brain self-repair after global ischemia*. Southern Medical University.

Li, S. Z., Zhu, F., Wang, J., He, G. N., Shi, K. S., Chen, J., et al. (2014). Compendium of Materia medica. *Nanchang 21st Century Press*.

Li, W., He, W., Gao, P., Zhang, C., Cai, H., Chen, Y., et al. (2016a). Preparation, *in vitro* and *in vivo* evaluations of compound calculus bovis sativus and Ornidazole film. *Biol. Pharm. Bull.* 39, 1588–1595. doi:10.1248/bpb.b16-00043

Li, W. H., and Li, C. Y. (2004). Influence of NiuHuang, ZhiZi and their compatibility on the content of nerve growth factor (NGF) in focal cerebral ischemia - reperfusion rats. *Med. J. Chin. People Heacth* 16, 129–131. doi:10.3969/j.issn.1672-0369.2004.03.001

Li, X. J., Li, X. Q., Li, X. J., Shang, F., Guo, S., Li, Z. X., et al. (2010). Effect of taurine on the expression of matrix metalloproteinases-3 in brain mantle of rats with diffuse brain injuries. *J. Xinxiang Med. Coll.* 27, 123–126. CNKI:SUN:XXYX.0.2010-02-006.

Li, X. X., Lu, S. F., Zhu, B. M., and Fu, S. P. (2016b). Ischemic Stroke,Excitatory amino acids toxicity and the adjustment of acupuncture intervention. *Acupunct. Res.* 41, 180–185. doi:10.13702/j.1000-0607.2016.02.016

Li, X. Y., Yao, Y. Q., Chen, M. L., Ding, H. R., Liang, C. R., Lv, L., et al. (2022). Comprehensive evaluation integrating omics strategy and machine learning algorithms for consistency of calculus bovis from different sources. *TALANTA* 237, 122873. doi:10.1016/j.talanta.2021.122873

Liao, S. Q. (2020). Clinical study of *in vitro* cultured bezoar combined with gastrodin injection in the treatment of acute ischemic stroke. *J. China Prescr. Drug* 18, 120–122. doi:10.3969/j.issn.1671-945X.2020.05.071

Liu, F. C., Li, L., Chen, J., Wu, Y., Cao, Y. B., and Zhong, P. (2021). A network pharmacology to explore the mechanism of calculus bovis in the treatment of ischemic stroke. *Biomed. Res. Int.* 2021, 6611018–6611020. doi:10.1155/2021/6611018

Liu, P., He, X. R., and Guo, M. (2010a). Single and combining effects of Calculus Bovis and zolpidem on inhibitive neurotransmitter of rat striatum corpora. *China J. Chin. Mater. Medica* 35, 904–907. doi:10.4268/cjcmm20100723

Liu, W. X., Cheng, X. L., Guo, X. H., Hu, X. R., Liu, Y., Wei, F., et al. (2019a). Progress in research on chemical constituents and quality control methods of calculus bovis and its substitutes. *Chin. Pharm. J.* 54, 597–602. doi:10.11669/cpj.2019.08.001

Liu, W. X., Cheng, X. L., Guo, X. H., Hu, X. R., Liu, Y., Wei, F., et al. (2019b). Progress in research on chemical constituents and quality control methods of calculus bovis and its substitutes. *Chin. Pharm. J. 0*.

Liu, X., Su, J., Du, P., Yao, W. L., Yang, Q. B., Lu, Y. M., et al. (2019c). Analysis of pharmacokinetic behavior of five components in qingkailing (lyophilized) for injection in normal rats and cerebral ischemia rats by UPLC-MS/MS. *Chin. J. Exp. Tradit. Med. Formulae* 25, 86–91. doi:10.13422/j.cnki.syfjx.20191452

Liu, X. Y., Peng, K. R., Yang, H. J., Wu, X. N., and Deng, B. M. (2010b). Clinical effect of cultured bezoar *in vitro* on acute cerebral infarction. *Chin. J. Inf. Tradit. Chin. Med.* 17, 66–67. doi:10.3969/j.issn.1005-5304.2010.05.031

Liu, Y. G., Tan, P., Liu, S. S., Shi, H., Feng, X., and Ma, Q. (2015). A new method for identification of natural, artificial and *in vitro* cultured Calculus bovis using high-performance liquid chromatography-mass spectrometry. *Pharmacogn. Mag.* 11, 304–310. doi:10.4103/0973-1296.153083

Liu, Y. X., Liu, T., Yang, X. Y., Li, H., Zhang, Y. Q., Li, H. W., et al. (2011a). Effects of Angong-niuhuang pill on expression of eNOS mRNA in spontaneously hypertensive rats after intracerebral hemorrhage. *Int. J. Tradit. Chin. Med.* 33. doi:10.3760/cma.j.issn.1673-4246.2011.02.004

Liu, Z. J., Wang, C. P., Yang, B., Wang, N., Wang, Q., Gu, J., et al. (2022). Protective effect and mechanism of Ershiwuwei Zhenzhu Pills on cerebral apoplexy rats. *China J. Chin. Mater. Medica* 47, 2064–2073. doi:10.19540/j.cnki.cjcmm.20211216.705

Liu, Z. T., Liu, J., Li, J. B., and Liu, Y. X. (2011b). Effects of Angong niuhuang wan on experimental cerebral ischemia of rats. *Chin. J. Exp. Tradit. Med. Formulae* 17, 188–191. doi:10.3969/j.issn.1005-9903.2011.23.056

Lu, F. B., Wang, L. F., Chen, Y. Y., Zhong, X. M., and Huang, Z. (2020). *In vitro* cultured calculus bovis attenuates cerebral ischaemia-reperfusion injury by inhibiting neuronal apoptosis and protecting mitochondrial function in rats. *J. Ethnopharmacol.* 263, 113168. doi:10.1016/j.jep.2020.113168

Luo, C., Guo, L. J., and Yin, G. F. (2004). Neuroprotective effect of taurine on focal cerebral ischemia in rats. *Chin. Pharmacol. Bull.*, 512–516. doi:10.3321/j.issn:1001-1978.2004.05.008

Marı´n-Teva, J. L., Dusart, I., Colin, C., Gervais, A., Van Rooijen, N., and Mallat, M. (2004). Microglia promote the death of developing purkinje cells. *Neuron* 41, 535–547. doi:10.1016/S0896-6273(04)00069-8

Mark, K. S., and Davis, T. P. (2002). Cerebral microvascular changes in permeability and tight junctions induced by hypoxia-reoxygenation. *Am. J. Physiol.-Heart Circ. Physiol.* 282, H1485–H1494. doi:10.1152/ajpheart.00645.2001

Melincovici, C. S., Boşca, A. B., Şuşman, S., Mărginean, M., Mihu, C., Istrate, M., et al. (2018). Vascular endothelial growth factor (VEGF) - key factor in normal and pathological angiogenesis. *Romanian J. Morphol. Embryol. Rev. Roum. Morphol. Embryol.* 59, 455–467.

Miao, C. M., Zhou, F. R., Zhang, X. M., and Li, J. (2013). Talk about bezoar and its substitutes. *Mod. Chin. Med.* 15, 1106–1108. CNKI:SUN:YJXX.0.2013-12-021.

Militante, J. D., and Lombardini, J. B. (2002). Treatment of hypertension with oral taurine: experimental and clinical studies. *Amino Acids* 23, 381–393. doi:10.1007/s00726-002-0212-0

Millikan, C. H. (1970). Stroke-A journal of cerebral circulation: a new journal. Editorial. *Stroke* 1, 1–2. doi:10.1161/01.str.1.1.1

Orellana-Urzúa, S., Rojas, I., Líbano, L., and Rodrigo, R. (2020). Pathophysiology of ischemic stroke: role of oxidative stress. *Curr. Pharm. Des.* 26, 4246–4260. doi:10.2174/1381612826666200708133912

Owolabi, M., Akarolo-Anthony, S., Akinyemi, R., Arnett, D., Gebregziabher, M., Jenkins, C., et al. (2015). The burden of stroke in Africa: a glance at the present and a glimpse into the future. *Cardiovasc. J. Afr.* 26, S27–S38. doi:10.5830/CVJA-2015-038

Pandey, A., Shrivastava, A. K., and Saxena, K. (2014). Neuron specific enolase and creactive protein levels in stroke and its subtypes: correlation with degree of disability. *Neurochem. Res.* 39, 1426–1432. doi:10.1007/s11064-014-1328-9

Park, S. J., Lee, H. Y., Choi, N. R., Kwon, Y. M., and Joo, J. C. (2013). Calculus bovis-fel uris-moschus pharmacopuncture's effect on regional cerebral blood flow and mean arterial blood pressure in rats. *J. Pharmacopuncture* 16, 30–35. doi:10.3831/KPI.2013.16.028

Peng, Hu L. Y., Xu, Y. C., Hu, X. L., Yin, D. G., Dong, X. X., Yin, X. B., et al. (2022). Discussion on quality standards of traditional Chinese medicine preparations containing moschus or bovis calculus in 2020 edition of Chinese Pharmacopoeia. *Chin. J. Exp. Tradit. Med. Formulae* 28, 205–213. doi:10.13422/j.cnki.syfjx.20220356

Peng, X. Q., Liang, F. M., and Pang, F. M. (2019). Clinical efficacy and safety observation of cultured bezoar *in vitro* for the treatment of stroke. *Lingnan J. Emerg. Med.* 24, 181–182. doi:10.3969/j.issn.1671-301X.2019.01.027

Przykaza, L. (2021). Understanding the connection between common stroke comorbidities, their associated inflammation, and the course of the cerebral ischemia/reperfusion cascade. *Front. Immunol.* 12, 782569. doi:10.3389/fimmu.2021.782569

Qi, Z. M., and Rao, M. R. (1995). Effects of the combination of tetrandrine and taurine on platelet aggregation and thrombosis. *Chin. Pharmacol. Bull.*, 398–400.

Qiao, X., Ye, M., Pan, D. L., Miao, W. J., Xiang, C., Han, J., et al. (2011). Differentiation of various traditional Chinese medicines derived from animal bile and gallstone: simultaneous determination of bile acids by liquid chromatography coupled with triple quadrupole mass spectrometry. *J. Chromatogr. A* 1218, 107–117. doi:10.1016/j.chroma.2010.10.116

Radak, D., Katsiki, N., Resanovic, I., Jovanovic, A., Sudar-Milovanovic, E., Zafirovic, S., et al. (2017). Apoptosis and acute brain ischemia in ischemic stroke. *Curr. Vasc. Pharmacol.* 15, 115–122. doi:10.2174/1570161115666161104095522

Raschke, P., Massoudy, P., and Becker, B. F. (1995). Taurine protects the heart from neutrophil-induced reperfusion injury. *Free Radic. Biol. Med.* 19, 461–471. doi:10.1016/0891-5849(95)00044-x

Ren, M. H., Li, Y., Chen, H., Chen, J. J., Li, H. Y., Xie, Q., et al. (2023). Protective effect of calculus bovis cultivated *in vitro* on cerebral ischemia-reperfusion injury in rats. *Chin. Tradit. Pat. Med.* 45, 1989–1997. doi:10.3969/j.issn.1001-1528.2023.06.041

Ri, H. Z. J., and Shang, Z. J. (2005). *Rihuazi herbal medicine*. Anhui Science and Technology Press.

Rodrigo, R., Fernández-Gajardo, R., Gutiérrez, R., Matamala, J. M., Carrasco, R., Miranda-Merchak, A., et al. (2013). Oxidative stress and pathophysiology of ischemic stroke: novel therapeutic opportunities. *CNS Neurol. Disord. Drug Targets* 12, 698–714. doi:10.2174/18715273113120500015

Rodrigues, C. M. P., Spellman, S. R., Solá, S., Grande, A. W., Linehan-Stieers, C., Low, W. C., et al. (2002). Neuroprotection by a bile acid in an acute stroke model in the rat. *J. Cereb. Blood Flow. Metab.* 22, 463–471. doi:10.1097/00004647-200204000-00010

Sarvari, S., Moakedi, F., Hone, E., Simpkins, J. W., and Ren, X. (2020). Mechanisms in blood-brain barrier opening and metabolism-challenged cerebrovascular ischemia with emphasis on ischemic stroke. *Metab. Brain Dis.* 35, 851–868. doi:10.1007/s11011-020-00573-8

Schaffer, S. W., Solodushko, V., and Kakhniashvili, D. (2002). Beneficial effect of taurine depletion on osmotic sodium and calcium loading during chemical hypoxia. *Am. J. Physiol. Cell Physiol.* 282, C1113–C1120. doi:10.1152/ajpcell.00485.2001

Seizer, P., Borst, O., Langer, H., Bültmann, A., Münch, G., Herouy, Y., et al. (2009). EMMPRIN (CD147) is a novel receptor for platelet GPVI and mediates platelet rolling via GPVI-EMMPRIN interaction. *Thromb. Haemost.* 101, 682–686. doi:10.1160/TH08-06-0368

Sekerdag, E., Solaroglu, I., and Gursoy-Ozdemir, Y. (2018). Cell death mechanisms in stroke and novel molecular and cellular treatment options. *Curr. Neuropharmacol.* 16, 1396–1415. doi:10.2174/1570159X16666180302115544

Selçuk, Ö., Yayla, V., Çabalar, M., Güzel, V., Uysal, S., and Gedikbaşi, A. (2014). The relationship of serum S100B levels with infarction size and clinical outcome in acute ischemic stroke patients. *Noro Psikiyatri Arsivi* 51, 395–400. doi:10.5152/npa.2014.7213

Shi, H. L. (2009). Hypoxia inducible factor 1 as a therapeutic target in ischemic stroke. *Curr. Med. Chem.* 16, 4593–4600. doi:10.2174/092986709789760779

Shi, Z. F., Fang, Q., Chen, Y., Xu, L. X., Wu, M., Jia, M., et al. (2021). Methylene blue ameliorates brain edema in rats with experimental ischemic stroke via inhibiting aquaporin 4 expression. *Acta Pharmacol. Sin.* 42, 382–392. doi:10.1038/s41401-020-0468-5

Shimada, K., Azuma, Y., Kawase, M., Takahashi, T., Schaffer, S. W., and Takahashi, K. (2013). Taurine as a marker for the identification of natural Calculus Bovis and its substitutes. *Adv. Exp. Med. Biol.* 776, 141–149. doi:10.1007/978-1-4614-6093-0_15

Steliga, A., Kowiański, P., Czuba, E., Waśkow, M., Moryś, J., and Lietzau, G. (2020). Neurovascular unit as a source of ischemic stroke biomarkers-limitations of experimental studies and perspectives for clinical application. *Transl. Stroke Res.* 11, 553–579. doi:10.1007/s12975-019-00744-5

Su, J., and Shang, Z. J. (2004). *Xinxiu Bencao*. Anhui Science and Technology Press.

Suh, S., Cho, Y. R., Park, M. K., Kim, D. K., Cho, N. H., and Lee, M. K. (2018). Relationship between serum bilirubin levels and cardiovascular disease. *PloS One* 13, e0193041. doi:10.1371/journal.pone.0193041

Sun, B. Z., Chen, L., Wu, Q., Wang, H. L., Wei, X. B., Xiang, Y. X., et al. (2014). Suppression of inflammatory response by flurbiprofen following focal cerebral ischemia involves the NF-κB signaling pathway. *Int. J. Clin. Exp. Med.* 7, 3087–3095.

Sun, M., Gu, Y., Zhao, Y. M., and Xu, C. (2011). Protective functions of taurine against experimental stroke through depressing mitochondria-mediated cell death in rats. *Amino Acids* 40, 1419–1429. doi:10.1007/s00726-010-0751-8

Sun, M., Zhao, Y. M., Gu, Y., and Xu, C. (2012). Anti-inflammatory mechanism of taurine against ischemic stroke is related to down-regulation of PARP and NF-κB. *Amino Acids* 42, 1735–1747. doi:10.1007/s00726-011-0885-3

Sun, M., Zhao, Y. M., and Xu, C. (2008). Taurine reduces the disorder of energy metabolism and oxidative damage due to focal cerebral ischemia in rats. *J. Apoplexy Nerv. Dis.*, 577–579.

Takahashi, K., Azuma, Y., Shimada, K., Saito, T., Kawase, M., and Schaffer, S. W. (2010). Quality and safety issues related to traditional animal medicine: role of taurine. *J. Biomed. Sci.* 17, S44. doi:10.1186/1423-0127-17-S1-S44

Tao, H. J. (1955). *Collection of herbal classics*. Shanghai Qunlian Press.

Tochitani, S. (2017). Functions of maternally-derived taurine in fetal and neonatal brain development. *Adv. Exp. Med. Biol.* 975 (Pt 1), 17–25. doi:10.1007/978-94-024-1079-2_2

Treda, C., Popeda, M., Ksiazkiewicz, M., Grzela, D. P., Walczak, M. P., Banaszczyk, M., et al. (2016). EGFR activation leads to cell death independent of PI3K/AKT/mTOR in an AD293 cell line. *PloS One* 11, e0155230. doi:10.1371/journal.pone.0155230

Tuo, Q. Z., Zhang, S. T., and Lei, P. (2022). Mechanisms of neuronal cell death in ischemic stroke and their therapeutic implications. *Med. Res. Rev.* 42, 259–305. doi:10.1002/med.21817

Tuttolomondo, A., Di Raimondo, D., di Sciacca, R., Pinto, A., and Licata, G. (2008). Inflammatory cytokines in acute ischemic stroke. *Curr. Pharm. Des.* 14, 3574–3589. doi:10.2174/138161208786848739

Vakili, A., Mojarrad, S., Akhavan, M. M., and Rashidy, P. A. (2011). Pentoxifylline attenuates TNF-α protein levels and brain edema following temporary focal cerebral ischemia in rats. *Brain Res.* 1377, 119–125. doi:10.1016/j.brainres.2011.01.001

Vaz, A. R., Cunha, C., Gomes, C., Schmucki, N., Barbosa, M., and Brites, D. (2015). Glycoursodeoxycholic acid reduces matrix metalloproteinase-9 and caspase-9 activation in a cellular model of superoxide dismutase-1 neurodegeneration. *Mol. Neurobiol.* 51, 864–877. doi:10.1007/s12035-014-8731-8

Vecsernyés, M., Fenyvesi, F., Bácskay, I., Deli, M. A., Szente, L., and Fenyvesi, É. (2014). Cyclodextrins, blood-brain barrier, and treatment of neurological diseases. *Arch. Med. Res.* 45, 711–729. doi:10.1016/j.arcmed.2014.11.020

Wan, T. C., Cheng, F. Y., Liu, Y. T., Lin, L. C., and Sakata, R. (2009). Study on bioactive compounds of *in vitro* cultured calculus suis and natural calculus bovis. *Anim. Sci. J. Nihon Chikusan Gakkaiho* 80, 697–704. doi:10.1111/j.1740-0929.2009.00689.x

Wang, G. H., Jiang, Z. L., Fan, X. J., Zhang, L., Li, X., and Ke, K. F. (2007a). Neuroprotective effect of taurine against focal cerebral ischemia in rats possibly mediated by activation of both $GABA_A$ and glycine receptors. *Neuropharmacology* 52, 1199–1209. doi:10.1016/j.neuropharm.2006.10.022

Wang, G. H., Jiang, Z. L., Li, X., Fan, X. J., and Shen, L. H. (2010). Combination of taurine and diazepam has neuroprotective effect on focal cerebral ischemia-reperfusion in rats. *Chin. J. Pathophysiol.* 26, 737–741. doi:10.3969/j.issn.1000-4718.2010.04.022

Wang, G. H., Lan, R., Zhen, X. D., Zhang, W., Xiang, J., and Cai, D. F. (2014). Angong Niuhuang Wan protects against cerebral ischemia induced apoptosis in rats: up-regulation of Bcl-2 and down-regulation of Bax and caspase-3. *J. Ethnopharmacol.* 154, 156–162. doi:10.1016/j.jep.2014.03.057

Wang, H., Chen, Y., Li, P., Huang, M., and Guo, Z. L. (2021). Clinical observation of in-vitro-cultured calculus bovis on ischemic apoplexy involving Zang and fu-viscera. *J. Hunan Univ. Chin. Med.* 41, 1098–1102. doi:10.3969/j.issn.1674-070X.2021.07.022

Wang, H. R., Li, J. S., and Yang, F. (2007b). Effects of taurine and zinc on the expression of HIF-1α mRNA in the cerebrum of acute hypoxic mice. *Chin. J. Pathophysiol.* 23, 160–162. doi:10.3321/j.issn:1000-4718.2007.01.036

Wang, L. J., and Xu, C. (2017). The efficacy of taurine on inflammatory reactions in ischemic brain tissue during focal cerebral ischemia-reperfusion damage in rats. *J. Clin. Exp. Med.* 16, 1564–1569. doi:10.3969/j.issn.1671-4695.2017.16.002

Wang, L. J., Zhao, L. Z., Zhou, X. M., and Niu, J. W. (2018). The efficacy of taurine on microglial activation in ischemic brain tissue of rats during cerebral ischemia reperfusion damage. *Chin. J. Difficult Complicat. Cases* 17, 71–74+79+109. doi:10.3969/j.issn.1671-6450.2018.01.018

Wang, Q., Fan, W. J., Cai, Y., Wu, Q. L., Mo, L. D., Huang, Z. W., et al. (2016). Protective effects of taurine in traumatic brain injury via mitochondria and cerebral blood flow. *Amino Acids* 48, 2169–2177. doi:10.1007/s00726-016-2244-x

Wang, W. (2021). Clinical efficacy of Tibetan medicine Zhenzhu-25 pill in the treatment of sequelae of cerebral thrombosis. *J. Med. Pharm. Chin. Minor.* 27, 16–18. doi:10.3969/j.issn.1006-6810.2021.03.009

Wang, Z., and Huang, Y. M. (2012). Oxidative stress and cerebral ischemia-reperfusion injury. *J. Clin. Pathol. Res.* 32, 343–346. doi:10.3969/j.issn.1673-2588.2012.04.014

Wang, Z. S., and Xue, H. (2008). *Shennong's herbal classic*. Sichuan Science and Technology Press.

Wang, Z. S., Zhao, X. C., Zheng, X. J., and Gao, F. (2013). Observation of the clinical efficacy on cerebral thrombosis sequela treated with twenty-five ingredients pearl pills. *World J. Integr. Tradit. West. Med.* 8, 165–167. doi:10.3969/j.issn.1673-6613.2013.02.020

Wen, H. R., Ji, Y. H., Han, X., Lyu, B. J., Wang, G. P., Liu, Y., et al. (2020). Determination of 18 kinds of amino acids in cultured bezoar and bovisc alculus using pre-column derivatization HPLC. *Mod. Chin. Med.* 22, 231–236. doi:10.13313/j.issn.1673-4890.20190305003

Wu, J. Y., and Prentice, H. (2010). Role of taurine in the central nervous system. *J. Biomed. Sci.* 17 (Suppl. 1), S1. doi:10.1186/1423-0127-17-S1-S1

Wu, R. (2019). *Chinese Materia medica, volume 22, daily Materia medica*. Beijing Huaxia Publishing House.

Wu, S., Wu, B., Liu, M., Chen, Z., Wang, W., Anderson, C. S., et al. (2019). Stroke in China: advances and challenges in epidemiology, prevention, and management. *Lancet Neurol.* 18, 394–405. doi:10.1016/S1474-4422(18)30500-3

Wu, X. Y., Guo, J. J., Shi, L., and Chen, T. (2023). Effect of cultured bezoar combined with intravenous thrombolytic therapy on prognosis of acute ischemic stroke patients with consciousness disturbance. *Chin. J. Gerontol.* 43, 2838–2840. doi:10.3969/j.issn.1005-9202.2023.12.006

Xiang, B., and Xiong, J. B. (2023). Clinical study of cultured bezoar *in vitro* on the recovery of nerve function after hemorrhagic stroke. *Chin. Sci. Technol. J. Database Cit. Ed. Med. Health*, 0183–0185.

Xiong, J., Wang, Z., Bai, J., Cheng, K., Liu, Q., and Ni, J. (2023). Calcitonin gene-related peptide: a potential protective agent in cerebral ischemia-reperfusion injury. *Front. Neurosci.* 17, 1184766. doi:10.3389/fnins.2023.1184766

Xu, F. (2015). Risk factors of early neurological deterioration in patients with acute ischemic stroke. *Pract. J. Card. Cereb. Pneumal Vasc. Dis.*, 11–14. doi:10.3969/j.issn.1008-5971.2015.07.003

Xu, J. X., and Luan, X. P. (2016). Protective effect and mechanism of Annao tablet on vascular dementia model rats. *Chin. J. Gerontol.* 36, 4693–4695. doi:10.3969/j.issn.1005-9202.2016.19.009

Xu, X. H., Zheng, X. X., Zhou, Q., and Li, H. (2007). Inhibition of excitatory amino acid efflux contributes to protective effects of puerarin against cerebral ischemia in rats. *Biomed. Environ. Sci. Bes.* 20, 336–342. doi:10.1111/j.1440-1584.2007.00912.x

Xu, X. L., Ma, C. Y., Wang, X. Q., Wang, G. L., Zhai, C. M., Yue, W. C., et al. (2017). Comparative study of cholic acid compounds of bezoar on anti-cerebral infarction and regulating endoplasmic reticulum stress. *Drug Eval. Res.* 40, 11–19. doi:10.7501/j.issn.1674-6376.2017.01.003

Yan, J. W., Chen, L., Wang, Y. Y., Gong, Q. H., Liu, J., Shi, J. S., et al. (2017). Protective effects of Angongniuhuang Wan on cerebral ischemia-reperfusion injury and traumatic brain injury in rats. *J. Zunyi Med. Univ.* 40, 249–253. doi:10.14169/j.cnki.zunyixuebao.2017.0055

Yan, S. K., Wu, Y. W., Liu, R. H., and Zhang, W. D. (2007). Comparative study on major bioactive components in natural, artificial and *in-vitro* cultured Calculus Bovis. *Chem. Pharm. Bull. (Tokyo)* 55, 128–132. doi:10.1248/cpb.55.128

Yang, H., and Luo, Y. (2015). Clinical efficacy of Annao tablet in the treatment of vascular dementia after stroke. *J. Kunming Med. Univ.* 36, 132–133. doi:10.3969/j.issn.1003-4706.2015.08.038

Yang, H. L., Hu, H. Y., and Zhang, D. (2006). Protective effect oftaurine on reperfusioninjury afterfocal cerebral ischemiain rats. *Med. J. Chin. People's Armed Police Force*, 26–29+83. doi:10.14010/j.cnki.wjyx.2006.01.012

Yang, M. Z., Chi, C., and Chi, P. (1996). Research trends of Chinese bezoar in 43 years (1949-1992). *Chin. J. Ethnomedicine Ethnopharmacy*, 27–35. CNKI:SUN:MZMJ.0.1996-01-016.

Yanguas-Casás, N., Barreda-Manso, M. A., Nieto-Sampedro, M., and Romero-Ramírez, L. (2017). TUDCA: an agonist of the bile acid receptor GPBAR1/TGR5 with anti-inflammatory effects in microglial cells. *J. Cell. Physiol.* 232, 2231–2245. doi:10.1002/jcp.25742

Ye, R. G., Wu, X. H., Wang, X. J., and Ma, C. M. (2016). Quantification of bile acids in traditional animal medicines and their preparations using ultra high-performance liquid chromatography-mass spectrometry in the multiple reaction monitoring mode. *Anal. Sci.* 32, 499–503. doi:10.2116/analsci.32.499

Yu, Z. J., Xu, Y., Peng, W., Liu, Y. J., Zhang, J. M., Li, J. S., et al. (2020). Calculus bovis: a review of the traditional usages, origin, chemistry, pharmacological activities and toxicology. *J. Ethnopharmacol.* 254, 112649. doi:10.1016/j.jep.2020.112649

Yuan, H. N., Yang, M. Z., and Shi, Q. S. (1992). Study on pharmacological effects of cultivated bezoar and natural bezoar. *Spec. Wild Econ. Anim. Plant Res.* 12-15, 64–65. doi:10.16720/j.cnki.tcyj.1992.02.006

Zang, Y. P., Liang, G. X., and Gao, H. M. (2011). Effects of taurine on the expression of neurons SCF mRNA of focal cerebral ischemia in rat. *Int. J. Tradit. Chin. Med.* 33, 689–691. doi:10.3760/cma.j.issn.1673-4246.2011.08.006

Zeng, Y. S. (2024). *Clinical observation on the treatment of aute ischemic stroke (wind phlegm stasis syndrome) with Huatan Tongluo Decoction and* in vitro *cultivation of bezoar*. Southern Medical University. doi:10.27003/d.cnki.gojyu.2023.000972

Zhang, D. (2006). *The protective effect of taurine against focal cerebral ischemia reperfusion injury in rats*. China Medical University.

Zhang, H., Liu, J., Liu, Y. Z., Su, C. H., Fan, G. Y., Lu, W. P., et al. (2020a). Hypertonic saline improves brain edema resulting from traumatic brain injury by suppressing the NF-κB/IL-1β signaling pathway and AQP4. *Exp. Ther. Med.* 20, 71. doi:10.3892/etm.2020.9199

Zhang, L., Li, Y. H., and Que, Y. M. (2013). Analysis of risk factors for early fever in patients with acute cerebral infarction. *Chin. J. Metall. Ind. Med.* 30, 411–412. doi:10.13586/j.cnki.yjyx1984.2013.04.089

Zhang, L. M., Liu, S., Ma, J., Wu, G. T., and Ren, Y. (2006). Effects of Ershiwuwei Zhenzhu wan on cerebral capillary permeability and cerebral water content in the experimental rats with cerebral ischemia. *China Pharm.* 17, 664–666. doi:10.3969/j.issn.1001-0408.2006.09.007

Zhang, R. W., Liu, Y. M., Feng, H. L., Yu, Q., and Wang, M. Y. (2016). Experiment of the neuroprotective effect of bilirubin on penumbra of cerebral ischemia-reperfusion rat models. *J. Chin. Pract. Diagn. Ther.* 30, 343–345. doi:10.13507/j.issn.1674-3474.2016.04.011

Zhang, S., Jiang, X., Wang, Y., Lin, K., Zhang, Z., Zhang, Z., et al. (2021). Protective effect of Angong niuhuang wan pre-treatment against experimental cerebral ischemia injury via regulating GSK-3β/HO-1 pathway. *Front. Pharmacol.* 12, 640297. doi:10.3389/fphar.2021.640297

Zhang, T. L., Xun, X. J., Xiang, J. Y., Shen, J., and Shi, H. (2008). Effect of taurine on the expression of nestin in neural stem cell after focal cerebral ischemia in rats. *J. Clin. Res.* 25, 414–416. doi:10.3969/j.issn.1671-7171.2008.03.011

Zhang, W. J., Cui, L. J., Niu, L. G., Liu, Y., Xue, W. X., Qin, J. L., et al. (2009). The effect of potassium channel on the dilation of pig coronary artery by taurine. *Chin. J. Integr. Med. Cardio-Cerebrovasc. Dis.* 7, 690–692. doi:10.3969/j.issn.1672-1349.2009.06.034

Zhang, X. Q., Ming, S. P., and Liu, L. (2015). Effect of cultured bezoar *in vitro* on patients with acute stroke with consciousness disturbance. *J. Math. Med.* 28, 216–217. doi:10.3969/j.issn.1004-4337.2015.02.039

Zhang, Y. (2019). *Effects and mechanisms of Annao tablets on cerebral ischemia-reperfusion injuries*. Peking Union Medical College, Chinese Academy of Medical Sciences.

Zhang, Y., Cao, M. Y., Wu, Y. M., Wang, J., Zheng, J., Liu, N. S., et al. (2020b). Improvement in mitochondrial function underlies the effects of Annao tablets on attenuating cerebral ischemia-reperfusion injuries. *J. Ethnopharmacol.* 246, 112212. doi:10.1016/j.jep.2019.112212

Zhang, Y., Ding, X. W., Miao, C. H., and Chen, J. W. (2019). Propofol attenuated TNF-α-modulated occludin expression by inhibiting Hif-1α/VEGF/VEGFR-2/ERK signaling pathway in hCMEC/D3 cells. *BMC Anesthesiol.* 19, 127. doi:10.1186/s12871-019-0788-5

Zhao, H., Chen, Z., Xie, L. J., and Liu, G. F. (2018a). Suppression of TLR4/NF-κB signaling pathway improves cerebral ischemia-reperfusion injury in rats. *Mol. Neurobiol.* 55, 4311–4319. doi:10.1007/s12035-017-0552-0

Zhao, Y. H. (2007). "Pharmacokinetics of bile acids in several traditional Chinese medicines and compound medicines," in *Academy of military medical sciences*. doi:10.7666/d.Y1201265

Zhao, Y. H., Kong, A. Y., Zhang, Z. Q., and Ruan, J. X. (2009). Pharmacokinetics of bile acid in natural calculus bovis and A200ngong niuhuang pills. *J. Beijing Univ. Tradit. Chin. Med.* 32, 344–348. doi:10.3321/j.issn:1006-2157.2009.05.015

Zhao, Y. M., Sun, M., and Xu, C. (2012). Protection of taurine against transient forebrain ischemia in gerbils. *Chin. J. Stroke* 7, 98–102. doi:10.3969/j.issn.1673-5765.2012.02.005

Zhao, Y. Y., Wang, H. H., Xu, J. X., Xu, L. S., Liu, Z., and Xu, C. (2018b). Antihypertensive effects and mechanism of indapamide and taurine in combination. *J. Shenyang Pharm. Univ.* 35, 1045–1051. doi:10.14066/j.cnki.cn21-1349/r.2018.12.009

Zheng, W., Niu, L. J., Zhu, C., Song, S. X., Hou, D. P., Chen, H. Z., et al. (2014). Experimental study on the mechanism of Angong niuhuang pill on blood brain barrier injury and brain edema after traumatic brain injury of rats. *Clin. Med. Eng.*, 1246–1247. doi:10.3969/j.issn.1674-4659.2014.10.1246

Zhou, F., Cheng, J. S., Li, F. F., Zhang, T., and Yan, Z. G. (2004). Effects of taurine on cerebral blood flow and infarct volume in rat model of acute focal cerebral ischemia. *Chin. J. New Drugs Clin. Remedies*, 266–268. doi:10.3969/j.issn.1007-7669.2004.05.003

Zhou, J. (2019). *Protective effects of taurine on injury of Hippocampus neural stem cells in rats induced by glutamate*. Shenyang Agricultural University.

Zhu, S. Z., Szeto, V., Bao, M. H., Sun, H. S., and Feng, Z. P. (2018). Pharmacological approaches promoting stem cell-based therapy following ischemic stroke insults. *Acta Pharmacol. Sin.* 39, 695–712. doi:10.1038/aps.2018.23

Zhu, X. L., Zhang, N., Li, P. T., Jiang, Y. F., and Xu, Y. (2004). Effects of cholic acid and jasminoidin on the levels of cerebral TNF-α, IL-1β and ICAM-1 in the rats undergone cerebral ischemia. *J. Beijing Univ. Tradit. Chin. Med.*, 26–29. doi:10.3321/j.issn:1006-2157.2004.06.010

Zhu, X. Y., Ma, P. S., Wu, W., Zhou, R., Hao, Y. J., Niu, Y., et al. (2016). Neuroprotective actions of taurine on hypoxic-ischemic brain damage in neonatal rats. *Brain Res. Bull.* 124, 295–305. doi:10.1016/j.brainresbull.2016.06.010

Glossary

8-OHdG	8-hydroxy-2′-deoxyguanosine
A.D.	Anno domini
AA	Arachidonic acid
ADP	Adenosine diphosphate
Asp	Aspartic acid
AUC	Area under curve
B.C.	Before christ
BBB	Blood-brain barrier
BDNF	Brain-derived neurotrophic factor
BR	Bilirubin
BV	Biliverdin
CA	Cholic acid
cAMP	Cyclic adenosine monophosphate
BCA	*Bovis Calculus artifactus*
CBF	Cerebral blood flow
BCS	*Bovis Calculus sativus*
CBC	Cultured *Bovis Calculus*
CDCA	Chenodeoxycholic acid
CI/RI	Cerebral ischemia/reperfusion injury
CPM	Chinese patent medicine
CRS-R	Coma Recovery Scale-Revised
CytC	Cytochrome C
DCA	Deoxycholic acid
EAA	Excitatory amino acids
ESR	Erythrocyte sedimentation rate
FIB	Fibrinogen
FOUR	Full Outline of UnResponsiveness
GABA	γ-aminobutyric acid
$GABA_AR$	γ-aminobutyric acid type a receptors
GCA	Glycocholic acid
GCDCA	Glycochenodeoxycholic acid
GCS	Glasgow Coma Scale
GDCA	Glycodeoxycholic acid
GDNF	Glia cell line-derived neurotrophic factor
Glu	Glutamic acid
Gly	Glycine
GSH	Glutathione
GSH-Px	Glutathione peroxidase
GUDCA	Glycoursodeoxycholic acid
HCT	Hematocrit
hs-CRP	High-sensitivity c-reactive protein
IAA	Inhibitory amino acids
ICAM-1	Intercellular adhesion molecule-1
IL-1β	Interleukin-1β
IL-6	Interleukin-6
IL-8	Interleukin-8
iNOS	Inducible nitric oxide synthase
IS	Ischemic stroke
LDL-C	Low-density lipoprotein cholesterol
MCP-1	Monocyte chemoattractant protein-1
MDA	Malondialdehyde
MMP-9	Matrix metalloproteinase-9
mRS	Modified Rankin Scale
NADPH	Nicotinamide adenine dinucleotide phosphate
NBC	Natural *Bovis Calculus*
NF-κB	Nuclear factor-κb
NIHSS	National Institute of Healthstroke scale
NMDA	N-methyl-D-aspartate
NO	Nitric oxide
NSE	Neuron specific enolase
NVU	Neurovascular unit
OD	Ornidazole
OGD/R	Oxygen-glucose deprivation/reperfusion
ROS	Reactive oxygen species
rt-PA	Recombinant tissue-type plasminogen activator
SOD	Superoxide dismutase
T-AOC	Total anti-oxidative capability
Tau	Taurine
TCA	Taurocholic acid
TCDCA	Taurochenodeoxycholic acid
TDCA	Taurodeoxycholic acid
TEER	Transepithelial electrical resistance
TG	Triglycerides
TGR5	Takeda g-protein receptor 5
TNF-α	Tumor necrosis factor-α
TUDCA	Tauroursodeoxycholic acid
UDCA	Ursodeoxycholic acid
VCAM-1	Vascular cell adhesion molecule-1
VEGF	Vascular endothelial growth factor
WBV	Whole blood viscosity
ZO-1	Zonula occludens-1
γ-GT	Gamma-glutamyl transferase

frontiers | Frontiers in Pharmacology

TYPE Original Research
PUBLISHED 13 March 2025
DOI 10.3389/fphar.2025.1528011

Check for updates

OPEN ACCESS

EDITED BY
Bin Yu,
Nanjing University of Chinese Medicine, China

REVIEWED BY
Caiyun Zhang,
Anhui University of Chinese Medicine, China
Jian Cui,
Nanjing University of Chinese Medicine, China

*CORRESPONDENCE
Jing Chen,
chenjing6385@163.com

RECEIVED 14 November 2024
ACCEPTED 26 February 2025
PUBLISHED 13 March 2025

CITATION
Liu S, Song X, Sun Y, Sun A, Li Y, Li Y and Chen J (2025) Kai-Xin-San ameliorates mild cognitive impairment in SAMP8 mice by inhibiting neuroinflammation and pyroptosis via NLRP3/Caspase-1 pathway modulation.
Front. Pharmacol. 16:1528011.
doi: 10.3389/fphar.2025.1528011

COPYRIGHT
© 2025 Liu, Song, Sun, Sun, Li, Li and Chen. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

Kai-Xin-San ameliorates mild cognitive impairment in SAMP8 mice by inhibiting neuroinflammation and pyroptosis via NLRP3/Caspase-1 pathway modulation

Shu Liu[1,2], Xiaochen Song[1], Yuefeng Sun[1], Ailin Sun[1], Yang Li[1], Yuyu Li[1] and Jing Chen[1]*

[1]College of Basic Medical and Sciences, Heilongjiang University of Chinese Medicine, Harbin, Heilongjiang, China, [2]The Faculty of Medicine, Qilu Institute of Technology, Jinan, Shandong, China

Mild Cognitive Impairment (MCI) represents a critical stage between normal aging and dementia, with limited effective interventions currently available. This study investigated the effects of Kai-Xin-San (KXS), a traditional Chinese herbal formula, on cognitive function, neuroinflammation, and pyroptosis in a senescence-accelerated prone 8 (SAMP8) mouse model of MCI. SAMP8 mice were treated with KXS for 8 weeks, followed by behavioral tests, biochemical analyses, and histological examinations. KXS significantly improved spatial memory, working memory, and executive function in SAMP8 mice. Furthermore, KXS treatment reduced β-amyloid (Aβ) deposition, attenuated neuroinflammation by decreasing pro-inflammatory cytokine levels (IL-1β, IL-18, IL-6, TNF-α), and inhibited microglia activation in the hippocampus. Notably, KXS suppressed pyroptosis by modulating the NLRP3/Caspase-1 signaling pathway, as evidenced by reduced expression of NLRP3, ASC, Caspase-1, and GSDMD. These effects were abolished by treatment with the NLRP3 inflammasome agonist Nigericin, suggesting that NLRP3 inhibition is a key mechanism of KXS action. Our findings reveal a novel mechanism by which KXS exerts neuroprotective effects in MCI, simultaneously targeting Aβ accumulation, neuroinflammation, and pyroptosis. This multi-target approach of KXS highlights its potential as a therapeutic strategy for MCI and warrants further investigation in clinical settings.

KEYWORDS

Kai-Xin-San, TCM, mild cognitive impairment, anti-inflammation function, NLRP3 inflammasome, pyroptosis

Highlights

- Kai-Xin-San (KXS) can prevent and treat mild cognitive impairment (MCI).
- KXS improved spatial and working memory and reduced brain injury in SAMP8 mice.
- KXS showed therapeutic potential for MCI in SAMP8 mice by reducing inflammation.
- KXS treated MCI in SAMP8 mice by inhibiting the NLRP3/Caspase-1 pyroptosis pathway.

1 Introduction

With the accelerating aging population worldwide, diseases related to cognitive impairments such as dementia have become a growing concern for global public health (Jongsiriyanyong and Limpawattana, 2018). Alzheimer's disease (AD), the predominant type of dementia, impacts countless people worldwide (Selkoe, 2002; Avila and Perry, 2021). It leads to deterioration of cognitive function, reduced quality of life, and loss of independence in advanced stages, posing a serious threat to the health and wellbeing of the elderly population.

Mild cognitive impairment (MCI), characterized by subtle declines in memory and thinking abilities without significant impact on daily activities, represents a critical juncture between normal aging and dementia (Chandra et al., 2019). The importance of focusing on MCI cannot be overstated, as it offers a potential window for intervention before the onset of irreversible AD, it will be irreversible, for which no curative treatments currently exist. This aligns with the traditional Chinese medicine principle of "treating the future disease," emphasizing early intervention and prevention.

Kai-Xin-San (KXS), a traditional Chinese medicine prescription recorded in the "Bei Ji Qian Jin Yao Fang" by Sun Simiao, a Tang Dynasty physician, has garnered attention for its potential to treat forgetfulness. It is composed of four traditional Chinese botanical drugs, namely, Wolfiporia cocos (F.A. Wolf) Ryvarden and Gilb [Polyporaceae], Panax ginseng C.A.Mey [Araliaceae], Polygala tenuifolia Willd [Polygalaceae], and Acorus tatarinowii Schott [Acoraceae], which is a representative formula for benefiting the intellect and tranquilizing the mind. Recent research has revealed its multifaceted pharmacological effects, including improvements in cognitive function and memory, along with antioxidant and anti-inflammatory capabilities, and enhancement of neurotrophic factors (Luo et al., 2020; Wang et al., 2020; Jiao et al., 2022; Su et al., 2023). The results of the mass spectrometry analysis demonstrated the presence of a total of 77 phyto ingredients, including 26 saponins, 13 triterpenoids, 20 oligosaccharides, 5 ketone compounds, and 13 other components, in the four botanical drugs of KXS. Among these bioactive natural components, 25 were derived from Polygala tenuifolia, primarily comprises oligosaccharides and ketone compounds; 28 were derived from Panax ginseng, predominantly saponins; 17 were derived from Poria cocos, primarily triterpenoid acids; and 5 were derived from Acorus tatarinowii, majorly β-as aryl ether, and other constituents (Li H. et al., 2022). In the analysis, LIN R. et al. employed the UPLC-Q-Orbitrap-MS technique in conjunction with a local database to determine different phytochemicals from KXS. A total of 211 compounds were identified from KXS, including 60 Panax ginseng, 40 Poria cocos, and 111 Poria cocos. Subsequently, 105 volatile compounds were recognized by GC-MS analysis, predominantly originating from the rhizome of Acorus tatarinowii (Lin et al., 2021). Furthermore, ginsenoside Rb1, ginsenoside Re, ginsenoside Rg1, Sibiricose A5, Sibiricose A6, 3,6′-Disinapoylsucrose, polygalaxanthone III, α-asarone, β-asarone, and Poria acid were identified in the serum of rats administered KXS groups (Gao and Lv, 2021). The aforementioned studies have collectively revealed that the components present in KXS, including polygala oligosaccharide esters, ginsenosides, and Acorus volatile oil, could serve as quality markers for KXS.

The pathogenesis of MCI and its progression to AD involve complex mechanisms, with β-amyloid (Aβ) deposition playing a central role. It is a significant contributor to the loss of neurons and the deterioration of cognitive abilities (Hardy and Selkoe, 2002). Excessive Aβ accumulation not only forms neurotoxic senile plaques but also triggers chronic neuroinflammation, creating a detrimental feedback loop that accelerates cognitive decline (Zhang et al., 2019; Li Z. et al., 2021). Furthermore, Aβ can activate NOD-like receptor protein 3 (NLRP3) inflammasome and drive pyroptosis, a recently recognized form of inflammatory cell death that exacerbates neuroinflammation and potentially hastens MCI progression (Yu et al., 2021).

Preliminary studies suggest that KXS may exert its cognitive-enhancing effects in senescence-accelerated prone 8 (SAMP8) mice by modulating Aβ production and catabolism, thereby reducing Aβ deposition and ameliorating pathological changes in brain tissue. Additionally, metabolomic and pharmacological investigations indicate that KXS can improve neuroinflammation and cognitive function by regulating inflammatory mediators (Tangalos and Petersen, 2018; Sun et al., 2021; Wang et al., 2021). However, the specific signaling pathway through which KXS inhibits neuroinflammation and pyroptosis in the context of MCI remains unexplored.

This study aimed to elucidate the capacity of KXS in reducing neuroinflammation and pyroptosis in MCI and to uncover its underlying mechanism. By focusing on the neuroprotective mechanisms underlying the cognitive benefits of KXS, our goal is to offer a thorough insight into its healing possibilities for MCI, bridging traditional wisdom with modern molecular insights.

2 Materials and methods

2.1 Materials

KXS were procured from Hebei Quantai Pharmaceutical Co., Ltd., (Hebei, China), adhering to the standards of the Pharmacopoeia of the People's Republic of China (2020 Edition), Part I and Part IV. Donepezil hydrochloride was obtained from Eisai (China) Pharmaceutical Co. Ltd., Fluoro-Jade C (FJC) kit and Congo red staining reagents were obtained from Beijing Solarbio Science and Technology Co., Ltd., (Beijing, China). Vectastain Elite ABC-HRP Immunohistochemistry (IHC) kit was acquired from VECTOR (California, United States). Terminal labeling (TUNEL) apoptosis kit was obtained from Roche (Basel, Switzerland). Hematoxylin, eosin reagent, and the BCA protein concentration determination kit were obtained from Beyotime Biotech. Inc., (Shanghai, China). Sources of ELISA kits for cytokines including

Abbreviations: KXS, Kai-Xin-San; MCI, mild cognitive impairment; AD, Alzheimer's disease; Aβ, β-amyloid; NLRP3, NOD-like receptor protein 3; SAMP8, senescence-accelerated prone 8; FJC, fluoro-Jade C; IHC, immunohistochemistry; TUNEL, terminal labeling; ELISA, enzyme-linked immunosorbent assay; GSDMD, gasdermin D; MWM, Morris water maze; NOR, novel object recognition; H&E, hematoxylin-eosin; TEM, transmission electron microscopy; Iba-1, ionized calcium-binding adaptor molecule-1; CNS, central nervous system; ASC, apoptosis-associated speck-like protein containing CARD; GSDMD-NT, GSDMD-N-terminal.

TABLE 1 Nesting-building score scale (Deacon, 2006).

Score	Scoring standard
1	There were no tear marks on the tissue paper
2	A small portion of tissue paper was torn
3	A large portion of tissue paper was torn, but no recognizable nests were formed
4	Most of the tissue paper was torn and had been built into flatter nests
5	The tissue paper was completely nested

IL-18, IL-6, IL-1β, and TNF-α were Elabscience Biotechnology Co., Ltd., and ABclonal Technology Co., Ltd., (both Wuhan, China). Primary antibodies: anti-ASC (cat. no. A16672), anti-Caspase-1 (cat. no. A0964), anti-IL-1β (cat. no. A19635), anti-IL-18 (cat. no. A20473), anti-β-actin (cat. no. AC026), and HPR goat anti-rabbit IgG (cat. no. AS014) from ABclonal Technology Co., Ltd. (Wuhan, China); Anti-Iba-1 (cat. no. DF6442), and anti-Gasdermin D (GSDMD) (cat. no. AF4012) from Affinity Biosciences Co., Ltd. (Jiangsu, China); Goat anti-rabbit IgG (cat. no. Ap132p) from Millipore (Massachusetts, United States); Anti-NLRP3 (cat. no. 381207) from Zen-Bioscience Co., Ltd., (Chengdu, China); Anti-Aβ1-42 (cat. no. BB02112732) from Beijing Biosynthesis Biotechnology Co., Ltd., (Beijing, China).

2.2 Preparation of KXS

KXS was prepared using Panax ginseng, Poria cocos, Polygala tenuifolia, and Acorus tatarinowii to crush into fine powder in the ratio of 2:1:1:1, ensuring that not less than 95% of the weight of the medicinal powder passed through a No. 6 sieve (100 mesh). The powders were mixed to create the final powder dosage form.

2.3 Animals and drug administration

Male SAMP8 and SAMR1 mice, aged 7 months and sharing the same genetic lineage (weight: 20 ± 2 g), were sourced from the Department of Laboratory Animal Science, Faculty of Medicine, Peking University [SCXK (Beijing) 2016–0,010]. Mice were housed individually in SPF-grade conditions at the Center for Evaluation of Pharmaceutical Safety of the Heilongjiang University of Chinese Medicine, with controlled room temperature (20.0 ~24.0)°C, humidity of (40.0 ~ 60.0)%, light/dark cycle of 12 h/12 h. All procedures conducted in the study adhered to the Declaration of Helsinki principles for animal research and complied with the Requirements for Ethical Experiments on Animals and the Guidelines for Experimental Animals and Code of Practice for the Use of Experimental Animals of the Heilongjiang University of Chinese Medicine.

All mice were acclimatized for 7 days and then trained in the Morris water maze. SAMP8 mice with an escape latency of >80 s were selected as MCI experimental animals (Hou et al., 2020) and randomly assigned to the following groups: KXS group (KXS was prepared in distilled water for oral administration at a daily dose of 0.58 g/kg), donepezil group (donepezil hydrochloride was prepared in distilled water and given orally at a daily dose of 0.65 mg/kg), KXS + Nigericin group (Nigericin was prepared in 0.9% saline solution for intraperitoneal injection at a dosage of 1.4 mg/kg, administered every 3 days, and at the same time, KXS was administered by gavage once a day, with an interval of half an hour between the two administrations), and SAMP8 group. The SAMR1 mice were kept in the control group. Mice in the SAMP8 and control groups received an equivalent volume of distilled water orally daily, and each group of mice was gavaged continuously for 28 days.

2.4 Behavioral tests

2.4.1 Morris water maze (MWM) test

MCI model animals were screened by the MWM test 7 days before the start of formal experiments. The apparatus consisted of a circular pool measuring 120 cm in diameter and 45 cm in depth, with black-painted interior walls. A video camera above the pool recorded swimming trajectories, which were analyzed by computer software to calculate escape latency, swimming distance, and speed. Each mouse underwent four separate trials daily, with a 10-min interval between each trial and a 90-s limit per trial. Following each trial, they were permitted to remain on the platform for 30 s.

The localization navigation experiment was conducted for 5 consecutive days, starting from the 21st day of administration. Randomly selected quadrants were used as starting points, where mice were released into the water to swim and locate the hidden platform. When a mouse located and climbed onto the platform within the 120-s timeframe, it was permitted to remain there for 10 s, and the latency was noted. In case of not finding the safe platform, the mouse was artificially guided to the platform for 10 s, so that it could memorize the platform's position, and the recording time was 120 s. The space exploration experiment was conducted the next day after the localization navigation experiment, and the duration of the experiment was 1 day. Then the safety platform was taken out of the pool, the mouse was put into the water. The frequency with which the mouse crossed the former platform's location within 120 s was documented, serving as a measure of its spatial memory capacity.

2.4.2 Nest-building test

Prior to the experiment, old bedding was removed from the cages. Each cage was then prepared with 20 g of fresh shavings, topped with 16 sheets of thin white tissue paper (4.5 cm × 4.5 cm). Mice were allowed 12 h for nest building, after which their nests were

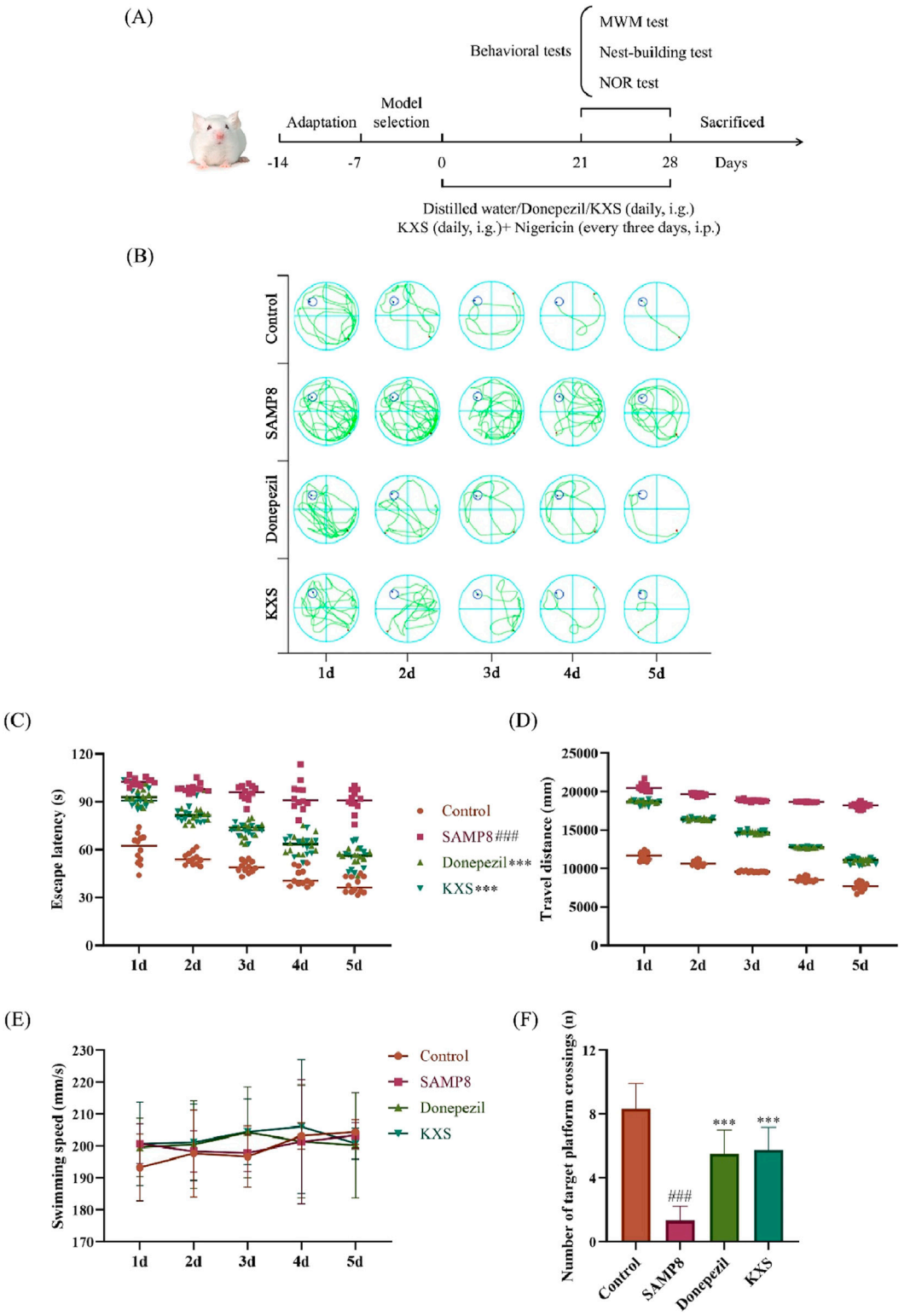


FIGURE 1
KXS improved learning and spatial memory abilities in SAMP8 mice (n = 12). **(A)** Experimental timeline. **(B)** Representative swimming trajectories during the localization navigation test. **(C)** Escape latency in the localization navigation test. **(D)** Travel distance in the localization navigation test. **(E)** Swimming speed in the localization navigation test. **(F)** Platform crossing in the spatial exploration test. Results are expressed as mean ± SD. $^{###}P < 0.001$ vs the control group; $^{***}P < 0.001$ vs. the SAMP8 group.

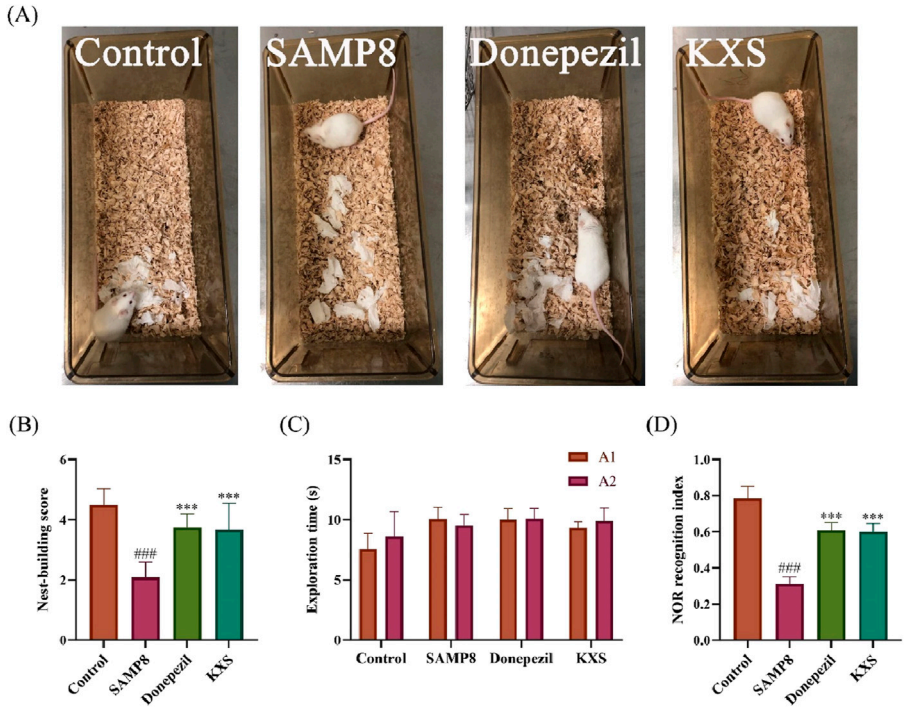


FIGURE 2
KXS ameliorated brain damage and enhanced working memory in SAMP8 mice (n = 12). **(A)** Representative nest-building test pictures. **(B)** The nest-building test scores. **(C)** Object exploration time during the familiarization period of the NOR test. **(D)** Recognition index during the test period of the NOR test. Results are expressed as mean ± SD. $^{###}P < 0.001$ vs. the control group; $^{***}P < 0.001$ vs. the SAMP8 group.

scored. The scoring criteria for the nest-building test are shown in Table 1. This test provides a measure of the mice's cognitive function and motivation, with higher scores indicating better performance.

2.4.3 Novel object recognition (NOR) test

The NOR test took place in a square arena (40 cm × 40 cm × 40 cm) over two consecutive days. A video camera positioned above the box recorded the mice's movements. On the first day, mice were permitted to adjust to the surroundings for 10 min before being returned to their home cages. On the second day, they underwent familiarization and testing phases. During familiarization, two identical items (A1 and A2) were positioned in the arena for a 5-min exploration period, during which the time spent interacting with each item was documented. In the test phase, 1 hour later, A2 was replaced with a new object (B) in the same spot. Mice were again allowed 5 min of exploration, with their interaction times recorded. The recognition index, determined by dividing the exploration time of the novel object B by the total exploration time for objects A1 and B, was utilized to evaluate object recognition memory.

2.5 Tissue collection

Following behavioral tests, mice were anesthetized with 1% sodium pentobarbital administered intraperitoneally, with dosage adjusted to body weight. Blood was collected via retro-orbital bleeding, centrifuged to separate serum, and then stored at −80°C for ELISA analysis. The hippocampi were extracted and sectioned into three parts: one portion was immersed in 4% paraformaldehyde at room temperature for subsequent paraffin embedding, another part was preserved in glutaraldehyde fixative at 4°C for transmission electron microscopy, and the remaining tissue was flash-frozen and stored at −80°C for Western blotting analysis.

2.6 Histological staining

Paraffin-embedded hippocampal tissues were sectioned at 4-μm-thickness. After deparaffinization and hydration, sections underwent three separate staining procedures: FJC staining, Congo red staining, and hematoxylin-eosin (H&E) staining. All staining procedures followed the respective kit instructions. After staining, sections were dehydrated, cleared, and mounted with neutral gum. Stained sections were stained and examined microscopically (BX53, OLYMPUS, Japan) for analysis.

2.7 TUNEL staining

Paraffin-embedded hippocampal sections were routinely deparaffinated and rehydrated. The sections were then treated successively with a proteinase K working solution and membrane stripping solution. The TUNEL method was followed as per the kit's protocol to perform TUNEL staining. Subsequently, nuclei were stained with DAPI. The stained sections were mounted using a

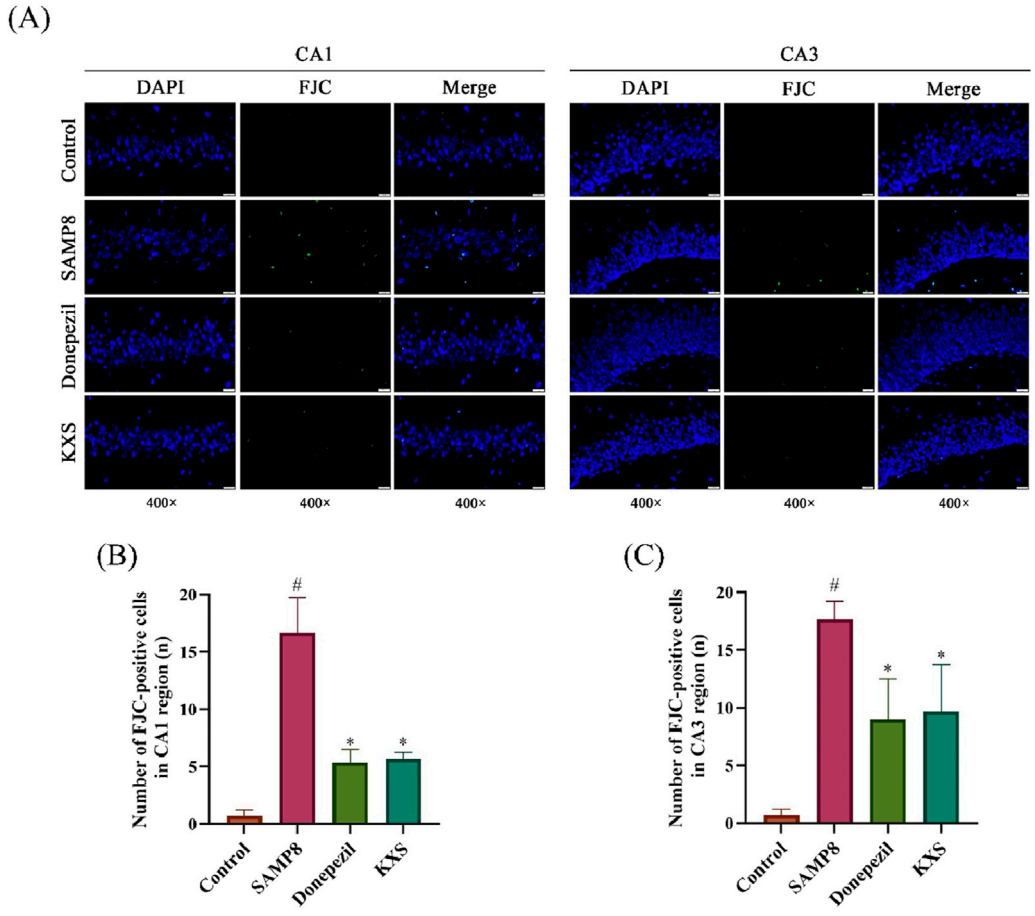


FIGURE 3
KXS reduced degeneration of hippocampal neurons in SAMP8 mice (n = 3). **(A)** Typical micrographs of FJC staining in the hippocampal CA1 and CA3 regions. Magnification, ×400. Scale bar, 20 μm. **(B)** Counting of FJC-positive cells in the CA1 region. **(C)** Counting of FJC-positive cells in the CA3 region. Results are expressed as mean ± SD. $^{\#}P < 0.05$ vs. the control group; $^{*}P < 0.05$ vs. the SAMP8 group.

fluorescence anti-quenching agent and examined under the microscope.

2.8 IHC

Hippocampal sections underwent deparaffinization, rehydration, and antigen retrieval, followed by blocking with a BSA solution and incubation with primary antibodies. Immunoreactivity was visualized using a DAB substrate solution followed by hematoxylin counterstaining. The stained sections were dehydrated and clarified, mounted with neutral gum, and analyzed microscopically.

2.9 Transmission electron microscopy (TEM)

The fixed tissue samples were dehydrated, infiltrated in a mixture of embedding agent and acetone, and embedded using an epoxy resin embedding agent to form a hard resin-embedded block. The resin-embedded blocks were stained after being cut into 7-μm-thick sections and finally observed under a TEM.

2.10 ELISA

Before the start of the test, all reagents were moved to room temperature to equilibrate for 30 min before use. According to the number of samples in the experiment, the required slats were taken from the aluminum foil pouch, and the remaining slats were stored at 4°C after sealing them well. IL-1β, IL-18, IL-6, and TNF-α concentrations in Response to question 1 of reviewer 3 the mouse serum were determined following the ELISA kit protocol.

2.11 Western blotting

The hippocampi were homogenized in lysis buffer, centrifuged at 12,000 rpm for 5 min, and protein concentrations were determined with a BCA kit. Samples were prepared with loading buffer and denatured at 100°C for 15 min. Proteins were resolved by SDS-PAGE, transferred to membranes, and blocked with 5% non-fat milk for 1 h at room temperature. They were then incubated with the primary antibody at 4°C overnight, followed by incubation with the secondary antibody for 2 h at room temperature. Bands were

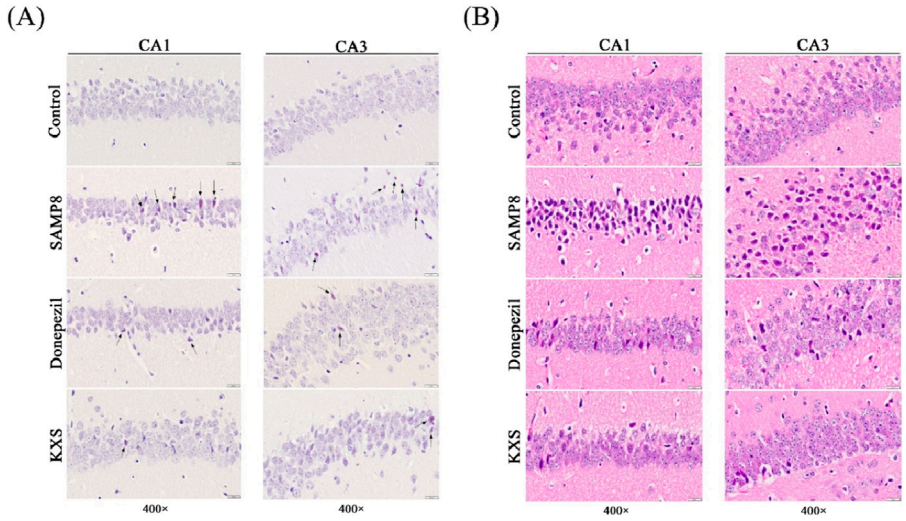


FIGURE 4
KXS reduced hippocampal amyloid deposition and neuropathologic damage in SAMP8 mice (n = 3). **(A)** Typical micrographs of Congo red staining in the hippocampal CA1 and CA3 regions. **(B)** Typical micrographs of H&E staining in the hippocampal CA1 and CA3 regions. Magnification, ×400. Scale bar, 20 μm.

detected with a chemiluminescent substrate and captured using an imaging system (Beijing SAIZ Technology Co., Ltd., Beijing, China).

2.12 Statistical analysis

Data analysis was conducted using SPSS 26.0 and GraphPad Prism 10.1.2. Results are presented as mean ± SD. Statistical evaluations were carried out with one-way ANOVA or suitable non-parametric tests. Significance was set at $P < 0.05$.

3 Results

3.1 KXS enhances cognitive function in SAMP8 mice

An animal model of MCI was established by using SAMP8 mice, which are characterized by a spontaneous rapid aging phenotype and short lifespan. SAMP8 mice of 7-month-old were selected to study the inflammatory response as well as pyroptosis of the hippocampus followed by KXS treatment.

First, we conducted a thorough assessment of KXS's impact on cognitive performance in SAMP8 mice through three behavioral assays: the MWM test, the nest-building test, and the NOR test, as outlined in Figure 1A. The MWM was utilized to evaluate the learning and spatial memory capabilities of the SAMP8 mice. As shown in the localization navigation experiment (Figures 1B–D), The SAMP8 mice exhibited notably extended escape latency and swimming distance compared to the SAMR1 control group, suggesting substantial deficits in spatial learning and memory among SAMP8 mice. Notably, KXS treatment markedly improved these deficits. After treatment with KXS, the escape latency and swimming distance in SAMP8 mice were considerably shortened. With no significant variation in swimming speed among the groups, this suggests that the experimental treatment did not lead to exhaustion or affect the motor function of the mice (Figure 1E). In the spatial exploration experiment, the frequency of platform crossings by SAMP8 mice was markedly lower than that of the control group. KXS treatment notably enhanced the number of platform crossings in SAMP8 mice, indicating improved spatial memory retention (Figure 1F).

Nest-building ability is an indicator of brain damage in mice (Deacon, 2012). As shown in the nest-building test (Figure 2A), SAMP8 mice exhibited significantly lower nest-building scores compared to SAMR1 controls. Remarkably, KXS treatment restored nest-building performance in SAMP8 mice to >90% levels of the control group, which is similar to that of the donepezil treatment (Figure 2B).

The NOR test revealed deficits in object discrimination memory in SAMP8 mice. During the familiarization phase, all groups showed similar exploration times for the identical objects A1 and A2, indicating no bias (Figure 2C). In the testing phase, mice in the SAMP8 group exhibited a markedly reduced recognition index for novel objects when compared to the control group. The recognition index of SAMP8 mice to novel objects after treatment with KXS and donepezil was significantly improved (Figure 2D), suggesting that KXS enhanced the discriminative memory of SAMP8 mice to novel objects.

3.2 KXS improves hippocampal neuronal degeneration in SAMP8 mice

To determine how KXS provides cognitive benefits through neuroprotection, we assessed several hippocampal neuropathological markers in SAMP8 mice. To assess the extent of neuronal damage, we employed FJC staining, a highly sensitive and specific method for detecting degenerating neurons (Ikenari et al., 2020). FJC staining revealed a significant increase of

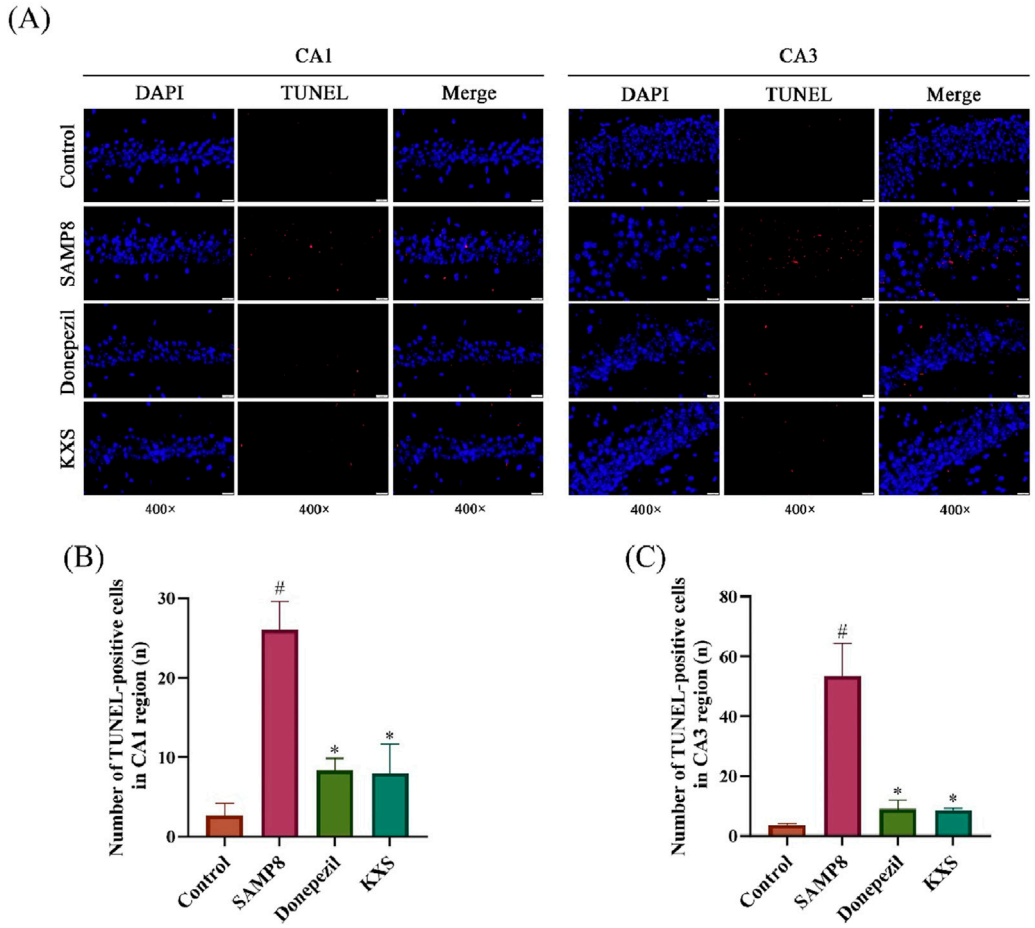


FIGURE 5
KXS decreased hippocampal neuronal apoptosis in SAMP8 mice (n = 3). **(A)** Typical micrographs of TUNEL staining in the hippocampal CA1 and CA3 regions. Magnification, ×400. Scale bar, 20 μm. **(B)** Counting of TUNEL-positive cells in the CA1 region. **(C)** Counting of TUNEL-positive cells in the CA3 region. Results are expressed as mean ± SD. $^{\#}P < 0.05$ vs. the control group; $^{*}P < 0.05$ vs. the SAMP8 group.

degenerated nerve cells (green fluorescence) in the hippocampal CA1 and CA3 areas of SAMP8 mice. Treatment with KXS significantly decreased the count of FJC-positive cells in these regions, and donepezil groups showed similar reduction levels (Figures 3A–C).

3.3 KXS reduces hippocampal amyloid deposition in SAMP8 mice

To visualize amyloid deposits, we used Congo red staining, which specifically binds to amyloid fibrils (Sarkar et al., 2020). Congo red staining showed extensive amyloid deposits with scattered distribution (reddish or date-red color) in the hippocampal CA1 and CA3 regions of SAMP8 mice, and nothing was presented in the control group (Figure 4A). After treatment with KXS and donepezil, an evident reduction of amyloid deposits was observed. Compared to the donepezil group, KXS seems to have a stronger effect (Figure 4A).

3.4 KXS attenuates hippocampal neuropathologic damage in SAMP8 mice

To evaluate overall tissue architecture and cellular morphology, we performed H&E staining, a classic histological technique. As shown in Figure 4B H&E staining showed that the cytoarchitecture of the CA1 and CA3 regions of the hippocampus of SAMP8 mice was blurred compared with that of the control group. Neuronal count was diminished, with cells haphazardly arranged and dispersed, alongside cytoplasmic expansion or distortion; various types of neuroglia were irregular in morphology and appeared to be markedly proliferated. KXS or donepezil treatment significantly ameliorated these changes, restoring neuronal density, organization, and morphology to near-normal levels. The number of neuronal cells increased significantly, evenly distributed, and the pathological changes such as pyknosis and displacement of the nucleus were greatly improved; the morphology of neuroglia tended to be normalized.

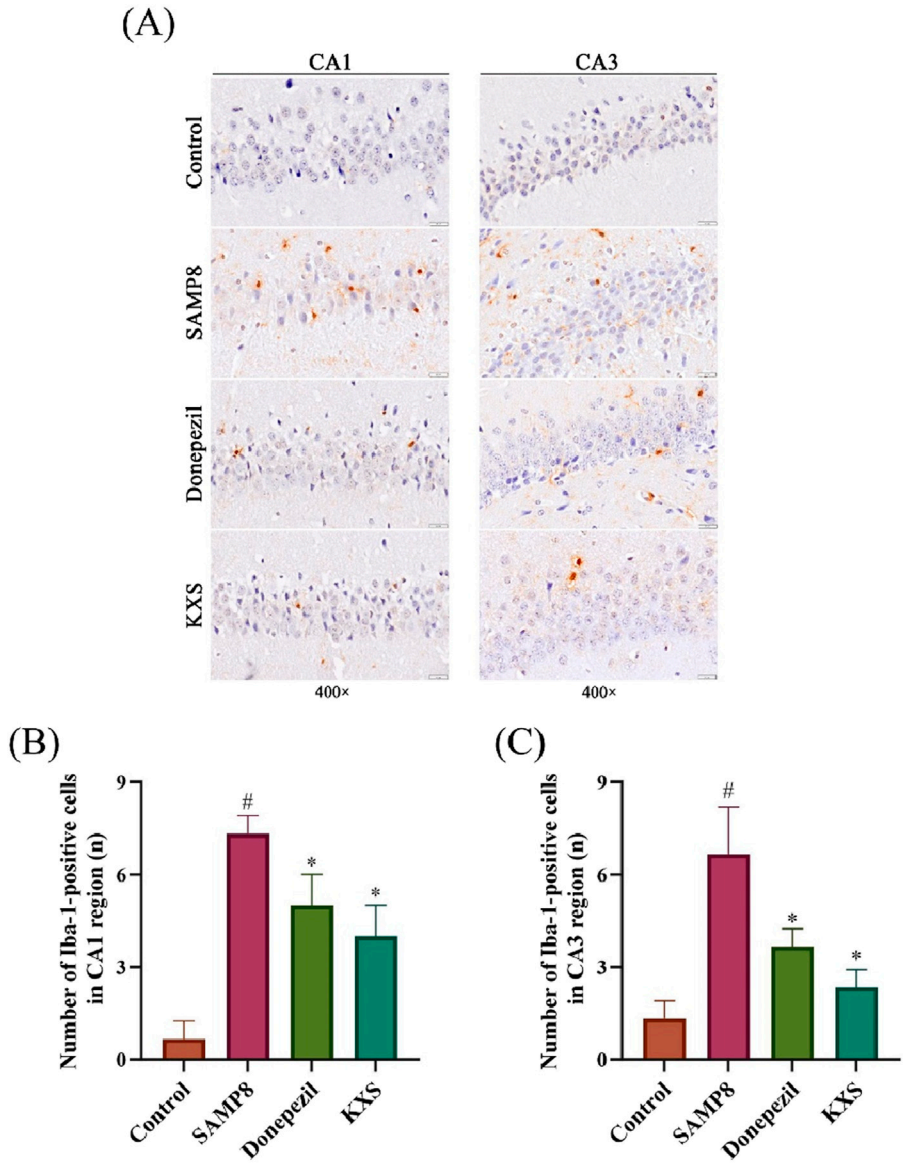


FIGURE 6
KXS reduced the activationof hippocampal microglia in SAMP8 mice (n = 3). **(A)** Typical micrographs of Iba-1 immunohistochemical staining in the hippocampal CA1 and CA3 regions. Magnification, ×400. Scale bar, 20 μm. **(B)** Counting of Iba-1-positive cells in the CA1 region. **(C)** Counting of Iba-1-positive cells in the CA3 region. Results are expressed as mean ± SD. $^{\#}P < 0.05$ vs. the control group; $^{*}P < 0.05$ vs. the SAMP8 group.

3.5 KXS reduces cell death in the hippocampus of SAMP8 mice

To investigate the extent of cell death, including both apoptosis and pyroptosis, we utilized TUNEL staining, which detects DNA fragmentation characteristic of dying cells. As shown in Figure 5A, the TUNEL staining indicated a substantial rise in the number of positive cells, as evidenced by red fluorescence, in the hippocampal CA1 and CA3 regions of SAMP8 mice when compared to the SAMR1 control group. Treatment with KXS significantly decreased TUNEL-positive cell counts in these hippocampal areas, with reductions comparable to those observed in the donepezil group (Figures 5A–C). These results suggest that KXS exerts neuroprotective effects by decreasing the pyroptosis of hippocampal cells and preventing hippocampal cell death.

3.6 KXS reduces the activation of hippocampal microglia in SAMP8 mice

Microglial activation, a hallmark of neuroinflammation, significantly contributes to cognitive deterioration associated with aging. To assess microglial activation, we performed immunohistochemical staining for Iba-1, a microglial calcium-binding protein unique to the central nervous system (CNS) microglia whose expression increases when microglia are

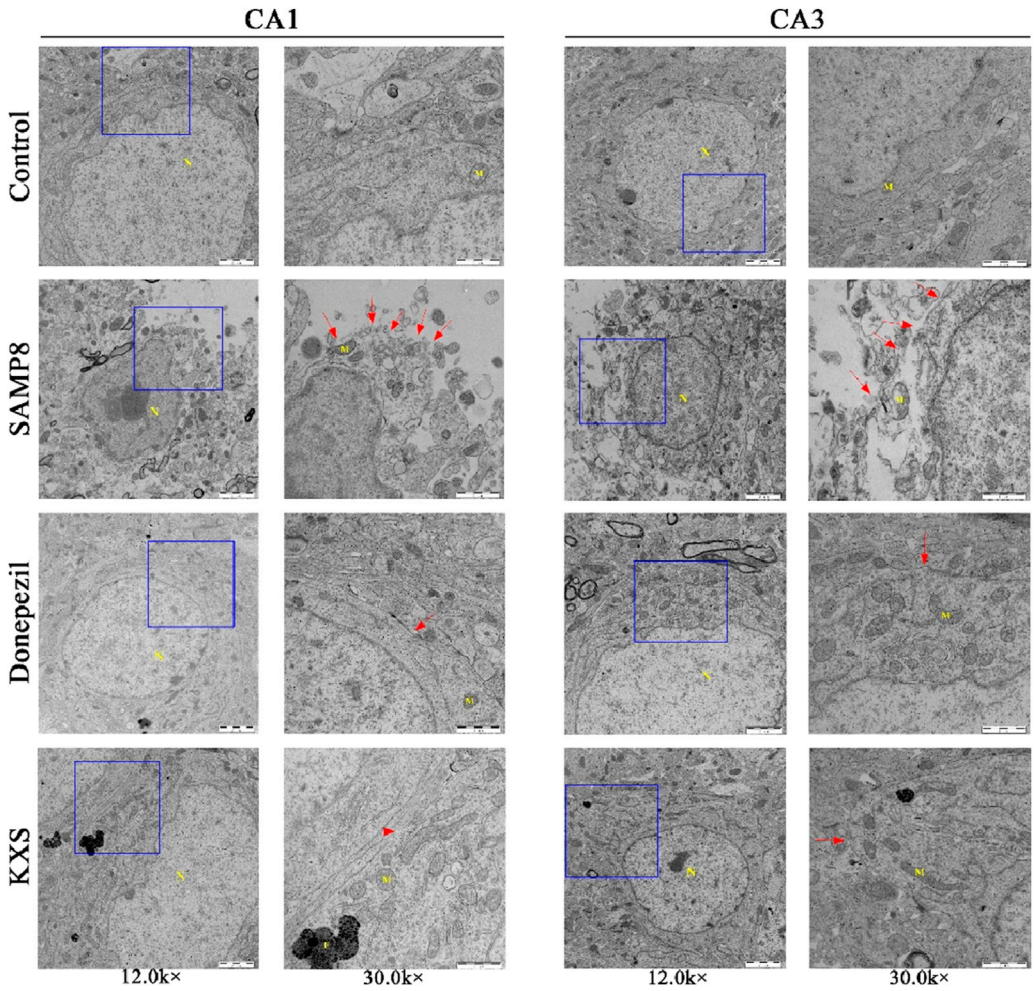


FIGURE 7
KXS attenuated the pyroptosis of hippocampal cells in SAMP8 mice (n = 3). Typical micrographs of TEM images of cells in the hippocampal CA1 and CA3 regions. N: nucleus, M: mitochondria, F: lysosomes. Magnification, 12.0 k ×, 30.0 k ×. Scale bars, 2 μm, 1 μm.

activated (Xiao et al., 2022). As depicted in Figure 6A, Iba-1-positive cell counts, characterized by rust or brownish-yellow coloration, were notably elevated in the hippocampal CA1 and CA3 regions of SAMP8 mice relative to the control group. Treatment with KXS or donepezil significantly lowered the quantity of Iba-1-positive cells in the hippocampal CA1 and CA3 areas, signifying an anti-neuroinflammatory effect (Figures 6A–C).

3.7 KXS reduces pyroptosis in SAMP8 mice hippocampal cells

To examine cellular changes at the subcellular level, we employed TEM, which provides high-resolution imaging of cellular ultrastructure. Examination via TEM revealed pronounced ultrastructural changes in the hippocampal CA1 and CA3 regions of SAMP8 mice when compared to the control SAMR1 group, including edema expansion, multiple fracture breaks in the cell membrane, and most of the organelles in the cytoplasm were broken or disappeared. These are the specific features of pyroptosis, which is manifested as swelling and expansion of cells accompanied by the formation of plasma membrane pores, leading to cell rupture and the release of pro-inflammatory cytokines and cellular contents, exacerbating the inflammatory response. KXS and donepezil treatment substantially improved cellular ultrastructure, with only occasional cell membrane abnormalities observed (Figure 7). The results suggest that the pyroptosis of hippocampal cells in SAMP8 mice was significantly improved after KXS treatment.

3.8 KXS modulates inflammatory and pyroptotic pathways in SAMP8 mice

To further investigate the molecular mechanisms fundamental to the neuroprotective effects of KXS, we examined inflammatory markers and key components of the NLRP3/Caspase-1 pyroptosis pathway.

SAMP8 mice exhibited significantly elevated serum levels of pro-inflammatory cytokines (IL-1β, IL-18, IL-6, and TNF-α). After treatment with KXS and donepezil, serum levels of all four inflammatory factors in SAMP8 mice were markedly reduced

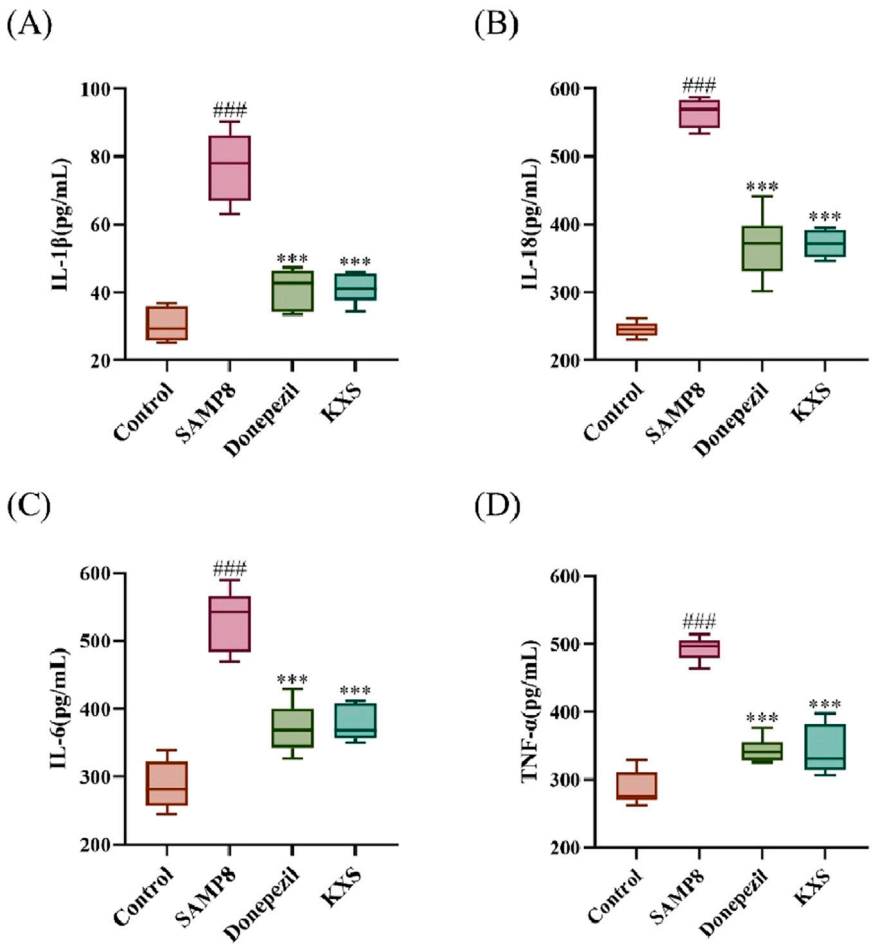


FIGURE 8
KXS reduced serum levels of inflammatory factors in SAMP8 mice (n = 10). **(A)** Concentration of IL-1β in serum. **(B)** Concentration of IL-18 in serum. **(C)** Concentration of IL-6 in serum. **(D)** Concentration of TNF-α in serum. Results are expressed as mean ± SD. $^{###}P < 0.001$ vs. the control group; $^{***}P < 0.001$ vs. the SAMP8 group.

(Figures 8A–D), demonstrating that KXS had a potent inhibitory effect on the inflammatory response of SAMP8 mice.

Western blot analysis revealed significant upregulation of NLRP3 inflammasome components and downstream effectors in the classical pyroptosis NLRP3/Caspase-1 signaling pathway in the hippocampal regions of SAMP8 mice (Figures 9A–K). These changes included increased expression of Aβ, NLRP3, ASC, and pro-Caspase-1; enhanced activation of Caspase-1 (increased cleaved Caspase-1); elevated levels of pyroptosis executioner protein GSDMD and its active form GSDMD-NT; and increased production and processing of pro-inflammatory cytokines (IL-1β and IL-18). Remarkably, the expression levels of the relevant proteins were significantly reduced after treatment with KXS and donepezil, indicating a comprehensive inhibition of the NLRP3/Caspase-1 pyroptosis pathway (Figures 9A–K).

To further elucidate the role of the NLRP3 inflammasome in KXS-mediated neuroprotection, we employed the NLRP3 activator Nigericin. Notably, Nigericin treatment abolished the suppressive effects of KXS on Aβ accumulation and NLRP3/Caspase-1 pathway activation (Figures 10A–K). These findings strongly suggest that KXS's neuroprotective action is exerted, at least partially, through modulation of the NLRP3/Caspase-1 signaling pathway.

4 Discussion

In our research, we investigated the impact of Kai-Xin-San (KXS) on cognitive function, neuroinflammation, and pyroptosis using the SAMP8 mouse model, which is relevant to Mild Cognitive Impairment (MCI). Our findings demonstrate that KXS significantly improves cognitive performance, reduces Aβ deposition, attenuates neuroinflammation, and inhibits pyroptosis through modulation of the NLRP3/Caspase-1 signaling pathway. These results offer novel perspectives on the mechanisms underlying the therapeutic efficacy of KXS in MCI.

Donepezil is a second-generation cholinesterase inhibitor and is the only AD treatment-approved drug for marketing by both the FDA and the UK Medicines Agency. It is mostly used for the symptomatic treatment of MCI and mild to moderate AD. In recent years, a large number of studies have focused on the anti-inflammatory effects of donepezil (Yoshiyama et al., 2010;

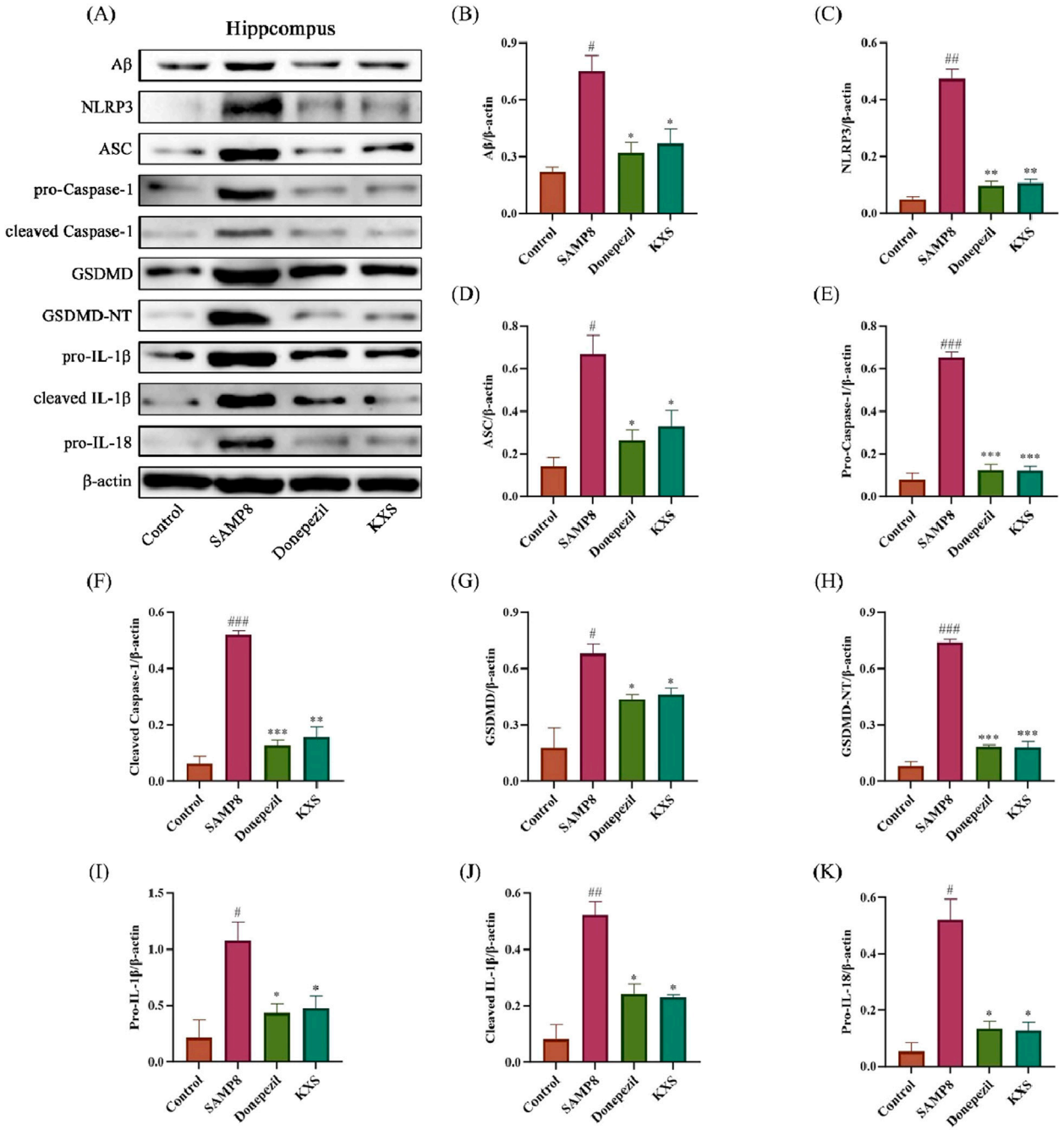


FIGURE 9
KXS modulated hippocampal Aβ protein and NLRP3/Caspase-1 signaling pathway-related protein expression in SAMP8 mice (n = 8). **(A)** Typical Western blot images showing protein expression of Aβ, NLRP3, ASC, pro-Caspase-1, cleaved Caspase-1, GSDMD, GSDMD-NT, pro-IL-1β, cleaved IL-1β, and pro-IL-18 from the hippocampus. **(B–K)** Quantification of relative protein levels. Results are expressed as mean ± SD. $^{###}P < 0.001$, $^{##}P < 0.01$, $^{#}P < 0.05$ vs. the control group; $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$ vs. the SAMP8 group.

Haraguchi et al., 2017; Goschorska et al., 2018; Maroli et al., 2019). Therefore, donepezil may not only exert its cholinesterase inhibitory effect but also inhibit the neurodegenerative pathological changes of MCI through its anti-inflammatory effect. Therefore, in the present study, donepezil hydrochloride tablets were chosen as the experimental positive control drug to conduct an in-depth study of KXS on the role and mechanism of the inhibition of inflammatory response and hippocampal neuronal pyroptosis.

The behavioral tests revealed that KXS treatment notably enhanced spatial memory, working memory, and executive function in SAMP8 mice. These results align with earlier findings that establish the cognition-enhancing properties of KXS across diverse models of cognitive deficits (Luo et al., 2020; Wang et al., 2020). The multi-domain cognitive improvements observed in our study suggest that KXS may have broad neuroprotective effects, potentially targeting multiple aspects of MCI pathology.

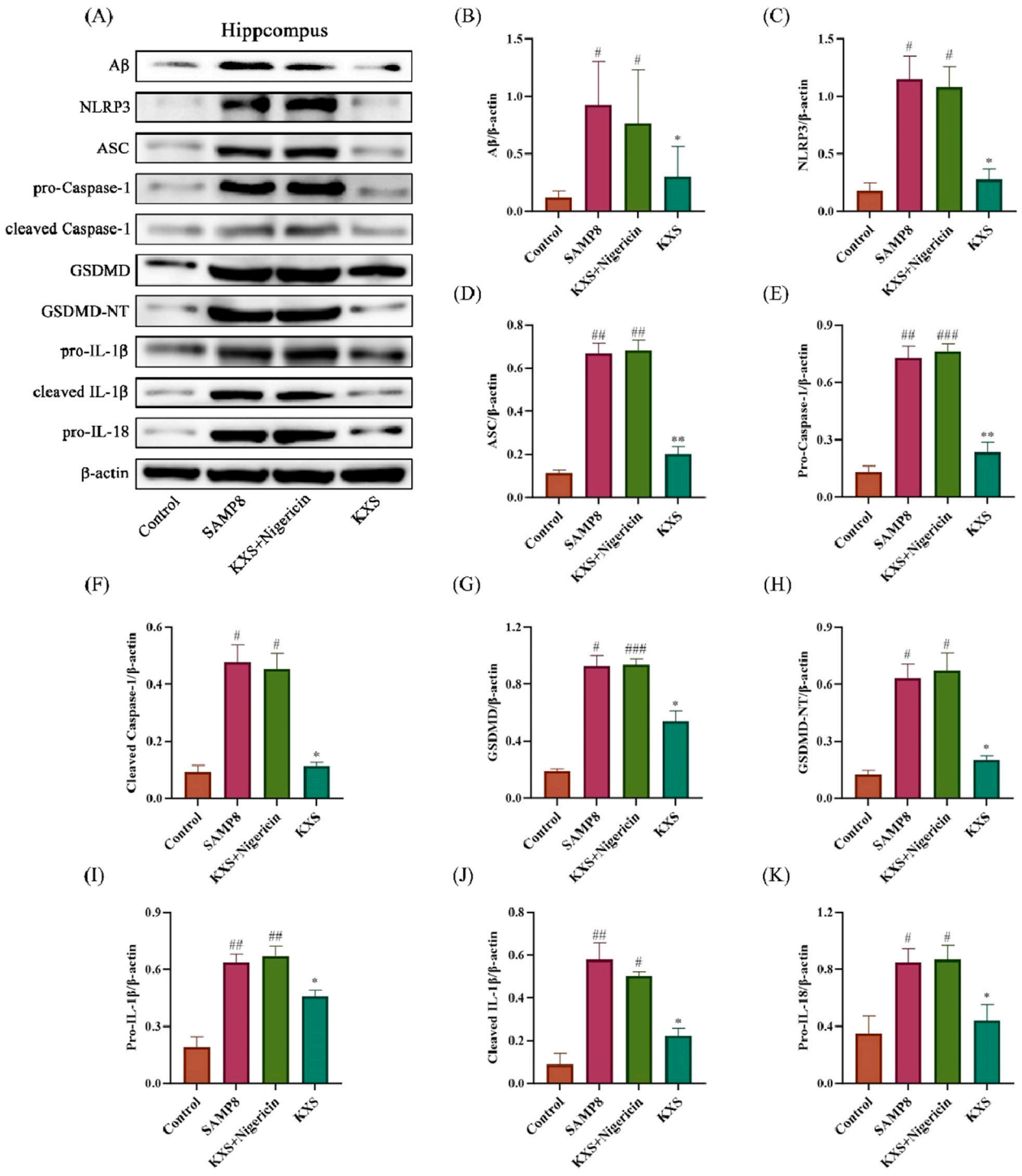


FIGURE 10
Effects of KXS on hippocampal Aβ protein and NLRP3/Caspase-1 signaling pathway-related protein expression in SAMP8 mice after Nigericin treatment (n = 8). **(A)** Typical Western blot images showing protein expression of Aβ, NLRP3, ASC, pro-Caspase-1, cleaved Caspase-1, GSDMD, GSDMD-NT, pro-IL-1β, cleaved IL-1β, and pro-IL-18 from the hippocampus. **(B–K)** Quantification of relative protein levels. Results are expressed as mean ± SD. $^{###}P < 0.001$, $^{##}P < 0.01$, $^{#}P < 0.05$ vs. the control group; $^{**}P < 0.01$, $^{*}P < 0.05$ vs. the SAMP8 group.

First, KXS reduces Aβ deposition and modulates neuroinflammation. A key finding of our study is the significance of hippocampal Aβ deposition following KXS treatment. This aligns with the growing body of evidence implicating Aβ accumulation as a central pathogenic mechanism in MCI and AD (Hardy and Selkoe, 2002). The ability of KXS to reduce Aβ deposition may be attributed to its effects on Aβ production and clearance pathways, as suggested by previous studies (Sun et al., 2021).

Neuroinflammation is the inflammatory response in the CNS that is usually caused by various pathological injuries including

infection, trauma, ischemia, and toxins. It is one of the important pathogenic mechanisms of MCI. In particular, chronic inflammation involving activated microglia increases the levels of pro-inflammatory factors that can penetrate the blood-brain barrier and aggravate brain inflammation. This would potentially amplify the amyloid cascade reaction, leading to Aβ deposition and neurotoxicity (Lee et al., 2021). In turn, excess deposited Aβ may accelerate the activation of microglia and exacerbate the inflammatory response. This vicious cycle of amyloid deposition and neuroinflammation leads to neuronal degeneration, which amplifies neuronal dysfunction and accelerates the pathological transformation of MCI to AD (Nordengen et al., 2019).

Here, we observed that KXS administration substantially lessened the deposition of hippocampal amyloid and decreased serum concentrations of pro-inflammatory cytokines (IL-1β, IL-18, IL-6, and TNF-α), and decreased microglial activation in the hippocampus. These findings suggest that KXS exerts potent anti-inflammatory effects, potentially breaking the vicious cycle between Aβ accumulation and chronic neuroinflammation that characterizes MCI progression. The ability of KXS to modulate both Aβ deposition and neuroinflammation simultaneously highlights its multi-target approach, which may be particularly advantageous in addressing the complex pathology of MCI.

Microglia, which are derived from yolk sac fetal macrophages, are major players in the neuroinflammatory response (Norris and Kipnis, 2019). By employing different regulatory networks, microglia exert important roles in the pyroptosis of nerve cells and immune monitoring (Ahmad et al., 2022; Morató et al., 2022). Activated microglia internalize pathogenic substances and degrade them through various intracellular pathways, which usually subside after the elimination of the immune stimulus (Subhramanyam et al., 2019). Microglial cells located in the MCI brain, however, are characterized by functional vulnerability and sustained activation, which may contribute to the initiation of MCI pathogenesis (Cai et al., 2022). In this research, we demonstrated that KXS can reduce the inflammatory activation of microglia, thereby attenuating the inflammatory response.

It has been shown that reactive microglia are tightly co-localized with amyloid plaques in the brains of MCI patients (Tan et al., 2020). Studies also found that pro-inflammatory cytokines produced by microglia can upregulate the level of β-secretase, suggesting microglia not only indirectly promote Aβ production, but also amyloid plaque formation (Leng and Edison, 2021). In addition, overactivation of NLRP3 inflammasome in microglia can exacerbate Tau protein hyperphosphorylation and neuro progenitor fiber tangles (Heneka, 2017; Li D.-D. et al., 2022). Our findings align with the above findings and show that KXS ameliorates the abnormal pathological changes in hippocampal cytopathic alterations and cell morphology in SAMP8 mice.

A novel finding of our study is the ability of KXS to inhibit pyroptosis in hippocampal neurons of SAMP8 mice. Pyroptosis is a distinct type of programmed cell death characterized by the emission of pro-inflammatory cytokines and subsequent inflammatory reactions (Kesavardhana et al., 2020). Activated microglia can trigger excessive NLRP3 inflammasome activation, which is underlying the activation of pyroptosis (Sbai et al., 2022). The NLRP3 inflammasome complex consists of three primary elements: the NLRP3 sensor, apoptosis-associated speck-like protein containing CARD (ASC), and effector protein Caspase-1. Once activated, Caspase-1 processes GSDMD, resulting in GSDMD-N-terminal (GSDMD-NT), which facilitates membrane pore formation for the secretion of mature IL-1β and IL-18, thereby triggering inflammation (Cai et al., 2021; Li M. et al., 2021). An increasing number of researchers have confirmed that the NLRP3 inflammasome significantly contributes to MCI pathogenesis. It has been shown that either recombinant $A\beta_{1-42}$ or Tau proteins can activate NLRP3 inflammasome, trigger pyroptosis, and induce IL-1β release (Stancu et al., 2019; Han et al., 2020). The involvement of pyroptosis in MCI and AD pathogenesis is an emerging area of research.

Our findings suggest that KXS was able to significantly reduce the development of pyroptosis by significantly ameliorating cell expansion and distension. KXS may exert its neuroprotective effects at least in part by inhibiting the NLRP3/Caspase-1-mediated pyroptosis pathway. KXS treatment significantly inhibited the assembly and activation of inflammatory, reduced the levels of NLRP3 inflammasome key components (NLRP3, ASC, pro-Caspase-1) and its downstream effectors (cleaved Caspase-1, GSDMD, GSDMD-NT), and decreased the maturation of Caspase-1 and the secretion of IL-1β, IL-18. This inhibition of the pyroptotic pathway corresponded to a decrease in the generation of pro-inflammatory cytokines (IL-1β, IL-18) and improved cellular ultrastructure. Interestingly, the impacts of KXS on the NLRP3/Caspase-1 pathway were abolished by treatment with Nigericin, a potent NLRP3 activator. This observation further supports the specificity of KXS action on this pathway and suggests that NLRP3 inhibition may be a critical mechanism underlying its therapeutic effects. We further clarified that the anti-inflammatory effect of KXS was mainly achieved by inhibiting the NLRP3/Caspase-1 signaling pathway.

5 Conclusion

In conclusion, our research offers novel perspectives on the mechanisms underlying the therapeutic effects of KXS in MCI. We propose a comprehensive mechanism for KXS action in MCI that integrates its effects on the reduction of Aβ deposition, attenuation of neuroinflammatory injury, and modulation of NLRP3/Caspase-1 signaling pathway to attenuate hippocampal pyroptosis. These findings not only expand our understanding of KXS action but also underscore the potential of traditional Chinese medicine approaches in addressing complex neurodegenerative disorders. While our research delivers meaningful findings of the mechanisms through KXS action in MCI, it is worth to mention that the specific bioactive components of KXS responsible for its observed effects remain to be identified. Future studies should focus on isolating and characterizing these components to optimize therapeutic strategies.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was approved by the Animal Experimental Ethics Committee of Heilongjiang University of Chinese Medicine (The ethics certification number is 2022012801). The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

SL: Data curation, Software, Writing–original draft, Writing–review and editing. XS: Conceptualization, Investigation, Writing–original draft. YS: Formal Analysis, Validation, Writing–original draft. AS: Methodology, Resources, Writing–original draft. YaL: Resources, Visualization, Writing–original draft. YuL: Investigation, Visualization, Writing–original draft. JC: Funding acquisition, Project administration, Supervision, Writing–review and editing.

Funding

The author(s) declare that financial support was received for the research and/or publication of this article. This study was supported by a grant from the National Natural Science Foundation of China (Project No. 82274395).

Acknowledgments

We are thankful to the Heilongjiang University of Chinese Medicine for their contribution to this work. We are grateful to the Natural Science Foundation of China for funding this work.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declare that no Generative AI was used in the creation of this manuscript.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

Ahmad, M. A., Kareem, O., Khushtar, M., Akbar, M., Haque, M. R., Iqubal, A., et al. (2022). Neuroinflammation: a potential risk for dementia. *Int. J. Mol. Sci.* 23, 616. doi:10.3390/ijms23020616

Avila, J., and Perry, G. (2021). A multilevel view of the development of alzheimer's disease. *Neuroscience* 457, 283–293. doi:10.1016/j.neuroscience.2020.11.015

Cai, M., Zhang, Y., Chen, S., Wu, Z., and Zhu, L. (2022). The past, present, and future of research on neuroinflammation-induced mild cognitive impairment: a bibliometric analysis. *Front. Aging Neurosci.* 14, 968444. doi:10.3389/fnagi.2022.968444

Cai, Y., Chai, Y., Liao, Y., and Yan, T. (2021). Research progress of pyroptosis-mediated pathogenesis of Alzheimer′s disease. *Chin. Pharm. J.* 56, 1701–1705. (in Chinese). doi:10.11669/cpj.2021.21.001

Chandra, A., Valkimadi, P.-E., Pagano, G., Cousins, O., Dervenoulas, G., Politis, M., et al. (2019). Applications of amyloid, tau, and neuroinflammation PET imaging to Alzheimer's disease and mild cognitive impairment. *Hum. Brain Mapp.* 40, 5424–5442. doi:10.1002/hbm.24782

Deacon, R. (2012). Assessing burrowing, nest construction, and hoarding in mice. *J. Vis. Exp.* e2607, e2607. doi:10.3791/2607

Deacon, R. M. J. (2006). Assessing nest building in mice. *Nat. Protoc.* 1, 1117–1119. doi:10.1038/nprot.2006.170

Gao, J., and Lv, S. (2021). Research progress in chemical constituents and pharmacological action of renshen(ginseng). *Guid. J. Traditional Chin. Med. Pharm.* 27, 127–130+137. doi:10.13862/j.cnki.cn43-1446/r.2021.01.030

Goschorska, M., Baranowska-Bosiacka, I., Gutowska, I., Tarnowski, M., Piotrowska, K., Metryka, E., et al. (2018). Effect of acetylcholinesterase inhibitors donepezil and rivastigmine on the activity and expression of cyclooxygenases in a model of the inflammatory action of fluoride on macrophages obtained from THP-1 monocytes. *Toxicology* 406–407, 9–20. doi:10.1016/j.tox.2018.05.007

Han, C., Yang, Y., Guan, Q., Zhang, X., Shen, H., Sheng, Y., et al. (2020). New mechanism of nerve injury in Alzheimer's disease: β-amyloid-induced neuronal pyroptosis. *J. Cell Mol. Med.* 24, 8078–8090. doi:10.1111/jcmm.15439

Haraguchi, Y., Mizoguchi, Y., Ohgidani, M., Imamura, Y., Murakawa-Hirachi, T., Nabeta, H., et al. (2017). Donepezil suppresses intracellular Ca2+ mobilization through the PI3K pathway in rodent microglia. *J. Neuroinflammation* 14, 258. doi:10.1186/s12974-017-1033-0

Hardy, J., and Selkoe, D. J. (2002). The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science* 297, 353–356. doi:10.1126/science.1072994

Heneka, M. T. (2017). Inflammasome activation and innate immunity in Alzheimer's disease. *Brain Pathol.* 27, 220–222. doi:10.1111/bpa.12483

Hou, Z., Qiu, R., Wei, Q., Liu, Y., Wang, M., Mei, T., et al. (2020). Electroacupuncture improves cognitive function in senescence-accelerated P8 (SAMP8) mice via the NLRP3/caspase-1 pathway. *Neural Plast.* 2020, 8853720. doi:10.1155/2020/8853720

Ikenari, T., Kurata, H., Satoh, T., Hata, Y., and Mori, T. (2020). Evaluation of fluoro-jade C staining: specificity and application to damaged immature neuronal cells in the normal and injured mouse brain. *Neuroscience* 425, 146–156. doi:10.1016/j.neuroscience.2019.11.029

Jiao, Y.-N., Zhang, J.-S., Qiao, W.-J., Tian, S.-Y., Wang, Y.-B., Wang, C.-Y., et al. (2022). Kai-xin-san inhibits tau pathology and neuronal apoptosis in aged SAMP8 mice. *Mol. Neurobiol.* 59, 3294–3309. doi:10.1007/s12035-021-02626-0

Jongsiriyanyong, S., and Limpawattana, P. (2018). Mild cognitive impairment in clinical Practice: a review article. *Am. J. Alzheimers Dis. Other Demen* 33, 500–507. doi:10.1177/1533317518791401

Kesavardhana, S., Malireddi, R. K. S., and Kanneganti, T.-D. (2020). Caspases in cell death, inflammation, and pyroptosis. *Annu. Rev. Immunol.* 38, 567–595. doi:10.1146/annurev-immunol-073119-095439

Lee, S., Cho, H.-J., and Ryu, J.-H. (2021). Innate immunity and cell death in alzheimer's disease. *ASN Neuro* 13, 17590914211051908. doi:10.1177/17590914211051908

Leng, F., and Edison, P. (2021). Neuroinflammation and microglial activation in Alzheimer disease: where do we go from here? *Nat. Rev. Neurol.* 17, 157–172. doi:10.1038/s41582-020-00435-y

Li, D.-D., Fan, H.-X., Yang, R., Li, Y.-Y., Zhang, F., and Shi, J.-S. (2022a). Dendrobium nobile lindl. Alkaloid suppresses NLRP3-mediated pyroptosis to alleviate LPS-induced neurotoxicity. *Front. Pharmacol.* 13, 846541. doi:10.3389/fphar.2022.846541

Li, H., Dong, P., Li, H., Xu, J., Wang, H., Cui, Y., et al. (2022b). UHPLC-Q-Exactive Orbitrap MS/MS-based rapid identification of chemical components in substance benchmark of Kaixin San. *China J. Chin. Materia Medica* 47, 938–950. doi:10.19540/j.cnki.cjcmm.20210823.304

Li, M., Zhang, G., Shang, T., Wang, Y., Wang, Z., and Sun, L. (2021a). Research progress of caspase-1/GSDMD pathway-dependent pyroptosis induced by β-amyloid and Alzheimer's disease. *Chin. J. Immunol.* 37, 3070–3074. (in Chinese). doi:10.3969/j.issn.1000-484X.2021.24.026

Li, Z., Song, Z., Li, F., He, C., Yang, M., Yu, W., et al. (2021b). High-fat diet impairs neural function of APPswe/PS1△E9 mice by enhancing inflammatory response and pyroptosis. *J. Sun Yat-sen Univ. Med. Sci.* 42, 686–693. (in Chinese). doi:10.13471/j.cnki.j.sun.yat-sen.univ(med.sci).2021.0506

Lin, R., Yin, J., Wu, M., Ding, H., Han, L., Yang, W., et al. (2021). Global identification and determination of the major constituents in Kai-Xin-San by ultra-performance liquid chromatography-quadrupole-Orbitrap mass spectrometry and gas chromatography-mass spectrometry. *J. Pharm. Biomed. Anal.* 206, 114385. doi:10.1016/j.jpba.2021.114385

Luo, Y., Li, D., Liao, Y., Cai, C., Wu, Q., Ke, H., et al. (2020). Systems pharmacology approach to investigate the mechanism of kai-xin-san in alzheimer's disease. *Front. Pharmacol.* 11, 381. doi:10.3389/fphar.2020.00381

Maroli, A., Di Lascio, S., Drufuca, L., Cardani, S., Setten, E., Locati, M., et al. (2019). Effect of donepezil on the expression and responsiveness to LPS of CHRNA7 and CHRFAM7A in macrophages: a possible link to the cholinergic anti-inflammatory pathway. *J. Neuroimmunol.* 332, 155–166. doi:10.1016/j.jneuroim.2019.04.012

Morató, X., Pytel, V., Jofresa, S., Ruiz, A., and Boada, M. (2022). Symptomatic and disease-modifying therapy pipeline for alzheimer's disease: towards a personalized polypharmacology patient-centered approach. *Int. J. Mol. Sci.* 23, 9305. doi:10.3390/ijms23169305

Nordengen, K., Kirsebom, B.-E., Henjum, K., Selnes, P., Gísladóttir, B., Wettergreen, M., et al. (2019). Glial activation and inflammation along the Alzheimer's disease continuum. *J. Neuroinflammation* 16, 46. doi:10.1186/s12974-019-1399-2

Norris, G. T., and Kipnis, J. (2019). Immune cells and CNS physiology: microglia and beyond. *J. Exp. Med.* 216, 60–70. doi:10.1084/jem.20180199

Sarkar, S., Raymick, J., Cuevas, E., Rosas-Hernandez, H., and Hanig, J. (2020). Modification of methods to use Congo-red stain to simultaneously visualize amyloid plaques and tangles in human and rodent brain tissue sections. *Metab. Brain Dis.* 35, 1371–1383. doi:10.1007/s11011-020-00608-0

Sbai, O., Djelloul, M., Auletta, A., Ieraci, A., Vascotto, C., and Perrone, L. (2022). AGE-TXNIP axis drives inflammation in Alzheimer's by targeting Aβ to mitochondria in microglia. *Cell Death Dis.* 13, 302. doi:10.1038/s41419-022-04758-0

Selkoe, D. J. (2002). Alzheimer's disease is a synaptic failure. *Science* 298, 789–791. doi:10.1126/science.1074069

Stancu, I.-C., Cremers, N., Vanrusselt, H., Couturier, J., Vanoosthuyse, A., Kessels, S., et al. (2019). Aggregated Tau activates NLRP3-ASC inflammasome exacerbating exogenously seeded and non-exogenously seeded Tau pathology *in vivo*. *Acta Neuropathol.* 137, 599–617. doi:10.1007/s00401-018-01957-y

Su, S., Chen, G., Gao, M., Zhong, G., Zhang, Z., Wei, D., et al. (2023). Kai-Xin-San protects against mitochondrial dysfunction in Alzheimer's disease through SIRT3/NLRP3 pathway. *Chin. Med.* 18, 26. doi:10.1186/s13020-023-00722-y

Subhramanyam, C. S., Wang, C., Hu, Q., and Dheen, S. T. (2019). Microglia-mediated neuroinflammation in neurodegenerative diseases. *Semin. Cell Dev. Biol.* 94, 112–120. doi:10.1016/j.semcdb.2019.05.004

Sun, Y., Sun, T., Li, M., and Wang, X. (2021). Research on modern pharmacological effects and mechanism of action of Kai-Xin-San. *J. Basic Chin. Med.* 27, 650–654. (in Chinese). doi:10.19945/j.cnki.issn.1006-3250.2021.04.030

Tan, Y.-L., Yuan, Y., and Tian, L. (2020). Microglial regional heterogeneity and its role in the brain. *Mol. Psychiatry* 25, 351–367. doi:10.1038/s41380-019-0609-8

Tangalos, E. G., and Petersen, R. C. (2018). Mild cognitive impairment in geriatrics. *Clin. Geriatr. Med.* 34, 563–589. doi:10.1016/j.cger.2018.06.005

Wang, B., Feng, X., Entzal, B., Zhao, H., and Li, Z. (2021). Pharmacological effects of Kaixin San on neuroinflammation and Aβ deposition in APP/PS1 mice. *Chin. Traditional Herb. Drugs* 52, 7511–7519. doi:10.7501/j.issn.0253-2670.2021.24.013

Wang, N., Jia, Y., Zhang, B., Li, Y., Murtaza, G., Huang, S., et al. (2020). Kai-xin-san, a Chinese herbal decoction, accelerates the degradation of β-amyloid by enhancing the expression of neprilysin in rats. *Evid. Based Complement. Altern. Med.* 2020, 3862342. doi:10.1155/2020/3862342

Xiao, S.-Y., Liu, Y.-J., Lu, W., Sha, Z.-W., Xu, C., Yu, Z.-H., et al. (2022). Possible neuropathology of sleep disturbance linking to alzheimer's disease: astrocytic and microglial roles. *Front. Cell Neurosci.* 16, 875138. doi:10.3389/fncel.2022.875138

Yoshiyama, Y., Kojima, A., Ishikawa, C., and Arai, K. (2010). Anti-inflammatory action of donepezil ameliorates tau pathology, synaptic loss, and neurodegeneration in a tauopathy mouse model. *J. Alzheimers Dis.* 22, 295–306. doi:10.3233/JAD-2010-100681

Yu, P., Zhang, X., Liu, N., Tang, L., Peng, C., and Chen, X. (2021). Pyroptosis: mechanisms and diseases. *Signal Transduct. Target Ther.* 6, 128. doi:10.1038/s41392-021-00507-5

Zhang, X., Xi, Y., Yu, H., An, Y., Wang, Y., Tao, L., et al. (2019). 27-hydroxycholesterol promotes Aβ accumulation via altering Aβ metabolism in mild cognitive impairment patients and APP/PS1 mice. *Brain Pathol.* 29, 558–573. doi:10.1111/bpa.12698

frontiers | Frontiers in Pharmacology

TYPE Original Research
PUBLISHED 10 April 2025
DOI 10.3389/fphar.2025.1545585

Check for updates

OPEN ACCESS

EDITED BY
Huazheng Liang,
Monash University - Southeast University Joint Research Institute (Suzhou), China

REVIEWED BY
Bilge Güvenç Tuna,
Yeditepe University, Türkiye
Yi Li,
China Pharmaceutical University, China

*CORRESPONDENCE
Qichun Zhang,
zhangqichun@njucm.edu.cn
Yingcong Yu,
43087693@qq.com

RECEIVED 15 December 2024
ACCEPTED 17 February 2025
PUBLISHED 10 April 2025

CITATION
Lin Y, Wang Y, Yang X, Ding Z, Hu M, Huang X, Zhang Q and Yu Y (2025) NMN reverses D-galactose-induced neurodegeneration and enhances the intestinal barrier of mice by activating the Sirt1 pathway.
Front. Pharmacol. 16:1545585.
doi: 10.3389/fphar.2025.1545585

COPYRIGHT
© 2025 Lin, Wang, Yang, Ding, Hu, Huang, Zhang and Yu. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

NMN reverses D-galactose-induced neurodegeneration and enhances the intestinal barrier of mice by activating the Sirt1 pathway

Yuxian Lin[1,2], Yajing Wang[3], Xinxin Yang[4], Ziwei Ding[3], Mingye Hu[1], Xianfeng Huang[3], Qichun Zhang[5]* and Yingcong Yu[1]*

[1]The Third Clinical Institute Affiliated to Wenzhou Medical University, Wenzhou, Zhejiang, China, [2]School of Pharmacy, Yantai University, Yantai, Shandong, China, [3]School of Pharmacy, Changzhou University, Changzhou, Jiangsu, China, [4]Department of Infectious Diseases, Wenzhou Third Clinical Institute Affiliated to Wenzhou Medical University, The Third Affiliated Hospital of Shanghai University, Wenzhou People's Hospital, Wenzhou, China, [5]School of Pharmacy, Nanjing University of Chinese Medicine, Nanjing, Jiangsu, China

Background: Age-related decline in nicotinamide adenine dinucleotide (NAD+)—a central regulator of cellular metabolism, DNA repair, and immune homeostasis—is strongly associated with physiological dysfunction. Nicotinamide mononucleotide (NMN), a potent NAD+ precursor, shows promise in counteracting aging-related pathologies, particularly neurodegenerative decline.

Methods: An aging model was established in mice through 8-week D-galactose (D-gal) exposure, followed by NMN oral supplementation. Behavioral outcomes (open field test, Morris water maze) were analyzed alongside oxidative stress markers (SOD, CAT, AGEs), inflammatory cytokines (TNF-α, IL-1β, IL-6, IL-10), and neurotransmitters (LC-MS/MS). Apoptotic activity (TUNEL, p16/p21), mitochondrial regulators (Sirt1, p-AMPK, PGC-1α), and intestinal barrier integrity (HE/AB-PAS staining) were evaluated. Sirt1 dependency was confirmed using inhibitor Ex527.

Results: NMN restored locomotor activity and spatial memory in D-gal mice without altering body weight. Mechanistically, NMN synergistically attenuated oxidative stress and systemic inflammation, elevating antioxidant enzymes (SOD, CAT) and IL-10 while suppressing pro-inflammatory cytokines (TNF-α, IL-6) and AGEs. Cortical/hippocampal analyses revealed reduced apoptosis (TUNEL$^+$ cells) and senescence markers (p16, p21), with enhanced mitochondrial function via Sirt1/AMPK/PGC-1α activation (Sirt1, p-AMPK). NMN concurrently preserved intestinal mucosal architecture, mitigating D-gal-induced barrier disruption. Crucially, all benefits were abolished by Sirt1 inhibition, confirming pathway specificity.

Conclusion: Our findings establish NMN as a multifaceted therapeutic agent that preserves neurocognitive function and intestinal homeostasis in aging models by

orchestrating antioxidative, anti-inflammatory, and antiapoptotic responses through Sirt1/AMPK/PGC-1α activation. This work provides translational insights into NAD+-boosting strategies for age-related disorders.

KEYWORDS

aging, NMN, oxidative stress, neuroinflammation, intestinal barrier, SIRT1

Introduction

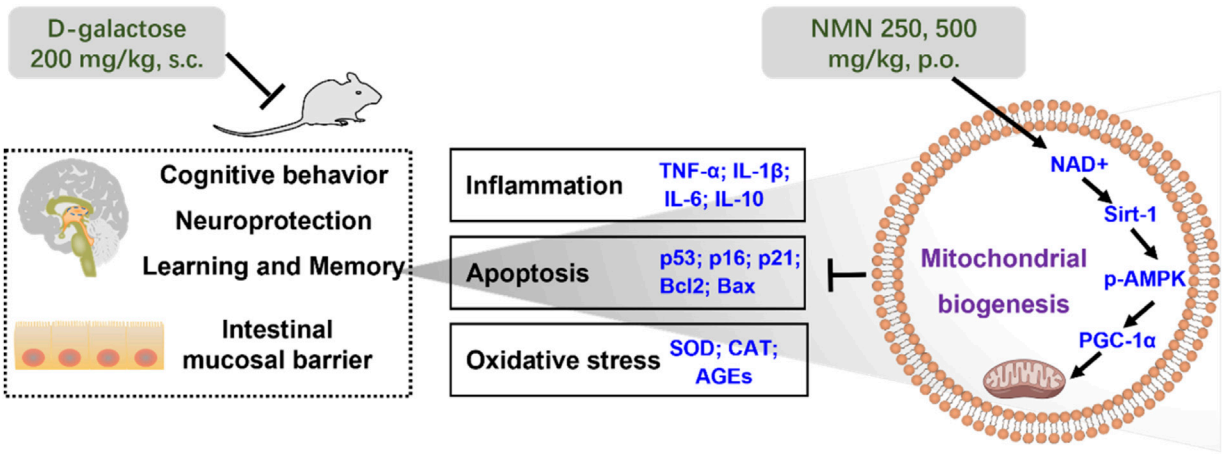
The global population aged 60 years and older is projected to double from 800 million in 2015 to 2 billion by 2050, emphasizing the urgent need for effective therapeutic strategies to mitigate age-related chronic diseases. While advancements in medicine have extended human life expectancy, they have also led to a rising prevalence of conditions such as Alzheimer's disease (AD), cardiovascular disease, and cancer-diseases that, despite their diverse clinical presentations, share common pathological features, including cellular senescence, mitochondrial dysfunction, and chronic inflammation (Farrelly and Daly, 2024; Ledford, 2024).

Non-resolving chronic inflammation, a hallmark of aging, has been identified as a major driver of pathological aging processes. It contributes to the dysfunction of hematopoietic stem cells, accelerates senescence, and perpetuates neuroinflammation, all of which are implicated in cognitive decline and neurodegeneration, particularly in Alzheimer's disease (Heneka et al., 2024; Riessland et al., 2024). Chronic neuroinflammation exacerbates tau protein hyperphosphorylation, disrupts mitochondrial function, and induces oxidative stress (OS), all of which accelerate neurodegeneration. Signaling pathways such as Sirt1 and cGAS/STING have been identified as central regulators of aging-associated inflammation and neurodegeneration, presenting attractive therapeutic targets (Kalinkovich and Livshits, 2024). Mitochondrial dysfunction further exacerbates chronic inflammation through the aberrant accumulation of metabolic intermediates, such as succinate and itaconate, derived from the tricarboxylic acid (TCA) cycle (Kong et al., 2024). Excess succinate, for instance, stabilizes hypoxia-inducible factor-1α (HIF-1α), inhibits prolyl hydroxylase, and promotes interleukin-1β (IL-1β) production, sustaining inflammatory signaling (Salminen et al., 2015). These mechanisms underscore the critical role of mitochondria not only as cellular energy providers but also as regulators of inflammation and aging.

The human brain, consisting of approximately 85 billion neurons, undergoes structural and functional changes with aging that culminate in cognitive decline and memory impairment. Neurons are highly reliant on mitochondrial ATP production; thus, mitochondrial dysfunction and the subsequent increase in ROS generation can significantly contribute to neurodegenerative processes (Sies and Jones, 2020). Age-related mitochondrial dysfunction involves the accumulation of mutations in mitochondrial DNA (mtDNA), decreased oxidative phosphorylation efficiency, and impaired antioxidant defenses, leading to oxidative damage and cellular aging (Byrns et al., 2024).

The sirtuin family, particularly Sirt1, has emerged as a critical regulator of aging and longevity due to its role as a NAD^+-dependent deacetylase. Sirt1 governs various cellular processes, including DNA repair, mitochondrial biogenesis, autophagy, and inflammation, primarily through the activation of downstream pathways such as AMPK and PGC-1α (Packer, 2020; Satoh et al., 2017). By modulating key proteins like p53, NF-κB, and the forkhead box O (FOXO) transcription factors, Sirt1 plays a pivotal role in promoting cellular resilience and neuroprotection (Fujita and Yamashita, 2018). Activation of the Sirt1/AMPK/PGC-1α axis has been shown to improve mitochondrial function, mitigate apoptosis, and enhance autophagy in neurodegenerative disease models, highlighting its therapeutic potential.

NAD^+, a vital coenzyme in cellular metabolism, is central to mitochondrial function, DNA repair, and the regulation of cellular aging. Age-related depletion of NAD^+ impairs these processes,



GRAPHICAL ABSTRACT

exacerbating the progression of neurodegenerative diseases and other age-associated pathologies (Covarrubias et al., 2021). Supplementation with NAD^+ precursors, such as nicotinamide mononucleotide (NMN), has emerged as a promising strategy to restore NAD^+ levels, thereby improving mitochondrial bioenergetics, activating longevity pathways like Sirt1, and mitigating chronic inflammation (Rajman et al., 2018). Recent studies have demonstrated the efficacy of NMN and other NAD^+ precursors in enhancing health span and reversing age-related functional decline (Wu et al., 2023).

The gastrointestinal (GI) tract, a critical interface for systemic health, harbors the body's largest population of immune cells and is a key component of the brain-gut axis. Aging compromises intestinal barrier integrity and increases gut permeability, allowing endotoxins to enter the systemic circulation and contribute to chronic inflammation and neuroinflammation (Connell et al., 2022). Disruption of the gut barrier may exacerbate systemic inflammatory responses, further implicating the brain-gut axis in aging-associated neurodegeneration. Recent studies suggest that NAD^+ precursors, such as NMN, may ameliorate intestinal barrier dysfunction, highlighting a potential avenue for modulating age-related systemic and neuroinflammatory processes (Li et al., 2024).

In the present study, we utilized a D-galactose (D-gal)-induced aging mouse model to investigate the potential of NMN in reversing age-related cognitive impairment, neuroinflammation, and intestinal barrier dysfunction. NMN supplementation significantly improved cognitive performance, reduced systemic and neuroinflammatory markers, and enhanced mitochondrial function while ameliorating oxidative stress. Importantly, the co-administration of Ex-527, a pharmacological Sirt1 inhibitor, attenuated these beneficial effects, confirming that NMN exerts its protective actions via activation of the Sirt1/AMPK/PGC-1α pathway.

Our findings provide compelling evidence that NMN supplementation mitigates age-related neurodegenerative symptoms and systemic dysfunction by restoring NAD^+ homeostasis and activating Sirt1-dependent pathways. This study highlights the therapeutic potential of NMN as a promising intervention to counteract cellular and molecular aging, offering translational relevance for combating age-associated neurodegenerative and inflammatory diseases.

Materials and methods

Animal model

In this study, we employed a male-specific pathogen-free (SPF) grade C57BL/6 murine model to evaluate the therapeutic effects of nicotinamide mononucleotide (NMN) on aging phenotypes induced by D-gal. A total of 60 male mice (6–8 weeks old, weighing 20 ± 1 g) were obtained from Hangzhou Medical College and acclimatized for 1 week under standardized conditions (temperature: 25°C ± 1°C; relative humidity: 60% ± 5%) with free access to food and water. Following acclimatization, animals were weighed, identified, and randomly assigned to five experimental groups: Group 1 (Normal), Group 2 (D-gal model), Group 3 (D-gal + NMN 250 mg/kg), Group 4 (D-gal + NMN 500 mg/kg), and Group 5 (D-gal + NMN 500 mg/kg + Ex527 5 mg/kg). The oral dosage of NMN was designed as previous literature (Yoshino et al., 2018). The aging model was induced by subcutaneous administration of D-gal (200 mg/kg/day) in all groups except the Control group. Groups 3 and 4 received NMN (purity 99.9%, Yuyao Lifespan Health Technology Co., Ltd., Zhejiang, China) by oral gavage, while Group 5 was co-administered NMN (500 mg/kg) and Ex527, a selective Sirt1 inhibitor, administered intraperitoneally at 5 mg/kg. The intervention period spanned 8 weeks, during which body weight was monitored longitudinally. All experimental procedures were conducted in strict compliance with the Guide for the Care and Use of Laboratory Animals (NIH Publication No. 80-23, revised 1978). Ethical approval for the study was obtained from the Animal Care and Use Committee of the Nanjing University of Chinese Medicine (Approval No. 202302A085).

Open-field test (OFT)

Following the 8-week treatment period, the open-field test (OFT) was conducted to evaluate locomotor activity and anxiety-like behavior in aging mice, as previously described (Ge et al., 2023). The OFT is a well-established behavioral assay that provides quantitative measurements of exploratory behavior, general locomotion, and anxiety-like responses in rodents. The test was performed using a mouse locomotor activity monitoring system (50 cm × 50 cm×40 cm, JLBehv-LAM-4; Jiliang Ltd., Shanghai, China) under standardized conditions. Prior to testing, the experimental chamber was thoroughly cleaned and disinfected to eliminate residual odors, particularly fecal or urinary traces, which could influence behavioral outcomes. Mice were acclimated to the testing room for at least 1 h before the experiment to minimize environmental stressors. Each mouse was carefully removed from its home cage with minimal handling stress, ensuring the animal's back was facing the experimenter during transfer, and gently placed in the center of the open field arena. Behavioral recording commenced immediately, capturing parameters such as total distance traveled and average velocity over a 5-minute observation period. To control for circadian influences on activity, all testing was conducted within a 24-hour time window under consistent lighting conditions. Post-assessment, mice were promptly returned to their home cages. The testing apparatus was disinfected with 70% ethanol and wiped clean between trials to prevent residual olfactory cues from confounding subsequent recordings. A quiet and undisturbed environment was maintained throughout the procedure to minimize external disturbances that could impact the behavioral outcomes.

Morris water maze (MWM)

Following the 8-week treatment regimen, spatial learning and memory performance in the mice was assessed using the Morris water maze (MWM) test, as previously described (Zhao et al., 2023). The MWM is a widely validated behavioral assay for evaluating hippocampus-dependent spatial navigation and memory in rodents. The specifications of the Morris water maze for mice are as follows:

circular pool diameter 120 cm, 50 cm height, platform diameter 8 cm in the fourth quadrant, and submersion level of 1.5 cm.

The MWM procedure consisted of a 5-day acquisition phase, followed by a probe trial on day 6. During the acquisition phase, mice underwent four training trials per day to locate a submerged escape platform hidden in a circular pool filled with water maintained at 20°C–22°C. At the start of each trial, mice were released from a randomized starting point, and the escape latency-defined as the time taken to reach the hidden platform-was recorded, with a maximum trial duration of 60 s. Mice that failed to locate the platform within the allotted time were gently guided to the platform and allowed to remain there for 60 s to facilitate spatial learning; their escape latency was recorded as 120 s. On the sixth day, a probe trial was conducted to assess memory retention in the absence of the escape platform. Mice were released into the pool and allowed to swim freely for 60 s. The parameters measured included the frequency of crossings over the target platform location, the time spent in the target quadrant, and the total distance traveled within the target quadrant. These parameters were quantified using an automated animal behavior analysis system (Zhenghua Ltd., Shanghai, China). To minimize variability, all experiments were conducted at consistent times of the day to control for diurnal influences on behavior. The water temperature was strictly maintained to reduce thermal stress and ensure consistency across trials. Metrics such as escape latency, path length, and swimming velocity were meticulously recorded to provide a robust evaluation of spatial learning and memory.

Determination of superoxide dismutase (SOD) and catalase (CAT)

Mice were anesthetized with 1% sodium pentobarbital at a dose of 50 mg/kg, and blood samples were collected from the orbital venous plexus using sterile capillary tubes. The collected blood was allowed to clot overnight at 4°C and subsequently centrifuged at 3,000 rpm for 10 min to isolate the serum. SOD and CAT activities were quantified using the nitroblue tetrazolium photoreduction assay and the guaiacol oxidation method, respectively, in accordance with the manufacturer's protocols (Huaying Ltd., Beijing, China).

Cytokines analysis

Mice were euthanized via cervical dislocation, and brain tissues were promptly harvested on ice to maintain tissue integrity and biochemical stability. The tissues were rinsed with ice-cold (4°C) saline to remove residual blood, and excess moisture was gently blotted. Brain tissues were weighed for accurate normalization, followed by homogenization in ice-cold saline at a mass-to-volume ratio of 1:9 (g/mL) using a mechanical homogenizer in an ice-water bath. The resulting homogenates were centrifuged at 3,500 rpm for 10 min at 4°C to remove cellular debris. The supernatant was carefully collected and stored at −80°C for subsequent biochemical analyses. Quantification of AGEs, tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), interleukin-6 (IL-6), and interleukin-10 (IL-10) was performed using validated radioimmunoassay kits (Huaying Ltd., Beijing, China), in accordance with the manufacturer's protocols. These analytes serve as critical biomarkers for evaluating neuroinflammatory and neurodegenerative processes. All procedures were conducted under rigorous quality control to ensure assay reliability and reproducibility.

TABLE 1 Ion pairs for neurotransmitters in MS.

Indicator	Q1→Q3/(m/z)	DP/V	CE /V
γ- aminobutyric acid (GABA)	104.0→87.2	36	13
Glutamate (Glu)	148.0→84.3	105	19
Dopamine (DA)	154.1→137.1	130	13
5-hydroxytryptamine(5-HT)	177.1→160.1	130	17
Epinephrine(E)	184.4→166.2	21	10
Norepinephrine (NE)	170.1→107.2	40	31

Neurotransmitter analysis

Following euthanasia via cervical dislocation, brain tissues were rapidly excised and placed on ice to preserve neurotransmitter integrity, as previously described (Ge et al., 2023). The tissues were accurately weighed and homogenized in a tenfold volume of ice-cold 0.1% formic acid solution to facilitate cellular disruption and neurotransmitter release. Homogenization was performed for 3 min under controlled conditions to ensure complete cell lysis. An aliquot of 100 μL of the homogenate was subsequently mixed with 400 μL of ice-cold 0.2% formic acid-acetonitrile solution, vortexed for 3 min to precipitate proteins and enhance neurotransmitter extraction. The mixture underwent centrifugation at 12,000 rpm for 10 min at 4°C, enabling separation of the supernatant containing the target analytes from cellular debris and insoluble components. The resulting supernatant was carefully transferred to a centrifugal concentrator and dried under vacuum to remove organic solvents, facilitating sample concentration. The dried residues were reconstituted in 0.1% formic acid to ensure solubility and optimal ionization for subsequent mass spectrometric analysis. The reconstituted samples were centrifuged at 12,000 rpm for 10 min at 4°C to remove residual particulates, and the resulting clear supernatants were filtered to ensure compatibility with the mass spectrometry system. Quantitative analysis of neurotransmitters was performed using an AB SCIEX Triple Quad™ 6500 triple quadrupole linear ion trap mass spectrometer. Targeted ion pairs and mass spectrometry parameters for neurotransmitter detection are summarized in Table 1. All procedures were conducted under stringent quality control to ensure analytical precision and reproducibility.

Western blotting

Following euthanasia via cervical dislocation, brain tissues were rapidly harvested on ice to preserve protein integrity. Tissues were homogenized in radioimmunoprecipitation assay (RIPA) buffer

supplemented with protease and phosphatase inhibitor cocktails to prevent protein degradation and dephosphorylation. The homogenates were centrifuged at 4°C to isolate the soluble protein fraction, and protein concentrations were determined using the bicinchoninic acid (BCA) assay, ensuring precise quantification of total protein content. For electrophoretic separation, protein samples were denatured under reducing conditions with dithiothreitol (DTT) and sodium dodecyl sulfate (SDS), ensuring uniform negative charges and size-dependent migration. Samples were resolved via SDS-PAGE on polyacrylamide gels. An initial voltage of 70 V was applied for 20 min to concentrate proteins within the stacking gel, followed by 110 V for 60 min to achieve separation in the resolving gel. Following electrophoresis, proteins were transferred onto polyvinylidene fluoride (PVDF) membranes using a wet-transfer system, ensuring efficient immobilization. Membranes were blocked with 5% non-fat milk in Tris-buffered saline containing 0.1% Tween-20 (TBST) to minimize nonspecific binding. Primary antibodies specific to SOD1 (1:10000), SOD2 (1:2000), Bax (1:5000), Bcl-2 (1:2000), Sirt1 (1:1000), p16 (1:5000), p21 (1:1000), p-AMPK (1:5000), AMPK (1:2000), PGC-1α (1:1000), and GAPDH (1:10000) were incubated overnight at 4°C to optimize antigen-antibody interactions. Antibodies against SOD1, SOD2, Bax, Bcl-2, Sirt1, p16, p21, p-AMPK, and AMPK were purchased from Abcam (United States); PGC-1α was sourced from Absin (China), and GAPDH from Cell Signaling Technology (CST, United States). After three washes in TBST to remove unbound primary antibodies, membranes were incubated for 2 h at room temperature with horseradish peroxidase (HRP)-conjugated secondary antibodies (CST, United States). Excess secondary antibodies were removed through additional TBST washes. Immunoreactive protein bands were visualized using enhanced chemiluminescence (ECL) substrates, and band intensities were quantified using ImageJ 22 software (National Institutes of Health, United States) to provide precise, semi-quantitative analysis of protein expression levels.

Terminal deoxynucleotidyl transferase dUTP nick-end labelling assay

Mice were anesthetized with an intraperitoneal injection of sodium pentobarbital (50 mg/kg). Following anesthesia, the chest cavity was carefully opened to expose the heart. The right atrium was incised, and a perfusion needle was inserted into the left ventricle. Initial perfusion was performed with saline to flush out blood, continuing until the effluent from the right atrium became clear. Subsequently, the mice were perfused with 4% paraformaldehyde for tissue fixation, with the process continuing until the liver tissue appeared white. The brain was then harvested and placed in 4% paraformaldehyde for a minimum of 24 h for post-fixation. Following fixation, the tissues underwent dehydration, and were subsequently embedded in paraffin, a standard procedure that preserves tissue morphology for further histological analysis. Paraffin-embedded tissues were sectioned into 5-μm-thick slices for further examination. To assess apoptosis, the tissue sections were subjected to the terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) assay, a widely used and highly sensitive method for detecting DNA fragmentation, a characteristic feature of apoptotic cell death. The TUNEL assay was conducted using a commercially available TUNEL kit (Saver, Wuhan, China), following the manufacturer's protocol. The TUNEL-positive cells, indicative of DNA fragmentation and apoptotic cell death, were visualized under a fluorescence microscope. Quantification of apoptotic cells was performed by counting the number of TUNEL-positive cells within the brain tissue sections, providing an assessment of apoptosis in the experimental model.

Intestinal H&E staining and Alcian Blue (AB)-Periodic Acid-Schiff (PAS) staining

Colonic tissues were harvested from mice fixed with 4% paraformaldehyde via left ventricle perfusion as described above. Following fixation, the tissues underwent a dehydration protocol and were subsequently embedded in paraffin. Thin sections (5 μm) were cut for histological analysis. For general tissue morphology, sections were stained with hematoxylin and eosin (H&E) using a standard procedure, allowing for detailed examination of the intestinal architecture. For Alcian Blue (AB)-Periodic Acid-Schiff (PAS) staining, sections were first incubated with Alcian blue (pH 2.5) for 30 min to visualize acidic mucopolysaccharides, which prominently stain goblet cells responsible for mucus secretion. After washing, PAS staining was performed to detect neutral mucopolysaccharides and eosinophils. This was achieved through periodic acid oxidation followed by Schiff's reagent application, and counterstaining with hematoxylin for nuclear visualization. This dual staining technique facilitates a comprehensive evaluation of the intestinal barrier, offering critical insights into its structural and functional integrity, which are pivotal for studies on gastrointestinal physiology and pathology.

Statistical analysis

Statistical analysis was performed using GraphPad Prism version 8.0.1. Data are presented as the mean ± standard error of the mean (SEM). To evaluate the significance of differences between groups, one-way analysis of variance (ANOVA) was conducted. Post-hoc analysis was carried out using Tukey's Honestly Significant Difference (HSD) test for multiple comparisons. Statistical significance was defined at a threshold of $p < 0.05$.

Results

Temporal Analysis of body weight

Body weight measurements were conducted at weeks 1, 3, 5, 7, and 8, with the results presented in Figures 1A–E. The trajectory of body weight changes over the 8-week experimental period is shown in Figure 1F. Analysis of the weight curves revealed no statistically significant differences between groups during the first 4 weeks of the study (Figures 1B, F). However, a divergence in weight gain became evident from week 5 (Figures 1C, F). Notably, in the group receiving NMN at a dose of 500 mg/kg in combination with Ex527 (5 mg/kg),

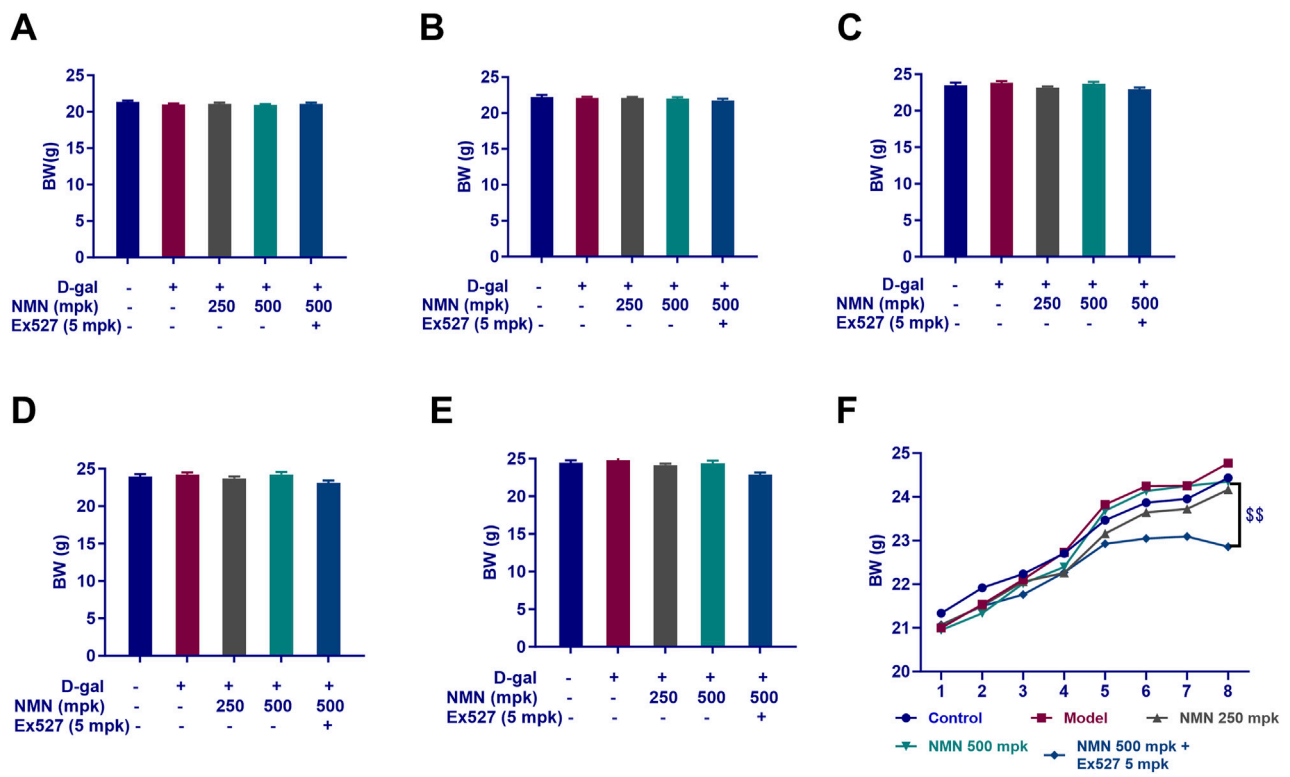


FIGURE 1
Temporal Analysis of Murine Body Weight. **(A–E)** The body weight measurements at distinct time points: Week 1 **(A)**, Week 3 **(B)**, Week 5 **(C)**, Week 7 **(D)**, and Week 8 **(E)**, following the corresponding treatment regimens; **(F)** A comprehensive summary of the body weight trajectory over the 8-week experimental period. Each data point represents the mean ± SEM, with n = 12; $$, $p < 0.01$, in comparison to the NMN 500 mg/kg group; mpk, mg/kg.

a significant reduction in mean body weight was observed by week 8 ($p < 0.01$), indicating a marked inhibitory effect of Ex527 on body weight gain in mice.

NMN ameliorates locomotor activity and enhances learning and memory in aging mice

To assess the effects of NMN on locomotor activity, as well as spatial learning and memory in aging mice, a comprehensive behavioral evaluation was performed using the OFT and MWM. As shown in the OFT, administration of NMN at doses of 250 mg/kg and 500 mg/kg resulted in a significant increase in locomotor activity, as evidenced by a greater total distance traveled (Figure 2A; $p < 0.05$ and $p < 0.01$) and higher moving speed (Figure 2B; $p < 0.01$) in aging mice, relative to the model group. These findings suggest that NMN enhances both motor function and locomotor activity in aged mice. In the MWM test, D-galactose-induced aging mice exhibited a significantly reduced number of target quadrant crossings ($p < 0.05$), shorter time spent in the target quadrant ($p < 0.01$), and decreased distance traveled within the target quadrant ($p < 0.01$), compared to the control group, indicating impaired spatial learning and memory. Administration of NMN at 250 mg/kg and 500 mg/kg increased the number of target quadrant crossings (Figure 2C) and residence time in the target quadrant (Figure 2D), suggesting an improvement in spatial memory, although these differences did not reach statistical significance. However, a significant increase in the total distance traveled ($p < 0.01$) in both the NMN 250 mg/kg and NMN 500 mg/kg groups was observed, indicating enhanced spatial learning (Figure 2E). Co-administration of Ex527, an inhibitor of Sirt1, appeared to attenuate the effects of NMN, although the difference did not achieve statistical significance. This suggests a potential interaction between NMN and Sirt1 signaling pathways in the regulation of aging and cognitive function. Collectively, these results indicate that NMN effectively mitigates age-related cognitive decline in D-gal-induced aging mice. The modulation of NMN's effects by Ex527 highlights the complexity of these interactions, warranting further investigation into the underlying mechanisms.

NMN reduces the levels of OS in aging mice

NMN has been shown to mitigate oxidative stress (OS) in aging mice, a key factor in the progression of aging and various degenerative and chronic conditions, including inflammation, cancer, arthritis, neurodegenerative diseases, and cardiovascular disorders. To quantitatively assess the impact of NMN on OS, we measured the expression levels of SOD and CAT in serum, and SOD1 and SOD2 in brain tissue from aging mice. Our results demonstrated a significant reduction in all these OS biomarkers

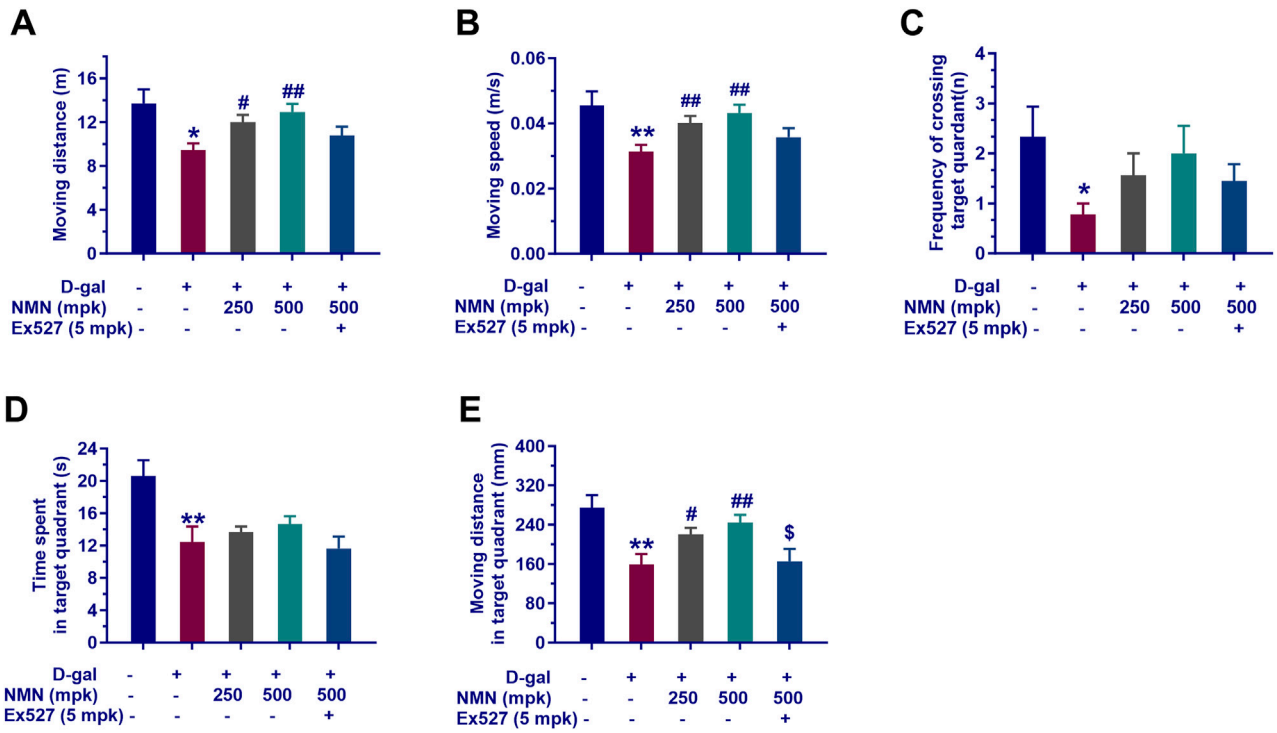


FIGURE 2
NMN ameliorates locomotor activity and enhances learning and memory in aging mice. **(A, B)** The locomotor moving distance and the velocity assessed over a 5-min period in the OFT; **(C–E)** The frequency of target quadrant crossings, residence time, and moving distance within the target quadrant of MWM, respectively. Each data point represents the mean ± SEM, with n = 9; *, $p < 0.05$, **, $p < 0.01$, compared to the Normal group; #, $p < 0.05$, ##, $p < 0.01$, versus the D-gal group; $, $p < 0.05$, in comparison to the NMN 500 mpk group.

following 8 weeks of D-gal administration, consistent with the accumulation of reactive oxygen species (ROS) commonly observed in aging models. Notably, administration of NMN at doses of 250 mg/kg and 500 mg/kg significantly increased serum SOD and CAT levels ($p < 0.01$, Figures 3A, B), and brain SOD1 and SOD2 expression ($p < 0.01$, Figures 3C, D), indicating a substantial enhancement in endogenous antioxidant defense mechanisms. These effects were notably attenuated by co-administration of Ex527, a Sirt1 inhibitor ($p < 0.01$), suggesting a potential role of the Sirt1 pathway in mediating the antioxidant effects of NMN. In summary, these findings suggest that NMN can effectively restore the balance between ROS and antioxidants, thereby alleviating the detrimental effects of oxidative stress in both serum and brain tissues of D-gal-induced aging mice.

NMN alleviates the inflammatory symptoms and reduces AGEs

In the context of aging research, nicotinamide mononucleotide (NMN) has emerged as a promising compound with potential anti-inflammatory effects. Our study rigorously assessed the impact of NMN on the attenuation of inflammatory markers and AGEs in aging mice. Following behavioral assessments, the cerebral cortex and hippocampus, key regions associated with cognitive function, were isolated and analyzed. We quantified AGEs and key inflammatory cytokines, including TNF-α, IL-1β, IL-6, and IL-10, using radioimmunoassay. NMN administration demonstrated a dose-dependent reduction in the expression of inflammatory markers in aging mice, including AGEs, TNF-α, IL-1β, and IL-6. The 500 mg/kg dose exhibited a particularly significant decrease in these markers ($p < 0.01$, Figures 4A–D), suggesting that NMN effectively mitigates the inflammatory response associated with aging. Concurrently, NMN at both 250 mg/kg and 500 mg/kg significantly upregulated the expression of the anti-inflammatory cytokine IL-10 ($p < 0.05$ and $p < 0.01$, Figure 4E), further attenuating inflammatory symptoms. Notably, co-administration of Ex527, a selective Sirt1 inhibitor, significantly inhibited the reduction in brain inflammation and AGEs induced by NMN at the 500 mg/kg dose ($p < 0.01$). This indicates a potential interaction between NMN and the Sirt1 signaling pathway in modulating inflammation and AGEs in aging mice. These findings highlight the potential of NMN in ameliorating inflammatory symptoms and reducing AGEs, both of which are critical factors in the pathogenesis of various age-related diseases.

Effects of NMN on neurotransmitters of aging mice

The impact of nicotinamide mononucleotide (NMN) on the neurotransmitter profile in aging mice was investigated, with a particular focus on the modulation of key neurochemicals implicated in various neurological conditions. Our findings

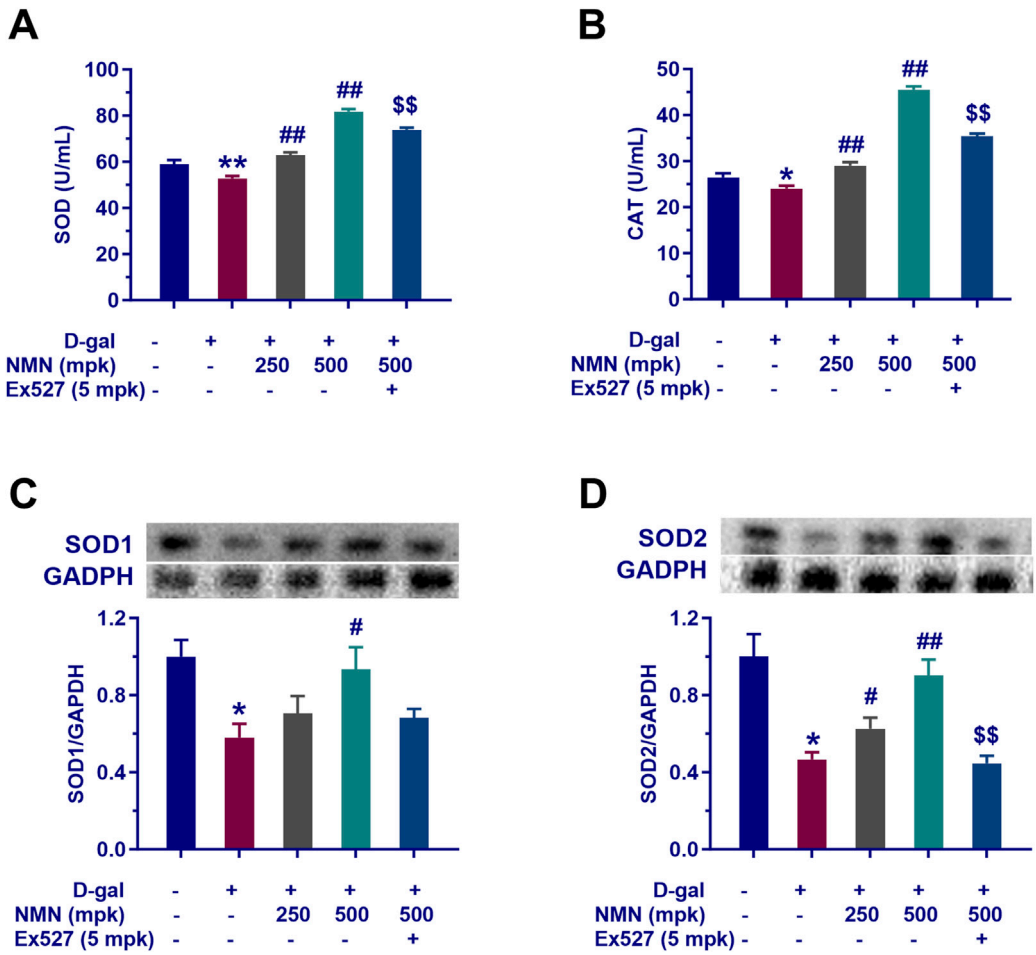


FIGURE 3
NMN attenuates oxidative stress of aging mice. **(A, B)** The activities of SOD and CAT in serum of mice determined via biochemical assay following the corresponding treatment regimens; **(C, D)** The expression levels of SOD1 and SOD2 in the brain of mice analysed by Western blotting. Each data point represents the mean ± SEM, with n = 12; *, $p < 0.05$, **, $p < 0.01$, compared to the Normal group; #, $p < 0.05$, ##, $p < 0.01$, versus the D-gal group; $$, $p < 0.01$, in comparison to the NMN 500 mpk group.

provide insights into the neuromodulatory effects of NMN, specifically on the levels of norepinephrine (NE), serotonin (5-hydroxytryptamine, 5-HT), glutamic acid, γ-aminobutyric acid (GABA), and dopamine (DA) in the brain.

In the aging model, NMN administration at dosages of 250 and 500 mg/kg resulted in a dose-dependent increase in the levels of NE, serotonin, glutamic acid, and GABA (Figures 5A–E), suggesting a potential enhancement of the balance between monoaminergic and excitatory/inhibitory neurotransmitter systems. Notably, NMN at 250 mg/kg significantly elevated the levels of NE and GABA ($p < 0.05$; Figures 5A, E), both of which are critical in the regulation of arousal, mood, and inhibitory neuronal signaling. Conversely, NMN treatment led to a reduction in DA levels (Figure 5F), a neurotransmitter central to motor control, reward mechanisms, and motivation. Furthermore, the positive effects of NMN on neurotransmitter levels were partially reversed by co-administration of Ex527, a selective Sirt1 inhibitor, suggesting that the modulation of neurotransmitter dynamics by NMN may involve Sirt1-mediated pathways. This observation provides important insights into the potential mechanisms underlying NMN's effects on cognitive and behavioral functions and underscores the role of Sirt1 in regulating neurochemical homeostasis in aging mice ($p < 0.01$). These findings contribute to our understanding of NMN's role in modulating neurotransmitter systems and its potential therapeutic implications for neurological aging and related disorders.

NMN regulates the aging-related genes in the brain of aging mice

In this study, we examined the regulatory effects of nicotinamide mononucleotide (NMN) on aging-associated genes in the brains of aging mice, with particular emphasis on p16 and p21, key regulators of cellular senescence. Our results demonstrated that NMN administration at dosages of 250 and 500 mg/kg significantly reduced the expression levels of p16 ($p < 0.05$; $p < 0.01$) and p21 ($p < 0.05$; $p < 0.01$) in brain tissue from aging mice (Figures 6A, B). This downregulation suggests a potential inhibitory effect of NMN on molecular markers of cellular aging, consistent with the well-

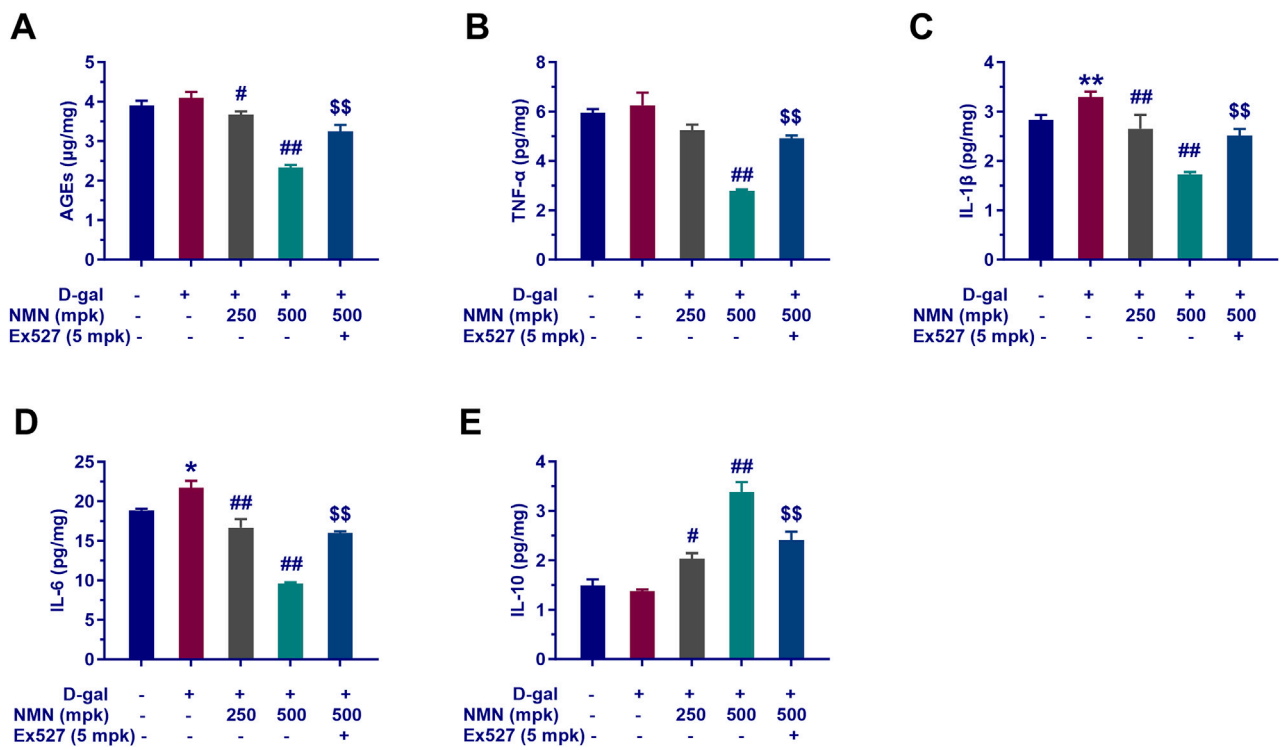


FIGURE 4
NMN exerts anti-inflammatory effects and modulates AGEs of aging mice. Panels A through E showcase the quantification of AGEs **(A)**, TNF-α **(B)**, IL-1β **(C)**, IL-6 **(D)**, and IL-10 **(E)** in the mice brains assayed with radioimmunoassay kit. Each data point represents the mean ± SEM, with n = 6; **, $p < 0.01$, compared to the Normal group; #, $p < 0.05$, ##, $p < 0.01$, versus the D-gal group; $$, $p < 0.01$, in comparison to the NMN 500 mpk group.

established roles of p16 and p21 in mediating cell cycle arrest and promoting cellular senescence. Notably, co-administration of Ex527, a selective Sirt1 inhibitor, effectively counteracted the suppressive effects of NMN on p16 and p21 expression, leading to a significant increase in the levels of these proteins ($p < 0.05$). These findings are in agreement with prior studies implicating Sirt1 in the regulation of cellular senescence, as well as the expression of senescence-associated proteins such as p16 and p21. The modulation of these proteins by NMN may contribute to its observed anti-aging effects and provides a molecular framework through which NMN could exert its neuroprotective and anti-senescence activities in the context of brain aging. These results underscore the potential of NMN as a modulator of cellular aging pathways, offering novel insights into its therapeutic mechanisms for age-related neurodegenerative conditions and cognitive decline.

Effect of NMN on mitochondria function in aging mice

In this study, we investigated the effects of nicotinamide mononucleotide (NMN) on mitochondrial function in aging mice, with particular focus on the Sirt1/AMPK/PGC-1α signaling pathway, a key regulator of mitochondrial biogenesis and energy metabolism. Our results demonstrated that NMN administration at dosages of 250 and 500 mg/kg significantly upregulated the expression of Sirt1, phosphorylated AMPK (p-AMPK), and PGC-1α in the brains of aging mice (Figures 7A–C; $p < 0.05$; $p < 0.01$). This upregulation suggests that NMN exerts a stimulatory effect on the Sirt1/AMPK/PGC-1α axis, which is central to the enhancement of mitochondrial biogenesis and cellular energy homeostasis. Furthermore, co-administration of Ex527, a selective Sirt1 inhibitor, abrogated the NMN-induced upregulation of the Sirt1/AMPK/PGC-1α pathway ($p < 0.05$; $p < 0.01$), indicating that the effects of NMN on mitochondrial function are, at least in part, mediated through Sirt1. These findings are consistent with previous studies that have highlighted the critical role of Sirt1 in regulating mitochondrial health and energy metabolism, particularly in the context of aging. In conclusion, our data suggest that NMN enhances mitochondrial function in aging mice through modulation of the Sirt1/AMPK/PGC-1α signaling pathway, providing molecular insights into its potential therapeutic effects on age-related mitochondrial dysfunction.

NMN attenuates apoptosis of the brain of aging model mice

Subsequently, the regulatory effects and underlying mechanisms of NMN on D-gal-induced neuronal apoptosis in aging mice was performed. First, TUNEL staining demonstrated that D-gal treatment significantly increased the number of TUNEL-positive cells in brain tissues ($p < 0.05$), whereas NMN administration at doses of 250 and 500 mg/kg effectively reduced apoptotic cell proportions (Figures 8A, B), directly confirming its neuroprotective efficacy. Complementing these morphological

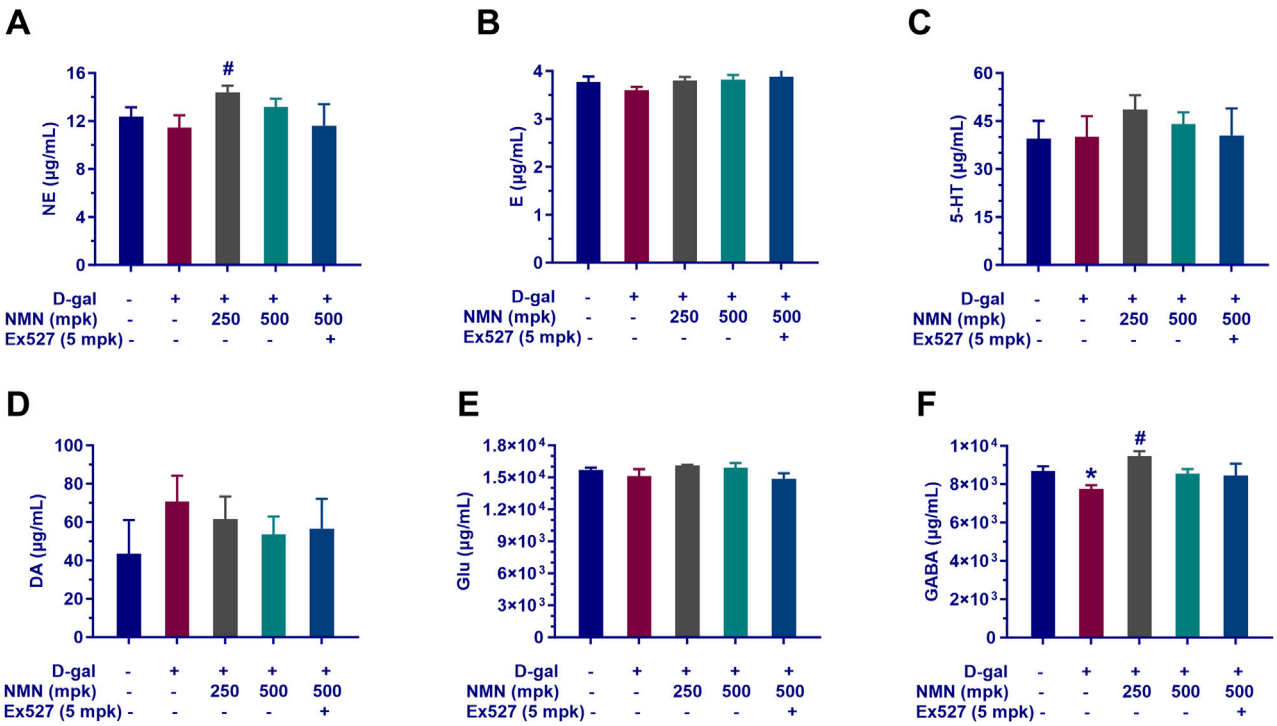


FIGURE 5
Influence of NMN on neurotransmitter levels in the brain tissue of aging mice. The key neurotransmitters in the brains of mice following the corresponding treatment regimens determined with LC-MS/MS assay indicated as **(A)**, Norepinephrine (NE); **(B)** Epinephrine; **(C)**, Serotonin (5-HT); **(D)**, Dopamine (DA); **(E)**, Glutamate (Glu); **(F)**, Gamma-Aminobutyric Acid (GABA). Each data point represents the mean ± SEM, with n = 5–6; *, $p < 0.05$, compared to the Normal group; #, $p < 0.05$, versus the D-gal group.

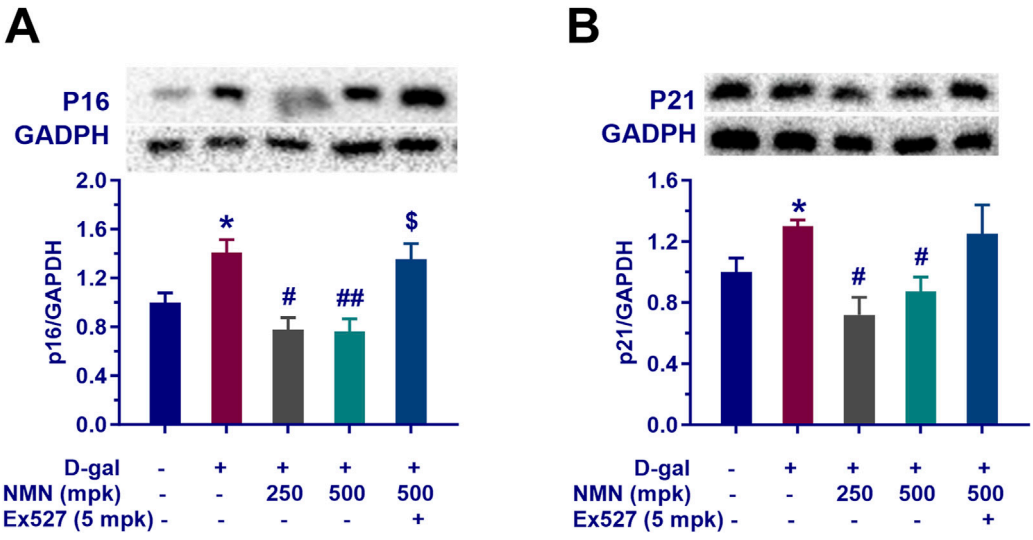


FIGURE 6
NMN mitigates the expression of senescence-associated proteins p16 and p21 of aging mice. Panels **(A, B)** indicate the protein levels of p16 and p21, respectively in the brains assayed by Western blotting with or without the treatment. Each data point represents the mean ± SEM, with n = 3; *, $p < 0.05$, compared to the Normal group; #, $p < 0.05$, ##, $p < 0.01$, versus the D-gal group; $, $p < 0.05$, in comparison to the NMN 500 mpk group.

findings, D-gal exposure markedly down-regulated the expression of the anti-apoptotic factor Bcl-2 while significantly elevating pro-apoptotic Bax levels (Figure 8C), resulting in a pro-apoptotic shift in the Bcl-2/Bax ratio ($p < 0.05$). Notably, NMN intervention not only reversed the decline in Bcl-2 expression but also suppressed the abnormal increase in Bax, thereby restoring the balance of

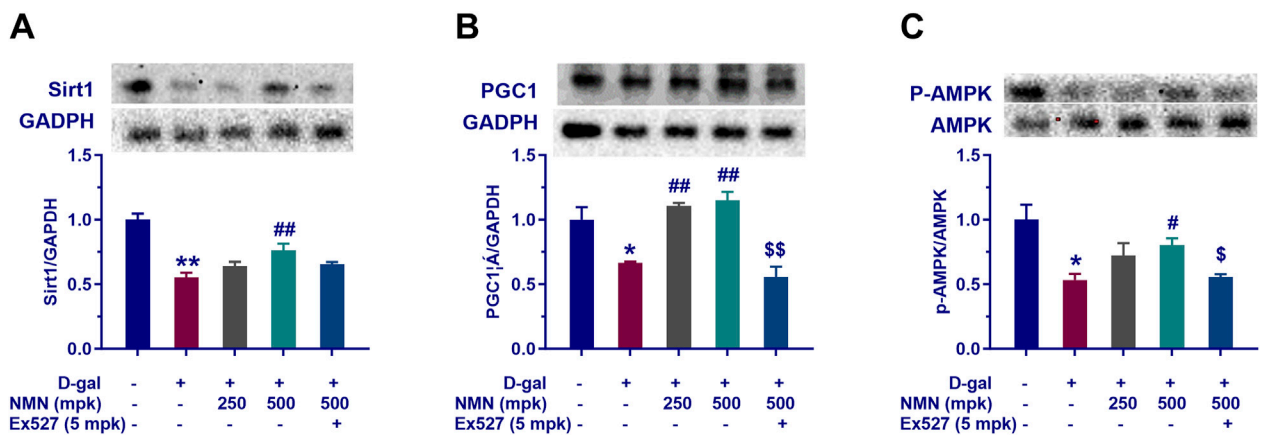


FIGURE 7
NMN reverses the decline of mitochondrial-related proteins tissue of aging mice. Panels **(A–C)** represent the protein expression levels of Sirt1, PGC1α, and phosphorylated AMPK (P-AMPK) in the brain assayed by Western blotting following the corresponding treatment, respectively. Each data point represents the mean ± SEM, with n = 3; *, $p < 0.05$, **, $p < 0.01$, compared to the Normal group; #, $p < 0.05$, ##, $p < 0.01$, versus the D-gal group; $, $p < 0.05$, $$, $p < 0.01$, in comparison to the NMN 500 mpk group.

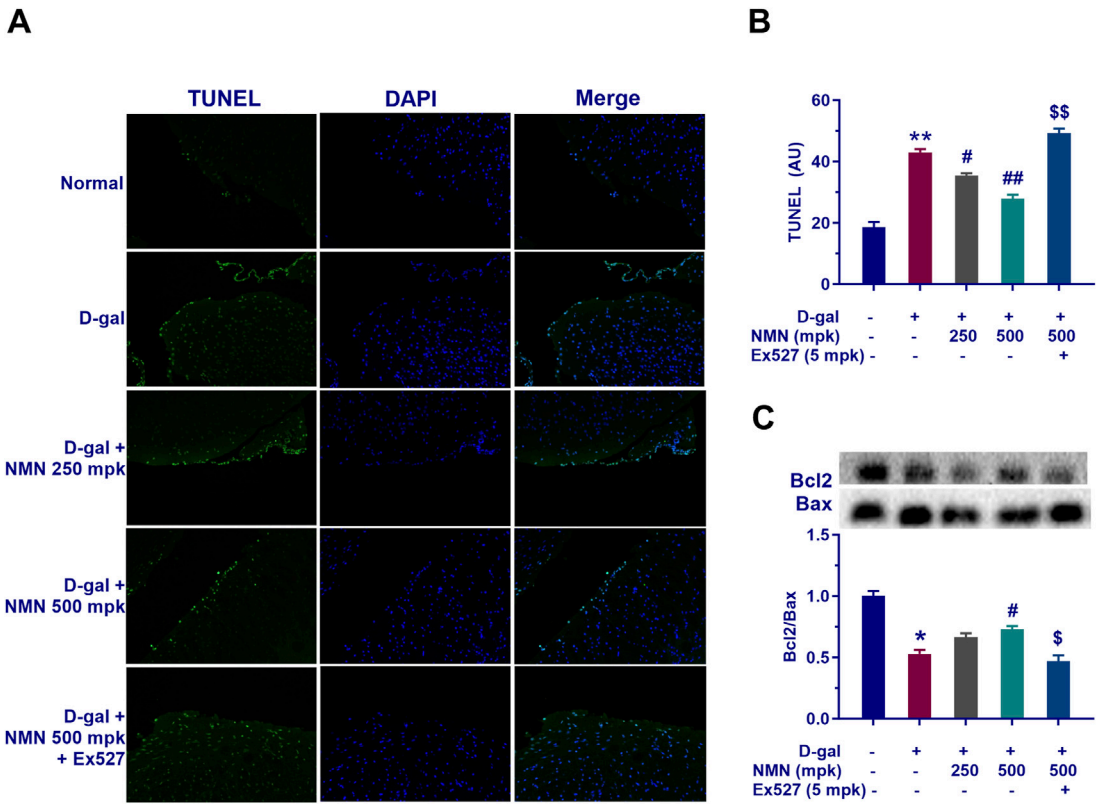


FIGURE 8
NMN mitigates apoptotic cell death in the brains of aging mice. **(A, B)** Representative images of TUNEL staining, a sensitive technique for detecting DNA fragmentation, a hallmark of apoptosis, along with corresponding statistical results in mice. **(C)** The protein levels of Bax and Bcl-2 as determined by Western blot analysis, along with their corresponding statistical results. Each data point represents the mean ± SEM, with n = 3; *, $p < 0.05$, **, $p < 0.01$, compared to the Normal group; #, $p < 0.05$, ##, $p < 0.01$, versus the D-gal group; $, $p < 0.05$, $$, $p < 0.01$, in comparison to the NMN 500 mpk group.

mitochondrial apoptotic pathways and preserving cellular integrity. Mechanistically, co-administration of the selective Sirt1 inhibitor Ex527 substantially counteracted ameliorative effects of NMN on both the Bcl-2/Bax ratio and TUNEL-positive cell counts ($p < 0.05$; $p < 0.01$), suggesting the potential involvement of Sirt1 signaling activation in anti-apoptotic actions of NMN. Collectively, these

results systematically illustrate the action of NMN in mitigating aging-associated neuronal apoptosis through modulating Bcl-2 family protein equilibrium and Sirt1-dependent pathways.

NMN improves the structure of the damaged colon in aging mice

Histological analysis of colonic tissues, using Hematoxylin and Eosin (H&E) staining, revealed distinct structural alterations in the model group treated with D-gal. The normal control group exhibited intact colonic wall architecture, characterized by well-organized mucosal microvilli, as well as normal crypt and gland structures. In contrast, the D-gal-treated group showed significant histopathological changes, including disorganized cellular arrangement in the colonic mucosa, partial crypt destruction, glandular atrophy, thinning of the submucosa, and pronounced eosinophilic infiltration, all of which are indicative of an inflammatory response and tissue damage. However, treatment with NMN at doses of 250 and 500 mg/kg preserved mucosal integrity, maintained the organization of goblet cells, and prevented epithelial cell shedding. Additionally, neutrophil infiltration was reduced, and there was a marked recovery in both crypt depth and villus height, suggesting a restorative effect of NMN on the colonic epithelial barrier (Figure 9A). Furthermore, Alcian Blue-Periodic Acid-Schiff (AB-PAS) staining demonstrated a dose-dependent increase in the number of goblet cells in the colons of NMN-treated mice. Goblet cells, essential for maintaining mucosal integrity and barrier function, were significantly enhanced (Figure 9A). Co-administration of Ex527, a selective Sirt1 inhibitor, abrogated the beneficial effects of NMN on D-gal-induced colonic degeneration, as evidenced by the reversal of these improvements (Figure 9B; $p < 0.01$). This suggests that the protective effects of NMN on colonic architecture may be partially mediated through the Sirt1 pathway, a key regulator of cellular health and longevity. These findings highlight the potential therapeutic utility of NMN in mitigating age-related intestinal dysfunction and inflammation.

Discussion

NAD^+ is a critical coenzyme involved in redox reactions and functions as a vital cofactor for non-redox enzymes, including sirtuins and poly (ADP-ribose) polymerases (PARPs), which are essential for maintaining tissue homeostasis and metabolic balance. A growing body of evidence underscores the causal relationship between cellular NAD^+ levels and the aging processes of various tissues, such as the skin, blood, liver, muscle, and brain (Covarrubias et al., 2021). Aging is characterized by a progressive decline in NAD^+ concentrations across multiple human tissues, which is considered a key contributor to the onset of age-related diseases. Disruptions in metabolic states associated with aging can result in the depletion of NAD^+ levels, which, in turn, exacerbate the inflammatory response through the upregulation of pro-inflammatory cytokines. This elevation in cytokines triggers cellular and DNA damage, further activating NAD^+-consuming enzymes such as CD38 and PARP, leading to an accelerated depletion of NAD^+. In aged brain tissue, oxidative stress, mitochondrial dysfunction, and impaired autophagy contribute to increased DNA damage. Accumulated DNA damage activates PARP, further depleting NAD^+ levels within the nucleus. The consequent reduction in NAD^+ leads to diminished Sirt1 activity, which impairs mitochondrial function and exacerbates oxidative stress, creating a feedback loop of cellular damage. Furthermore, axonal degeneration, a precursor to numerous neurodegenerative diseases, is closely linked to a rapid decline in NAD^+ levels, attributed to decreased expression of the NAD^+ biosynthetic enzyme nicotinamide mononucleotide adenylyl transferase 2 (NMNAT2). Supplementation with nicotinamide mononucleotide (NMN) has been shown to increase NAD^+ levels, activate the Sirt1 pathway, and enhance the expression of the mitotic checkpoint kinase BubR1, extending lifespan in murine models (North et al., 2014). Similarly, a one-week NMN intervention in aged mice improved mitochondrial oxidative metabolism, restored mitochondrial homeostasis, and reversed skeletal muscle dysfunction, bringing muscle health to levels comparable to those of young mice (Gomes et al., 2013). These findings highlight the potential of NMN as a therapeutic strategy for combating age-related declines in NAD^+ and mitigating tissue dysfunction. In the current study, C57BL/6 mice were treated with D-gal for 8 weeks, inducing age-related phenotypic changes and confirming the successful establishment of an aging model. Administration of NMN over the same period ameliorated the senescence phenotype in D-gal-treated mice. Notably, body weight, a critical indicator of overall health, did not exhibit significant fluctuations in the NMN-treated groups, suggesting that long-term supplementation at a dose of 500 mg/kg/day was well-tolerated. However, the co-administration of NMN (500 mg/kg) and Ex527, a Sirt1 inhibitor, resulted in the slowest weight gain among the aging mice. This outcome is consistent with reports by Jonathan et al., who found that Ex527-induced Sirt1 inhibition increased p53 acetylation without affecting cell viability or gene expression in etoposide-treated cells (Solomon et al., 2006). Similarly, Kundu et al. demonstrated that Ex527 administration in high-fat diet-induced diabetic rats significantly reduced elevated blood glucose levels and kidney weight (Kundu et al., 2020). The precise effects of Ex527 on body weight remain to be elucidated. However, NMN supplementation notably improved neurological function and mitigated cognitive deficits induced by D-gal, with Sirt1-mediated pathways playing a pivotal role in the neuroprotective effects of NMN. However, we acknowledge that the AMPK-PGC-1α axis may crosstalk with other pathways (e.g., Nrf2/ARE, NF-κB). The potential synergistic involvement of other pathways cannot be excluded (Cheng et al., 2024; Salminen et al., 2011).

The elevation of NAD^+ levels has become a primary focus in aging research, with a growing recognition of its potential to slow tissue aging and preserve functional integrity. NAD^+ can be synthesized through three distinct pathways: the *de novo* synthesis pathway (also known as the kynurenine pathway), the Preiss-Handler pathway, and the salvage pathway. The *de novo* pathway, which occurs primarily in the liver, begins with dietary tryptophan. The Preiss-Handler pathway converts dietary niacin to NAD^+, while the salvage pathway is the principal route for NAD^+ biosynthesis in most mammalian cells. Through this pathway, nicotinamide (NAM) is converted into NMN by nicotinamide

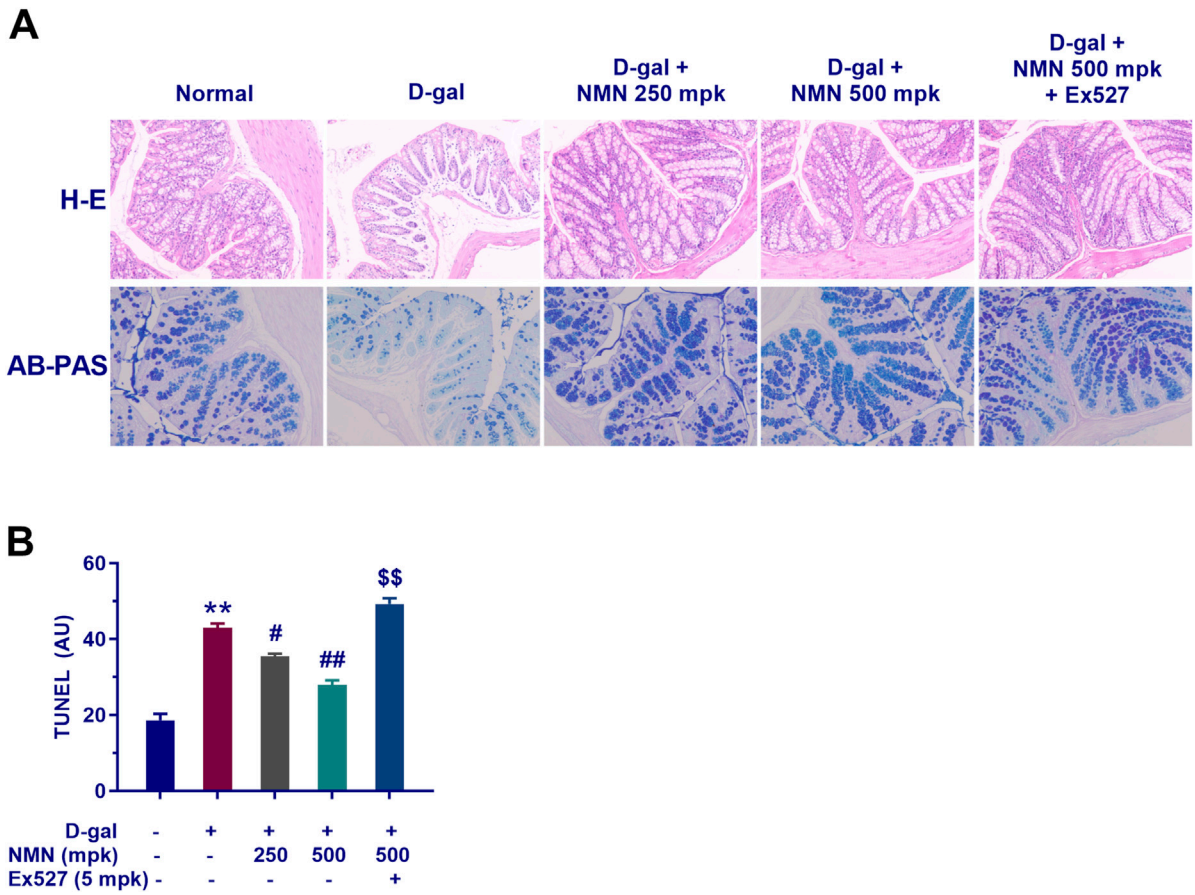


FIGURE 9
NMN exerts a protective effect on the intestinal mucosal barrier in aging mice. **(A)** Haematoxylin and eosin (HE) staining and Alcian Blue-Periodic Acid-Schiff (AB-PAS) staining of mouse colon sections, respectively. **(B)** The corresponding statistical results of AB-PAS staining. Each data point represents the mean ± SEM, with n = 3; **, $p < 0.01$, compared to the Normal group; #, $p < 0.05$, ##, $p < 0.01$, versus the D-gal group; $$, $p < 0.01$, in comparison to the NMN 500 mpk group.

phosphoribosyltransferase (NAMPT), and NMN is subsequently converted into NAD^+ by NMNAT. Therefore, direct supplementation with NMN is an effective strategy to enhance the salvage pathway (Song et al., 2023). NMN, a natural NAD^+ intermediate, is widely found in foods such as vegetables, fruits, and meats, with particularly high concentrations in plant-based foods such as soybeans, avocados, broccoli, cabbage, and cucumbers. While physiological amounts of NMN can be absorbed through dietary sources, supplemental NMN is often necessary under pathological conditions such as aging to counteract the decline in intracellular NAD^+ levels. The safety of NMN supplementation has been evaluated in both preclinical and clinical studies. In rodent models, administration of 500 mg/kg/day of NMN for 91 days did not result in adverse events, while a higher dose of 2000 mg/kg/day caused weight loss and reduced food intake (Turner et al., 2021). Long-term studies have shown that NMN supplementation in mice for 12 months is safe, inhibits age-related weight gain, improves energy metabolism, enhances physical activity, and increases insulin sensitivity (Mills et al., 2016). Similarly, nicotinamide riboside (NR), another NAD^+ precursor, has been shown to delay neural stem cell aging and extend lifespan in mice (Zhang et al., 2016). A Phase I clinical trial found that oral NR supplementation was safe and resulted in minor clinical improvements in Parkinson's disease patients (Brakedal et al., 2022). Furthermore, NR supplementation activates the mitochondrial unfolded protein response and enhances neurogenesis in amyotrophic lateral sclerosis (ALS) mouse models (Zhou et al., 2020). Beyond NAD^+ precursors, NAMPT activators such as P7C3 and its analog P7C3-A20 have been explored for their ability to enhance NAD^+ salvage pathway activity. These activators increase intracellular NAD^+ levels and offer neuroprotective effects in various animal models of neurodegeneration and injury (Wang et al., 2014). Additionally, natural compounds such as triterpenes from Panax notoginseng and flavonoids like apigenin have demonstrated neuroprotective effects by modulating NAD^+ levels (Xie et al., 2020; Escande et al., 2013). *Rehmannia glutinosa*, a traditional Chinese medicine, has also been shown to increase NAD^+ levels and enhance energy metabolism, contributing to its neuroprotective properties (Han et al., 2022).

Cognitive and memory decline associated with aging was reversed by NMN supplementation, as evidenced by OFT and MWM performance, consistent with previous reports (Hosseini et al., 2019; Liu et al., 2022). These findings suggest that NMN may exert neuroprotective effects and enhance cognitive function in aging mice, likely through modulation of sirtuin pathways and

NAD^+ levels. Neurodegenerative diseases, including AD, Parkinson's disease, and ALS, are characterized by oxidative stress, mitochondrial dysfunction, and neuroinflammation, all of which are exacerbated by aging. AGE formation, either through endogenous processes or dietary intake, is associated with cellular aging and plays a role in the pathogenesis of age-related diseases, contributing to oxidative stress and neuroinflammation. NMN has been shown to mitigate these effects by modulating oxidative stress markers (e.g., SOD, CAT) and inflammatory cytokines (e.g., TNF-α, IL-1β, IL-6), thus underscoring its potential in protecting against neurodegenerative diseases.

In addition to its effects on cognitive function, NMN supplementation ameliorates intestinal barrier dysfunction, a common age-related change, suggesting its broader impact on aging in both the gastrointestinal and central nervous systems (Huang et al., 2022). NMN enhances mitochondrial function by increasing NAD^+ levels, promoting cellular energy production, and maintaining intestinal epithelial integrity. This mechanism may be particularly beneficial in conditions such as inflammatory bowel disease, where impaired intestinal barrier function exacerbates inflammation. NMN-mediated activation of sirtuins further attenuates inflammation by deacetylating histones and transcription factors, thereby downregulating pro-inflammatory genes (Kiss et al., 2020). Moreover, NMN influences microbial metabolism and gut microbiota diversity, which plays a crucial role in immune modulation and gastrointestinal homeostasis. NMN supplementation has been shown to alter gut microbial composition and metabolome, including shifts in the abundance of beneficial bacteria such as *Helicobacter*, Lachnospiraceae, and *Faecalibacterium*, which are associated with improved nicotinamide metabolism (Niu et al., 2021).

Furthermore, NMN supplementation corrected age-related declines in neurotransmitter levels, particularly norepinephrine (NE), which plays a key role in regulating both neural function and intestinal barrier integrity. NE, as the primary neurotransmitter of the sympathetic nervous system, is crucial for maintaining the intestinal barrier, while GABAergic signaling in the enteric system promotes intestinal defense and immunity (Deng et al., 2023; Lee et al., 2005; You et al., 2021). These findings highlight the intricate interplay between neurotransmitters, NAD^+ metabolism, and intestinal health, suggesting that NMN may offer therapeutic benefits through these interconnected pathways.

The study demonstrates that NMN supplementation in D-gal-induced aging mice improves cognitive function, mitochondrial health, and intestinal barrier integrity, largely via Sirt1 activation and NAD^+ restoration. Translating these findings to humans requires cautious optimism, considering both conserved biological pathways and interspecies differences. NAD^+ metabolism and sirtuin pathways are evolutionarily conserved, suggesting similar benefits in humans. Clinical trials with NAD^+ precursors like NR have shown safety and modest efficacy in neurodegenerative diseases (e.g., Parkinson's) and metabolic disorders, supporting the potential of NMN (Brakedal et al., 2022). D-gal-induced aging mimics accelerated oxidative stress and inflammation but does not fully replicate natural aging. Human aging involves multifactorial processes (e.g., epigenetics, chronic diseases), which may alter NMN's efficacy. Phase I trials with NR show promise, but NMN-specific human data are limited. Although the clinical trial reported NR's safety in Parkinson's patients, hinting at NMN's potential. However, long-term studies are needed to confirm benefits on cognition and longevity. NMN is naturally present in foods (e.g., broccoli, avocado), suggesting low toxicity, but synthetic supplementation's long-term effects remain unproven. Regulatory approval and standardization (e.g., purity, bioavailability) are critical for clinical translation. Human equivalents require careful scaling, as high doses in mice (2000 mg/kg) caused adverse effects, though lower doses in humans (e.g., 250–1,000 mg/day) have been tolerated in early trials (Yoshino et al., 2018).

Long-term NMN supplementation is more necessary for addressing age-related clinical symptoms. A 12-month NMN study in mice inhibited age-related weight gain, improved insulin sensitivity, and enhanced physical activity without adverse effects, indicating sustained benefits (Mills et al., 2016). Similarly, NR extended lifespan in mice by delaying (Zhang et al., 2016). These findings suggest that prolonged NMN use may amplify benefits, particularly in mitigating chronic age-related pathologies. High-dose NMN (2000 mg/kg) in rodents caused weight loss and reduced food intake, underscoring the need for dose optimization (Turner et al., 2021). Chronic use might theoretically downregulate endogenous NAD^+ synthesis (e.g., NAMPT activity), though no evidence currently supports this. Aging in humans spans decades, necessitating decades-long studies to assess NMN's impact on longevity and age-related diseases. Short-term human trials (≤12 weeks) show NAD^+ elevation and metabolic improvements, but longer trials are needed to evaluate cognitive preservation, cancer risk, and organ-specific effects (Chen et al., 2024). NMN's modulation of gut microbiota (e.g., increased Faecalibacterium) and intestinal barrier function may have systemic anti-aging effects (Huang et al., 2022). Long-term studies should explore whether these changes reduce inflammation-driven pathologies like Alzheimer's or inflammatory bowel disease. Co-administration with NAMPT activators (e.g., P7C3) or lifestyle interventions (e.g., exercise, caloric restriction) could synergize with NMN to enhance NAD^+ levels, warranting investigation in extended studies (Das et al., 2018; Khaidizar et al., 2021; Wang et al., 2021).

Despite the promising results, the current study has certain limitations. The exact mechanisms by which NMN crosses the blood-brain barrier and its direct effects on neuronal cells require further investigation. Additionally, long-term clinical studies are needed to explore the dose-response relationships, potential side effects, and individual variability in response to NMN supplementation. These studies will be critical in optimizing the clinical application of NMN for aging-related diseases.

In conclusion, NMN supplementation improves cognitive function and mitigates age-related intestinal barrier dysfunction in mice. These effects are likely mediated through the correction of oxidative stress, reduction of inflammation, and modulation of senescence and apoptosis-related pathways. The present study suggests that NMN holds significant therapeutic potential for the treatment of aging-associated diseases, including neurodegenerative diseases and impaired intestinal barrier function. However, the regulation of related signaling pathways by Sirt1 underscores the need for further exploration of these mechanisms to fully leverage the therapeutic potential of NMN.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was approved by Animal Care and Use Committee of the Nanjing University of Chinese Medicine. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

YL: Investigation, Writing–original draft. YW: Investigation, Writing–original draft. XY: Writing–original draft, Data curation, Formal Analysis. ZD: Data curation, Formal Analysis, Writing–review and editing. MH: Data curation, Formal Analysis, Writing–review and editing, Investigation. XH: Data curation, Formal Analysis, Investigation, Writing–review and editing. QZ: Investigation, Writing–original draft. YY: Conceptualization, Funding acquisition, Supervision, Validation, Writing–review and editing.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. This project was sponsored by the "Leading Goose" R&D Program of Zhejiang (2022C03145) and the Postgraduate Research & Practice Innovation Program of Jiangsu Province (SJCX22_1314).

Acknowledgments

We also thank Yuyao Lifespan Health Technology Co., Ltd. for providing NMN.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declare that no Generative AI was used in the creation of this manuscript.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

Brakedal, B., Dolle, C., Riemer, F., Ma, Y., Nido, G. S., Skeie, G. O., et al. (2022). The NADPARK study: a randomized phase I trial of nicotinamide riboside supplementation in Parkinson's disease. *Cell Metab.* 34 (3), 396–407 e6. doi:10.1016/j.cmet.2022.02.001

Byrns, C. N., Perlegos, A. E., Miller, K. N., Jin, Z., Carranza, F. R., Manchandra, P., et al. (2024). Senescent glia link mitochondrial dysfunction and lipid accumulation. *Nature* 630 (8016), 475–483. doi:10.1038/s41586-024-07516-8

Chen, F., Zhou, D., Kong, A. P., Yim, N. T., Dai, S., Chen, Y. N., et al. (2024). Effects of nicotinamide mononucleotide on glucose and lipid metabolism in adults: a systematic review and meta-analysis of randomised controlled trials. *Curr. Diab Rep.* 25 (1), 4. doi:10.1007/s11892-024-01557-z

Cheng, D., Zhang, M., Zheng, Y., Wang, M., Gao, Y., Wang, X., et al. (2024). α-Ketoglutarate prevents hyperlipidemia-induced fatty liver mitochondrial dysfunction and oxidative stress by activating the AMPK-pc-1α/Nrf2 pathway. *Redox Biol.* 74, 103230. doi:10.1016/j.redox.2024.103230

Connell, E., Le Gall, G., Pontifex, M. G., Sami, S., Cryan, J. F., Clarke, G., et al. (2022). Microbial-derived metabolites as a risk factor of age-related cognitive decline and dementia. *Mol. Neurodegener.* 17 (1), 43. doi:10.1186/s13024-022-00548-6

Covarrubias, A. J., Perrone, R., Grozio, A., and Verdin, E. (2021). NAD(+) metabolism and its roles in cellular processes during ageing. *Nat. Rev. Mol. Cell Biol.* 22 (2), 119–141. doi:10.1038/s41580-020-00313-x

Das, A., Huang, G. X., Bonkowski, M. S., Longchamp, A., Li, C., Schultz, M. B., et al. (2018). Impairment of an endothelial NAD(+)-H(2)S signaling network is a reversible cause of vascular aging. *Cell* 173 (1), 74–89 e20. doi:10.1016/j.cell.2018.02.008

Deng, Z., Li, D., Yan, X., Lan, J., Han, D., Fan, K., et al. (2023). Activation of GABA receptor attenuates intestinal inflammation by modulating enteric glial cells function through inhibiting NF-κB pathway. *Life Sci.* 329, 121984. doi:10.1016/j.lfs.2023.121984

Escande, C., Nin, V., Price, N. L., Capellini, V., Gomes, A. P., Barbosa, M. T., et al. (2013). Flavonoid apigenin is an inhibitor of the NAD+ ase CD38: implications for cellular NAD+ metabolism, protein acetylation, and treatment of metabolic syndrome. *Diabetes* 62 (4), 1084–1093. doi:10.2337/db12-1139

Farrelly, A., and Daly, L. (2024). Older persons' experiences of frailty: a systematic review. *Int. J. Older People Nurs.* 19 (3), e12611. doi:10.1111/opn.12611

Fujita, Y., and Yamashita, T. (2018). Sirtuins in neuroendocrine regulation and neurological diseases. *Front. Neurosci.* 12, 778. doi:10.3389/fnins.2018.00778

Ge, P. Y., Qu, S. Y., Ni, S. J., Yao, Z. Y., Qi, Y. Y., Zhao, X., et al. (2023). Berberine ameliorates depression-like behavior in CUMS mice by activating TPH1 and inhibiting IDO1-associated with tryptophan metabolism. *Phytother. Res.* 37 (1), 342–357. doi:10.1002/ptr.7616

Gomes, A. P., Price, N. L., Ling, A. J., Moslehi, J. J., Montgomery, M. K., Rajman, L., et al. (2013). Declining NAD(+) induces a pseudohypoxic state disrupting nuclear-mitochondrial communication during aging. *Cell* 155 (7), 1624–1638. doi:10.1016/j.cell.2013.11.037

Han, G., Zhen, W., Dai, Y., Yu, H., Li, D., and Ma, T. (2022). Dihuang-yinzi alleviates cognition deficits via targeting energy-related metabolism in an alzheimer mouse model as demonstrated by integration of metabolomics and network pharmacology. *Front. Aging Neurosci.* 14, 873929. doi:10.3389/fnagi.2022.873929

Heneka, M. T., van der Flier, W. M., Jessen, F., Hoozemanns, J., Thal, D. R., Boche, D., et al. (2024). Neuroinflammation in alzheimer disease. *Nat. Rev. Immunol.* doi:10.1038/s41577-024-01104-7

Hosseini, L., Farokhi-Sisakht, F., Badalzadeh, R., Khabbaz, A., Mahmoudi, J., and Sadigh-Eteghad, S. (2019). Nicotinamide mononucleotide and melatonin alleviate aging-induced cognitive impairment via modulation of mitochondrial function and apoptosis in the prefrontal cortex and Hippocampus. *Neuroscience* 423, 29–37. doi:10.1016/j.neuroscience.2019.09.037

Huang, P., Wang, X., Wang, S., Wu, Z., Zhou, Z., Shao, G., et al. (2022). Treatment of inflammatory bowel disease: potential effect of NMN on intestinal barrier and gut microbiota. *Curr. Res. Food Sci.* 5, 1403–1411. doi:10.1016/j.crfs.2022.08.011

Kalinkovich, A., and Livshits, G. (2024). The cross-talk between the cGAS-STING signaling pathway and chronic inflammation in the development of musculoskeletal disorders. *Ageing Res. Rev.* 104, 102602. doi:10.1016/j.arr.2024.102602

Khaidizar, F. D., Bessho, Y., and Nakahata, Y. (2021). Nicotinamide phosphoribosyltransferase as a key molecule of the aging/senescence process. *Int. J. Mol. Sci.* 22 (7), 3709. doi:10.3390/ijms22073709

Kiss, T., Nyul-Toth, A., Balasubramanian, P., Tarantini, S., Ahire, C., Yabluchanskiy, A., et al. (2020). Nicotinamide mononucleotide (NMN) supplementation promotes neurovascular rejuvenation in aged mice: transcriptional footprint of SIRT1 activation, mitochondrial protection, anti-inflammatory, and anti-apoptotic effects. *Geroscience* 42 (2), 527–546. doi:10.1007/s11357-020-00165-5

Kong, X., Lyu, W., Lin, X., Lin, C., Feng, H., Xu, L., et al. (2024). Itaconate alleviates anesthesia/surgery-induced cognitive impairment by activating a Nrf2-dependent anti-neuroinflammation and neurogenesis via gut-brain axis. *J. Neuroinflammation* 21 (1), 104. doi:10.1186/s12974-024-03103-w

Kundu, A., Richa, S., Dey, P., Kim, K. S., Son, J. Y., Kim, H. R., et al. (2020). Protective effect of EX-527 against high-fat diet-induced diabetic nephropathy in Zucker rats. *Toxicol. Appl. Pharmacol.* 390, 114899. doi:10.1016/j.taap.2020.114899

Ledford, H. (2024). Human body's ageing 'clock' ticks faster after heat stress. *Nature* 636, 534. doi:10.1038/d41586-024-04007-8

Lee, J., Song, H. O., Jee, C., Vanoaica, L., and Ahnn, J. (2005). Calcineurin regulates enteric muscle contraction through EXP-1, excitatory GABA-gated channel, in *C. elegans*. *J. Mol. Biol.* 352 (2), 313–318. doi:10.1016/j.jmb.2005.07.032

Li, C., Zhao, X., Xu, H., Liu, X., He, Y., and Gu, J. (2024). NMN synbiotics: a multifaceted therapeutic approach for alzheimer's disease. *Neurochem. Res.* 49 (10), 2888–2896. doi:10.1007/s11064-024-04210-z

Liu, X., Dilxat, T., Shi, Q., Qiu, T., and Lin, J. (2022). The combination of nicotinamide mononucleotide and lycopene prevents cognitive impairment and attenuates oxidative damage in D-galactose induced aging models via Keap1-Nrf2 signaling. *Gene* 822, 146348. doi:10.1016/j.gene.2022.146348

Mills, K. F., Yoshida, S., Stein, L. R., Grozio, A., Kubota, S., Sasaki, Y., et al. (2016). Long-term administration of nicotinamide mononucleotide mitigates age-associated physiological decline in mice. *Cell Metab.* 24 (6), 795–806. doi:10.1016/j.cmet.2016.09.013

Niu, K. M., Bao, T., Gao, L., Ru, M., Li, Y., Jiang, L., et al. (2021). The impacts of short-term NMN supplementation on serum metabolism, fecal microbiota, and telomere length in pre-aging phase. *Front. Nutr.* 8, 756243. doi:10.3389/fnut.2021.756243

North, B. J., Rosenberg, M. A., Jeganathan, K. B., Hafner, A. V., Michan, S., Dai, J., et al. (2014). SIRT2 induces the checkpoint kinase BubR1 to increase lifespan. *EMBO J.* 33 (13), 1438–1453. doi:10.15252/embj.201386907

Packer, M. (2020). Longevity genes, cardiac ageing, and the pathogenesis of cardiomyopathy: implications for understanding the effects of current and future treatments for heart failure. *Eur. Heart J.* 41 (39), 3856–3861. doi:10.1093/eurheartj/ehaa360

Rajman, L., Chwalek, K., and Sinclair, D. A. (2018). Therapeutic potential of NAD-boosting molecules: the *in vivo* evidence. *Cell Metab.* 27 (3), 529–547. doi:10.1016/j.cmet.2018.02.011

Riessland, M., Ximerakis, M., Jarjour, A. A., Zhang, B., and Orr, M. E. (2024). Therapeutic targeting of senescent cells in the CNS. *Nat. Rev. Drug Discov.* 23 (11), 817–837. doi:10.1038/s41573-024-01033-z

Salminen, A., Hyttinen, J. M., and Kaarniranta, K. (2011). AMP-activated protein kinase inhibits NF-κB signaling and inflammation: impact on healthspan and lifespan. *J. Mol. Med. Berl.* 89 (7), 667–676. doi:10.1007/s00109-011-0748-0

Salminen, A., Kauppinen, A., and Kaarniranta, K. (2015). 2-Oxoglutarate-dependent dioxygenases are sensors of energy metabolism, oxygen availability, and iron homeostasis: potential role in the regulation of aging process. *Cell Mol. Life Sci.* 72 (20), 3897–3914. doi:10.1007/s00018-015-1978-z

Satoh, A., Imai, S. I., and Guarente, L. (2017). The brain, sirtuins, and ageing. *Nat. Rev. Neurosci.* 18 (6), 362–374. doi:10.1038/nrn.2017.42

Sies, H., and Jones, D. P. (2020). Reactive oxygen species (ROS) as pleiotropic physiological signalling agents. *Nat. Rev. Mol. Cell Biol.* 21 (7), 363–383. doi:10.1038/s41580-020-0230-3

Solomon, J. M., Pasupuleti, R., Xu, L., McDonagh, T., Curtis, R., DiStefano, P. S., et al. (2006). Inhibition of SIRT1 catalytic activity increases p53 acetylation but does not alter cell survival following DNA damage. *Mol. Cell Biol.* 26 (1), 28–38. doi:10.1128/MCB.26.1.28-38.2006

Song, Q., Zhou, X., Xu, K., Liu, S., Zhu, X., and Yang, J. (2023). The safety and antiaging effects of nicotinamide mononucleotide in human clinical trials: an update. *Adv. Nutr.* 14 (6), 1416–1435. doi:10.1016/j.advnut.2023.08.008

Turner, J., Licollari, A., Mihalcea, E., and Tan, A. (2021). Safety evaluation for Restoring® NMN, a NAD+ precursor. *Front. Pharmacol.* 12, 749727. doi:10.3389/fphar.2021.749727

Wang, G., Han, T., Nijhawan, D., Theodoropoulos, P., Naidoo, J., Yadavalli, S., et al. (2014). P7C3 neuroprotective chemicals function by activating the rate-limiting enzyme in NAD salvage. *Cell* 158 (6), 1324–1334. doi:10.1016/j.cell.2014.07.040

Wang, Y. J., Paneni, F., Stein, S., and Matter, C. M. (2021). Modulating sirtuin biology and nicotinamide adenine diphosphate metabolism in cardiovascular disease-from bench to bedside. *Front. Physiol.* 12, 755060. doi:10.3389/fphys.2021.755060

Wu, W., Yuan, S., Tang, Y., Meng, X., Peng, M., Hu, Z., et al. (2023). Effect of exercise and oral niacinamide mononucleotide on improving mitochondrial autophagy in alzheimer's disease. *Nutrients* 15 (13), 2851. doi:10.3390/nu15132851

Xie, W., Zhu, T., Zhou, P., Xu, H., Meng, X., Ding, T., et al. (2020). Notoginseng leaf triterpenes ameliorates OGD/R-Induced neuronal injury via SIRT1/2/3-foxo3a-MnSOD/PGC-1α signaling pathways mediated by the NAMPT-NAD pathway. *Oxid. Med. Cell Longev.* 2020, 7308386. doi:10.1155/2020/7308386

Yoshino, J., Baur, J. A., and Imai, S. I. (2018). NAD(+) intermediates: the biology and therapeutic potential of NMN and NR. *Cell Metab.* 27 (3), 513–528. doi:10.1016/j.cmet.2017.11.002

You, X. Y., Zhang, H. Y., Han, X., Wang, F., Zhuang, P. W., and Zhang, Y. J. (2021). Intestinal mucosal barrier is regulated by intestinal tract neuro-immune interplay. *Front. Pharmacol.* 12, 659716. doi:10.3389/fphar.2021.659716

Zhang, H., Ryu, D., Wu, Y., Gariani, K., Wang, X., Luan, P., et al. (2016). NAD⁺ repletion improves mitochondrial and stem cell function and enhances life span in mice. *Science* 352 (6292), 1436–1443. doi:10.1126/science.aaf2693

Zhao, X., Ge, P., Lei, S., Guo, S., Zhou, P., Zhao, L., et al. (2023). An exosome-based therapeutic strategy targeting neuroinflammation in alzheimer's disease with berberine and palmatine. *Drug Des. Devel Ther.* 17, 2401–2420. doi:10.2147/DDDT.S417465

Zhou, Q., Zhu, L., Qiu, W., Liu, Y., Yang, F., Chen, W., et al. (2020). Nicotinamide riboside enhances mitochondrial proteostasis and adult neurogenesis through activation of mitochondrial unfolded protein response signaling in the brain of ALS SOD1(G93A) mice. *Int. J. Biol. Sci.* 16 (2), 284–297. doi:10.7150/ijbs.38487

frontiers | Frontiers in Pharmacology

TYPE Original Research
PUBLISHED 17 April 2025
DOI 10.3389/fphar.2025.1574323

Check for updates

OPEN ACCESS

EDITED BY
Huazheng Liang,
Monash University - Southeast University Joint Research Institute (Suzhou), China

REVIEWED BY
Zeliha Selamoglu,
Niğde Ömer Halisdemir University, Türkiye
Liz Girardi Müller,
Regional Community University of Chapecó, Brazil

*CORRESPONDENCE
Sajad Fakhri,
pharmacy.sajad@yahoo.com,
sajad.fakhri@kums.ac.ir
Javier Echeverría,
javier.echeverriam@usach.cl

RECEIVED 10 February 2025
ACCEPTED 31 March 2025
PUBLISHED 17 April 2025

CITATION
Zarneshan SN, Fakhri S, Kiani A, Abbaszadeh F, Hosseini SZ, Mohammadi-Noori E and Echeverría J (2025) Polydatin attenuates Alzheimer's disease induced by aluminum chloride in rats: evidence for its antioxidant and anti-inflammatory effects.
Front. Pharmacol. 16:1574323.
doi: 10.3389/fphar.2025.1574323

COPYRIGHT
© 2025 Zarneshan, Fakhri, Kiani, Abbaszadeh, Hosseini, Mohammadi-Noori and Echeverría. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

Polydatin attenuates Alzheimer's disease induced by aluminum chloride in rats: evidence for its antioxidant and anti-inflammatory effects

Seyede Nazanin Zarneshan[1], Sajad Fakhri[2]*, Amir Kiani[2,3], Fatemeh Abbaszadeh[4], Seyede Zahra Hosseini[1], Ehsan Mohammadi-Noori[2] and Javier Echeverría[5]*

[1]Student Research Committee, Kermanshah University of Medical Sciences, Kermanshah, Iran, [2]Pharmaceutical Sciences Research Center, Health Institute, Kermanshah University of Medical Sciences, Kermanshah, Iran, [3]Regenerative Medicine Research Center, Health Technology Institute, Kermanshah University of Medical Sciences, Kermanshah, Iran, [4]Neurobiology Research Center, Institute of Neuroscience and Cognition, Shahid Beheshti University of Medical Sciences, Tehran, Iran, [5]Departamento de Ciencias del Ambiente, Facultad de Química y Biología, Universidad de Santiago de Chile, Santiago, Chile

Background: Considering the complex pathophysiological mechanisms behind Alzheimer's disease (AD), a few drugs for managing related cognitive symptoms have been approved. The phytochemical resveratrol has shown promising anti-inflammatory and antioxidant effects in AD, but it has low bioavailability. Chemical modification of resveratrol to its glycosylated form, polydatin (PD), significantly increases its bioavailability and bioactivity.

Purpose: The study aimed to investigate the therapeutic potential and mechanisms of action of PD against AD in rats.

Material and method: AD was caused by an intraperitoneal (i.p.) administration of aluminum chloride ($AlCl_3$). Six groups of six rats each were defined as sham, negative control ($AlCl_3$), positive control (Donepezil), and treatments (PD 5, 10, and 20 mg/kg, i.p.). On days 7, 8, 14, and 15, the rats' behavioral changes were assessed by the open field, Y-maze test, passive avoidance test, and elevated plus maze tests. At the end of the study, the blood samples were collected to assess the levels of glutathione (GSH), catalase (CAT), and nitrite, as well as the activity of matrix metalloproteinases (MMPs). Furthermore, hippocampal brain tissue was removed and used for histological investigations.

Results and discussion: The findings revealed that PD injections at three different doses (5, 10, and 20 mg/kg) improved cognitive and other behavioral impairments. Furthermore, PD improved the antioxidant capacity by increasing GSH and CAT while decreasing serum nitrite levels. PD showed anti-inflammatory effects by reducing the activity of inflammatory MMP-9, while elevating the activity of anti-inflammatory MMP-2. PD also modulated pathogenic changes in the hippocampal brain tissue.

Conclusion: PD alleviated cognitive and other behavioral impairments in AD rats by enhancing antioxidant defenses and reducing neuroinflammation.

KEYWORDS

Alzheimer's disease, aluminum chloride, polydatin, inflammation, matrix metalloproteinase, oxidative stress, catalase, glutathione

1 Introduction

Dementia is a clinical situation defined as gradual dysfunction in memory, behavior, language, cognitive/visuospatial function, and personality, leading to the impairment of the ability to perform daily tasks (Crous-Bou et al., 2017). As the most prevalent dementia reason, Alzheimer's disease (AD) has been a critically costly, burdensome, and fatal disease of the 21st century (Scheltens et al., 2021). AD is a very sophisticated and advanced neurodegenerative condition with multiple pathophysiological mechanisms (Querfurth and LaFerla, 2010). Amyloid beta (Aβ) and amyloid precursor protein (APP) are transmembrane proteins generally found in synapses. They are cleaved by α-secretase, followed by γ-secretase, and processed and removed appropriately in healthy non-amyloidogenic situations. Tau acts as a microtubule-stabilizing protein in neuronal axons in the healthy condition (Eratne et al., 2018). Extracellular Aβ plaque aggregates and intracellular neurofibrillary tangles (NFTs), which are made of hyperphosphorylated tau linked with microtubules, are thought to be the histological features of AD (Goedert, 2015). Aβ pathology is in a near interconnection with the inflammatory hypothesis in AD. Matrix metalloproteinases (MMPs) are among the proteins receiving increased attention for their function in neuroinflammation and neurodegeneration. MMP-2 and MMP-9 break Aβ monomers and oligomers, causing neurotoxicity (Iranpanah et al., 2024). AD has been related to iron accumulation and brain iron dysregulation, which interact with tau and Aβ pathologies, resulting in oxidative damage and reactive oxygen species, promoting neurodegeneration. Free radical buildup leads to synaptic dysfunction, inflammation, mitochondrial damage, oxidative stress, neurotransmitter deficits (particularly cholinergic), microglial activation, and, finally, neuronal death (Eratne et al., 2018). Critical neuroprotective mechanisms of active compounds have also been shown to be connected with antioxidant defense mechanisms (Ishak et al., 2024). Regulation of oxidative/antioxidative mediators (e.g., nitric oxide synthase) is also correlated with protective effects against brain and peripheral damage (Selamoglu-Talas et al., 2015; Sureda et al., 2023).

Substantial studies have focused on the possible involvement of aluminum (Al) in the AD process, owing to its recognized numerous and powerful neurotoxic effects. Al is the most common natural neurotoxic metal to which we are exposed (Bhattacharjee et al., 2014). As a result, identifying pharmaceuticals or natural compounds that protect against Al-mediated neuronal cell death is a potent method for neurodegenerative disorder prevention and treatment (Anwar et al., 2021).

The amount of fatalities from AD has climbed dramatically during the last 20 years, yet a cure is still unachievable. FDA-approved therapies for AD have not demonstrated a curative impact, but generally neither do they slow the pace of disease progression (Singh et al., 2024). For patients with AD dementia, cholinesterase inhibitors (e.g., physostigmine) and *N*-methyl-D-aspartate (NMDA) antagonists (e.g., memantine) are approved drugs (Mossello and Ballini, 2012). The currently authorized medications operate on a single target, however, importance is being given to numerous therapeutic techniques for designing and developing treatments that can impact several targets (Athar et al., 2021). As a result, there is a growing demand for innovative multi-target medicines. Numerous natural chemicals derived from edible and medicinal plants have been studied for possible pharmacological use. Natural substances are high in polyphenolic substances such as stilbenoids with antioxidant potentials (Fakhri et al., 2021a). Natural stilbenes have received far less attention (Freyssin et al., 2020). The antioxidant capacity of stilbene polyphenols has been linked with neuroprotective mechanisms (Sureda et al., 2023). However, several stilbenes polyphenols have been characterized solely in terms of their *in vitro* actions. In contrast, resveratrol has been extensively studied for its benefits both *in vitro* and *in vivo*, in murine and rat models, and even in clinical trials of AD (Witte et al., 2014; Moussa et al., 2017; Cicero et al., 2019; Ma et al., 2019; Broderick et al., 2020). However, because resveratrol undergoes substantial hepatic metabolism and is quickly eliminated by the kidneys, its bioavailability is limited (Gambini et al., 2015). Due to resveratrol's limited bioavailability, newer molecules like its homolog (3,4′,5-trihydroxystilbene-3-β-D-glucoside, commonly called polydatin (PD), should be investigated. PD, is a glucoside derivative of resveratrol. The substitution of a hydroxyl group for a glucose unit distinguishes it from resveratrol (Tang, 2021). PD's putative protein targets have been discovered. PD can penetrate the blood-brain barrier (BBB), which is critical in the treatment of brain disorders (Pan et al., 2018; Tang and Tan, 2019). In comparison to its counterpart, resveratrol, research on PD's preventive function against AD is limited (Tang, 2021). We also previously reported the neuroprotective effects of PD amphiphilic chitosan nanocarriers in a rat model of AD (Zarneshan et al., 2025).

This research aims to investigate the potential mechanisms of action of PD against AD in an aluminum chloride-induced AD rat model.

2 Materials and methods

2.1 Animals

Male Albino Wistar rats (weighing 220–250 g) were obtained from the central animal house at Kermanshah University of Medical Sciences (KUMS). They were kept in an environment with an *ad libitum* supply of food and water, a standard light/dark cycle (i.e., 12 h/12 h), a relative temperature of 24°C ± 2°C, and a proper humidity (i.e., 60% ± 5%). All the experimental protocols were done based on the guidelines of the institutional animal care and ethics of KUMS (IR.KUMS.AEC.1401.050).

For 14 days, thirty-six rats were split up into six groups of six rats each. The sample size was calculated using the modified resource equation method (Arifin and Zahiruddin, 2017). The sham group just received intraperitoneal (i.p.) saline. The negative control group received $AlCl_3$ (100 mg/kg, i.p.) and i.p. saline. The positive control group received donepezil (10 mg/kg, i.p.) after $AlCl_3$ (100 mg/kg,

Abbreviations: Aβ, amyloid beta; AD, Alzheimer's disease; $AlCl_3$, aluminum chloride; BBB, blood-brain barrier; CAT, catalase; GSH, glutathione; H&E, Hematoxylin and Eosin; i.p., intraperitoneal; ITL, initial transfer latency time; MMPs, matrix metalloproteinase; NFTs, neurofibrillary tangles; PD, polydatin; STL, step through latency time.

i.p.). Groups IV, V, and VI received $AlCl_3$ (100 mg/kg, i.p.) followed by three doses of PD (i.e., 5, 10, and 15 mg/kg, i.p.). The animals' behavioral analyses were all done during the study timeline. The rats were subsequently sacrificed, and blood samples were used to measure serum levels of nitrite, glutathione (GSH), and catalase (CAT). In addition, the activity of MMP-2 and MMP-9 was measured, and hippocampal brain tissue was extracted and utilized for histopathological studies. The rats were assigned to groups using randomization and blinding in animal assignments, behavioral assessments, and biochemical analyses (Zarneshan et al., 2025).

2.2 Chemicals

Polydatin, donepezil, ammonium molybdate, aluminum chloride ($AlCl_3$), sodium nitrite, *N*-(1-naphthyl) ethylenediamine dihydrochloride (NED), sodium dodecyl sulfate (SDS), 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB), coomassie brilliant blue G-250, acrylamide, and Triton X-100 were purchased from Sigma-Aldrich (St. Louis, MO).

2.3 Behavioral studies

2.3.1 Open field test

The open field test is a common laboratory method designed to evaluate general locomotion and anxiety-related behavior (Lu et al., 2019). Depending on the animal being tested, it consists of a wall enclosed (high sufficient to prevent escape), circular, square, or rectangular, unknown space that is large enough to provide the impression of openness. It is possible to assess a range of activities, such as the number of squares that the rats traversed, rearing, and grooming (Vuralli et al., 2019).

2.3.2 Passive avoidance test

According to the association formed between a particular unpleasant event and a certain environmental setting, the passive avoidance task is a fear-motivated test used to evaluate fear-based conditioned avoidance learning and memory/behavior in rats (Giménez De Béjar et al., 2017). In this project, normal animals come to the recognition that staying away from a specific location will help them prevent an unpleasant event. This test is carried out in two compartments of similar size, one of which is bright (white and lit by a 24 V-10 W bulb), and the other of which is dark (black and dark), both of which are divided by a gate. The acquisition (training) phase and the test phase were the two stages of testing. Throughout the initial training phase, animals were placed in the illuminated box and permitted a maximum of 5 min to pass the gate and enter the dark box. Animals accessing the dark box with the gate closed suffered a 2-s foot shock. Following the foot shock, each animal was allowed 20 s in the dark cage to acclimate (0.5 mA). After 20 s, the animals were taken away from the apparatus and placed back in their cages. On day two of the test phase, testing was conducted in the same manner as on day one, but without the use of a foot shock in the dark box (Wu et al., 2020).

2.3.3 Y-maze test

The Y-maze is a standard method for testing spatial memory. Three arms diverge at a 120° angle from the device's center. Throughout the test, the baffles of two randomly selected arms were opened. The start arm is preferred by the two, and the new arm is allocated to the arm with the baffle still attached (Shi et al., 2021). Beginning by assigning the letters A, B, and C to the maze's three arms, then start recording. Next, consider the overall number of arm entries and alternations recorded. Finally, use the following formula to determine the percent (%) alternation: % Alternation (SAP) = (Number of Alternations/ [Total number of arm entries − 2]) × 100 (Kraeuter et al., 2019).

2.3.4 Elevated plus maze test

The elevated plus-maze is the most frequently employed task to study anxiety-like behavior overall, and in transgenic models of AD in particular (Pentkowski et al., 2021). The testing apparatus looks like a cross and has two arms. The arm enclosed by walls is known as the contained arm, and the arm without walls is known as the open arm (MacLellan et al., 2009). Rats were placed at the center of defined arms and allowed to explore for 5 min. Spent time in open arms, overall distance traveled, transfer latency, and initial transfer latency (ITL) were recorded (Zarneshan et al., 2025).

2.4 Biochemical tests

2.4.1 Glutathione assay

GSH was determined using Ellman's assay (Ellman, 1959). In this research, each well received 50 mL of phosphate buffer with a pH of 7.4 and 60 mL of rat serum sample. Following the addition of 100 mL of DTNB, a yellow complex was seen, which was then incubated for 10 min at 37°C before the absorbance at 412 nm was detected using an ELISA reader. Lastly, the percentage of optical absorption groups that differed from the reference group (healthy rats) was reported.

2.4.2 Catalase assay

In this experiment, CAT changes using the Aebi method were evaluated by measuring hydrogen peroxide (Aebi, 1980). First 20 mL of this study's serum samples from the rats in each group were added to each well, followed by the addition of 100 mL of hydrogen peroxide at a concentration of 65 mM, 4 min of incubation at 25°C, and the addition of 100 mL of ammonium molybdate at a concentration of 32.4 mM to terminate the reaction. Finally, using an ELISA reader, the yellow molybdate and hydrogen peroxide complex were quantified at 405 nm. Eventually, the control group's optical absorption % was detected to be different from the other groups (Zarneshan et al., 2025).

2.4.3 Nitrite assay

Nitrite was measured using the Griess method (Granger et al., 1995; 1996) through a deproteinization by adding zinc sulfate powder. First, sulfanilamide (in 5% HCl) and naphthyl ethylene diamine dihydrochloride (NEDD; 0.1% in distilled water) were prepared. In a 96-well plate, 50 µL of sulfanilamide solution was combined with 100 µL of the prepared serum samples and incubated in the dark for 5 min. After that, 50 µL of NEDD solution was added, and incubation was done at 37°C for 30 min. After that, the purple complex was formed, and its absorbance was measured at 540 nm using a plate reader. Lastly, the concentration of nitrite in the serum of rats was reported in mM (Zamani et al., 2025).

2.5 Zymography

Gelatin zymography was used to measure the gelatinolytic activity of MMPs. Polyacrylamide gels containing sodium dodecyl sulphate were copolymerized with 0.1% gelatin. Following the loading of blood samples (equal to 100 μg of total protein content as determined by the Bradford assay), electrophoresis was performed using a mini-gel slab device called the Mini Protean III (Bio-Rad, Hercules, CA) at a fixed voltage of 150 V. The gel was shaken for an hour while being washed in renaturation buffer with 2.5% Triton X-100 (in 50 mM Tris-HCl). Incubation buffer containing 10 mM $CaCl_2$, 0.02% NaN_3, and 0.15 M NaCl in 50 mM Tris-HCl was then used to incubate the gel for 18 h at 37°C. After incubation, the gels were stained for 40 min with Coomassie brilliant blue G-250 (0.5% in 10% acetic acid and 30% methanol) and then destained with a solution of distilled water, glacial acetic acid, and methanol for 60 min. ImageJ® software (NIH) was used to analyze images and assess MMP-9 and MMP-2 activity by evaluating the intensity of clear bands against colored backgrounds (Fakhri et al., 2021b; Hashemi et al., 2024).

2.6 Histopathological assessments

All animals were given 100 mg/kg of ketamine and 10 mg/kg of xylazine for scarification after the behavioral tests. After being carefully removed, the hippocampal tissue was stored in 200 mL of 0.1 M PBS and 4% paraformaldehyde. Tissues were sliced to a 5 mm thickness, and Hematoxylin and Eosin (H&E) stain was then applied. The histological changes were examined under a light microscope, and data were gathered at ×400 magnification (Zarneshan et al., 2025).

2.7 Statistical analysis

Regarding analyzing the data, Prism 8.4.3 software (GraphPad Software Inc., San Diego, CA, USA) was employed. To evaluate the normality of the data distribution, the Shapiro-Wilk test was performed. One-way and two-way analyses of variance (ANOVA) were conducted, followed by Tukey or Bonferroni *post hoc* analysis. The data were presented as mean ± standard error of the mean (SEM), with a statistical significance of $P < 0.05$.

3 Results

3.1 Behavioral tests

3.1.1 Polydatin effects on the open field test

Figure 1 represents the general locomotion and anxiety-related behaviors in a novel setting as indicated by the open field test. On every day of the open field test, a statistically significant ($P < 0.001$) reduction was noted in the quantity of crossing, rearing, and grooming in the $AlCl_3$ group as compared to the control group. Treatment with PD in doses of 5, 10, and 20 mg/kg significantly increased the movement of rats compared with the $AlCl_3$ group ($P < 0.001$). This increase in crossing in PD 10 mg/kg and donepezil is more significant ($P < 0.001$). Also, treatment with PD caused a noteworthy increase compared to the $AlCl_3$ group in the number of rearing and grooming in rats ($P < 0.01$ and $P < 0.05$). Therefore, the use of PD improved movement (locomotion) and anxiety behavior caused by $AlCl_3$.

3.1.2 Polydatin effects on passive avoidance test

Figure 2 presents the results of the passive avoidance test for the avoidance learning, memory, and behavior of rats. The purpose of this test was to see if the animal could learn to react quickly to unpleasant stimuli like electric shocks. Because they are nocturnal creatures, rodents, like rats, prefer the dark. After training on the first day (learning), the rat is supposed to recall the negative stimulus in this test, which is an electric shock, and not go into the dark room the following day. The initial transfer latency time (ITL) on days 7 and 14, and step through latency time (STL) to enter the dark section were recorded on days 8 and 15. Considering the inherent tendency of rats to be in the dark part, the amount of ITL time in the $AlCl_3$ group increased compared to the control group on days 7 and 14 ($P < 0.001$). While the consumption of PD in doses of 5, 10, and 20 mg/kg causes a noteworthy reduction in ITL time in comparison with the $AlCl_3$ group ($P < 0.05$, $P < 0.001$, and $P < 0.001$). In the PD-receiving groups, the ITL time reduction in the group receiving 5 mg/kg PD is less than the other treatment groups ($P < 0.05$), and there was no considerable difference between the PD 10 and 20 groups and the group receiving donepezil. In the $AlCl_3$ group compared to the control group, a noteworthy decrease in STL time was observed ($P < 0.001$), which indicates memory deterioration in the $AlCl_3$ group. Treatment with PD in doses of 5, 10, and 20 mg/kg causes significant improvement and an increase in STL in comparison with the $AlCl_3$ group ($P < 0.001$). Among treatment groups with PD, memory improvement in PD 10 mg/kg was more than in other groups. In addition, there was no major difference between groups receiving PD 10 mg/kg and the group receiving donepezil ($P < 0.001$).

3.1.3 Polydatin effects on Y-maze test

Figure 3 presents the Y-maze results for the spatial memory of rats. The results of this test show an important defect in spatial memory and a decrease in general activity in the $AlCl_3$ group compared to the control group, with a decrease in the percentage of non-repetitive intervals and the total number of entering the arm (SAP%) ($P < 0.001$). Also, treatment with PD in different doses increased the SAP% in comparison with the $AlCl_3$ group, especially at the 10 mg/kg and 20 mg/kg dosage, which was significantly higher than that of the $AlCl_3$ group ($P < 0.001$ and $P < 0.01$) and able to recover short-term memory and general activity in rats and averts memory loss caused by $AlCl_3$. Additionally, there was no significant difference in %SAP between the NPD and donepezil groups ($P < 0.001$).

3.1.4 Polydatin effects on the elevated plus maze test

Figure 4 indicates how PD affects anxiety-like behavior, such as the transfer latency of rats given $AlCl_3$ treatment in the elevated plus-maze. The purpose of this test was to measure the rats' post-learning memory. In this test, the time required to enter the arm for each rat on day 7 (learning) as the ITL, and the time required to enter the arm on days 8 and 14 (memory) as the transfer latency time, was recorded.

10.3389/fphar.2025.1574323

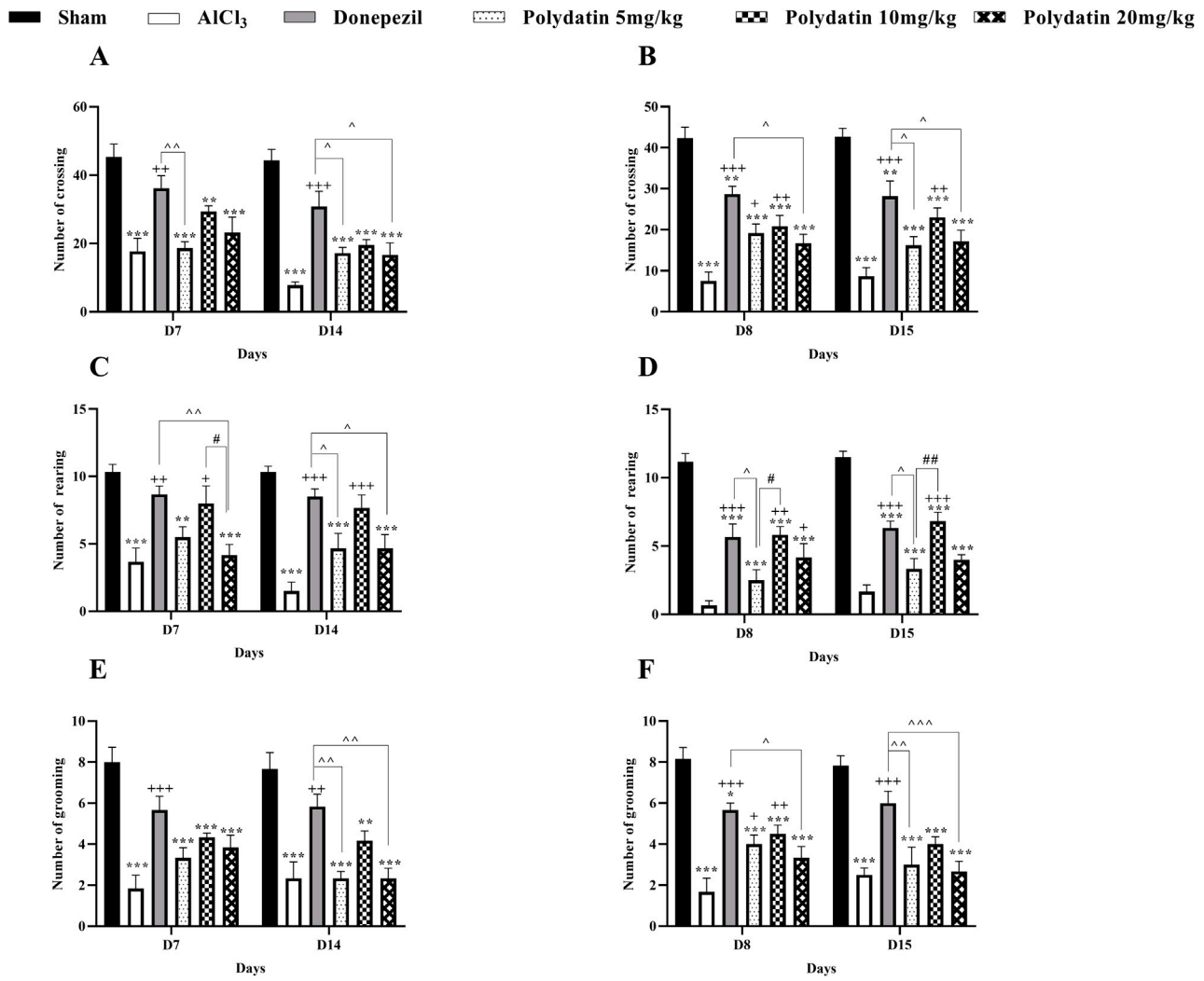


FIGURE 1
Polydatin attenuated anxiety-related behaviors in an $AlCl_3$-induced rat model of AD. Number of **(A, B)** Crossing, **(C, D)** Rearing, and **(E, F)** Grooming. The data passed the Shapiro-Wilk test for normality. The two-way ANOVA with Bonferroni *post hoc* analysis was employed, and data were presented as mean ± SEM (n = 6). $^{**}P < 0.01$, $^{***}P < 0.001$ vs. Sham group; $^{+}P < 0.05$, $^{++}P < 0.01$, $^{+++}P < 0.001$ vs. $AlCl_3$ group; $^{\wedge}P < 0.05$, $^{\wedge\wedge}P < 0.01$, $^{\wedge\wedge\wedge}P < 0.001$ vs. Donepezil group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs. Polydatin 10 mg/kg groups.

Reduction in transfer latency time after determining the ITL time indicates the improvement of the rats' diagnostic memory. In this test, a significant difference was observed in the learning time between the group receiving $AlCl_3$ and the control group ($P < 0.01$).

Similarly, in this test, the group receiving $AlCl_3$ showed an increase in the transfer latency time of the first and second transfer (memory) on days 8 and 14, respectively, according to the time ITL on the 7th day, compared to the control group, treatment with PD showed a memory enhancement ($P < 0.05$). This effect is more visible at the dose of 10 mg/kg PD.

3.2 Biochemical tests

3.2.1 Polydatin effects on catalase assay

The amount of serum CAT was measured as an antioxidative stress factor, and it is desirable not to decrease it. The results in Figure 5A reveal that consuming $AlCl_3$ induces an enormous decrease in serum CAT levels ($P < 0.001$); whereas the PD groups have a considerably lower difference in serum CAT levels than the sham group ($P < 0.01$ and $P < 0.001$). CAT quantity improvement in PD 10 mg/kg was more than in other PD groups ($P < 0.05$), and there was no statistically significant distinction between rats receiving PD 10 mg/kg and those receiving donepezil.

3.2.2 Polydatin effects on glutathione assay

Serum GSH has been investigated as an antioxidative stress component and should not be decreased. The results in Figure 5B show that the consumption of $AlCl_3$ causes a major decrease in the serum GSH level ($P < 0.01$), while the treatment groups showed a significant difference in the serum GSH level compared to the sham group exclusively 10 mg/kg, thus causing neuroprotection by inhibiting oxidative stress ($P < 0.05$).

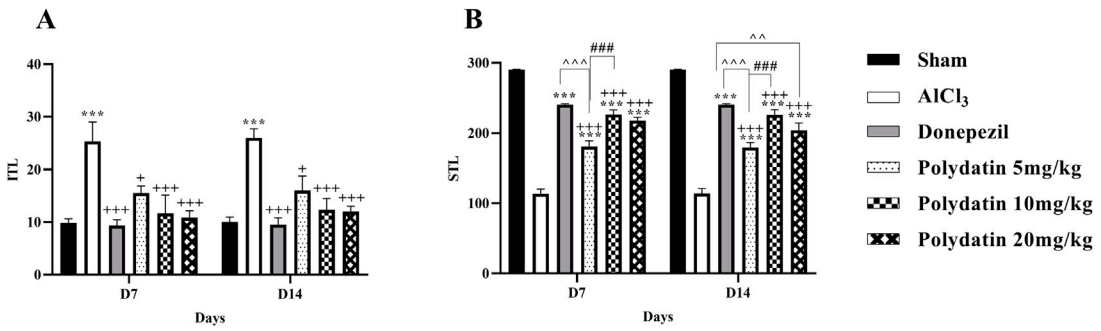


FIGURE 2
Polydatin regulated related behaviors in the passive avoidance test during an $AlCl_3$-induced rat model of AD. **(A)** Initial transfer latency (ITL) and **(B)** Step-through latency (STL). The data passed the Shapiro-Wilk test for normality. The two-way ANOVA with Bonferroni *post hoc* analysis was employed, and data were presented as mean ± SEM (n = 6). ***P < 0.001 vs. Sham group; ^{+}P < 0.05, ^{+++}P < 0.001 vs. $AlCl_3$ group;$^{\wedge\wedge}P$ < 0.01,$^{\wedge\wedge\wedge}P$ < 0.001 vs. Donepezil group; $^{\#\#\#}P$ < 0.001 vs. Polydatin 10 mg/kg groups.

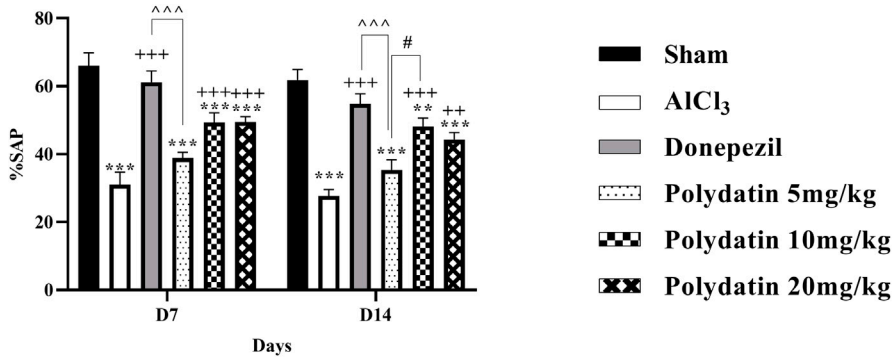


FIGURE 3
Polydatin modulated spatial memory in an $AlCl_3$-induced rat model of AD. The data passed the Shapiro-Wilk test for normality. The two-way ANOVA with Bonferroni *post hoc* analysis was employed and data was presented as mean ± SEM (n = 6). **P < 0.01, ***P < 0.001 vs. Sham group; ^{++}P < 0.01, ^{+++}P < 0.001 vs. $AlCl_3$ group; $^{\wedge\wedge\wedge}P$ < 0.001 vs. Donepezil group; $^{\#}P$ < 0.05 vs. Polydatin 10 mg/kg groups.

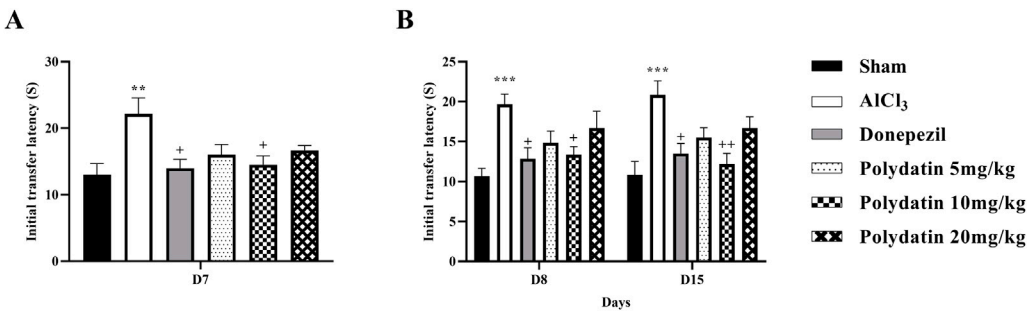


FIGURE 4
Polydatin effects on anxiety-like behavior and memory in an $AlCl_3$-induced rat model of AD. **(A)** Initial transfer latency (ITL-D7) and **(B)** ITL (D8-D15). The data passed the Shapiro-Wilk test for normality. The one-way and two-way ANOVA with Tukey and Bonferroni *post hoc* analysis was employed, and data were presented as mean ± SEM (n = 6). **P < 0.01, ***P < 0.001 vs. Sham group; ^{+}P < 0.05, ^{++}P < 0.01 vs. $AlCl_3$ group.

3.2.3 Polydatin effects on nitrite assay

The level of serum nitrite has been identified as an oxidative stress factor and should not be increased. The results in Figure 5C demonstrate that intake of $AlCl_3$ resulted in an important reduction in serum nitrite level compared to the sham group (P < 0.001), whereas consumption of PD resulted in a substantial decrease in NO level compared to the group (P < 0.001). As a result, the usage of PD could result in neuroprotection by decreasing oxidative stress,

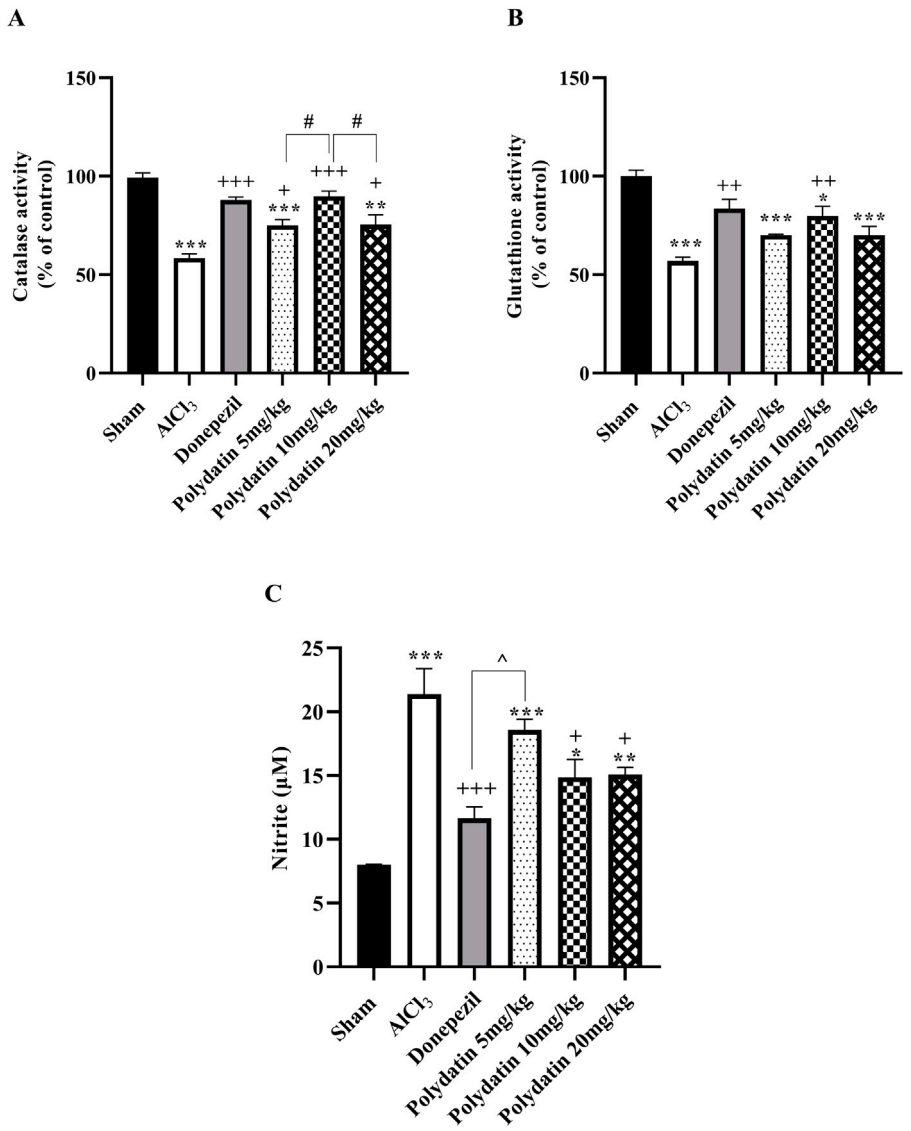


FIGURE 5
Polydatin regulatory roles on oxidative stress in a rat model of AD induced by $AlCl_3$. **(A)** Catalase, **(B)** Glutathione, and **(C)** Nitrite. The data passed the Shapiro-Wilk test for normality. The one-way ANOVA with Tukey *post hoc* analysis was employed, and data were presented as mean ± SEM (n = 6). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. Sham group; $^{+}P < 0.05$, $^{++}P < 0.01$, $^{+++}P < 0.001$ vs. $AlCl_3$ group; $^{\wedge}P < 0.05$, $^{\wedge\wedge}P < 0.01$ vs. Donepezil group; $^{\#}P < 0.05$ vs. Polydatin 10 mg/kg groups.

particularly in a dose of 10 mg/kg ($P < 0.001$). Crucially, there were no discernible variations in these parameters between the donepezil and PD treatment groups ($P < 0.01$).

3.3 Polydatin effects on MMP-2 and MMP-9

The assessment of MMP gelatinase activity across different groups indicated that MMP-9 activity was significantly elevated in the $AlCl_3$ group compared to the sham group ($P < 0.05$, Figure 6A). Treatment with PD effectively reduced MMP-9 gelatinase activity ($P < 0.001$). Interestingly, the 10 mg dose of PD not only decreased MMP-9 activity but also showed effects comparable to those of donepezil (positive control). Moreover, the $AlCl_3$ group demonstrated markedly lower MMP-2 levels than the sham group ($P < 0.05$, Figure 6B). Conversely, the 5 mg/kg PD-treated group exhibited a partial increase in MMP-2 levels when compared to the $AlCl_3$ group.

3.4 Polydatin effects on histopathological assessments

H&E staining was used to investigate morphological changes in the hippocampus CA2, CA4, and DG regions of experimental rats (Figure 7). The sham group's neurons showed typical properties such as distinct nuclei, large nucleoli, and abundant cytoplasm, indicating their health. In contrast, treatment with $AlCl_3$ resulted in a significant reduction in viable neurons ($P < 0.001$). The group that received 10 or 20 mg/kg of PD showed fewer pathogenic changes in

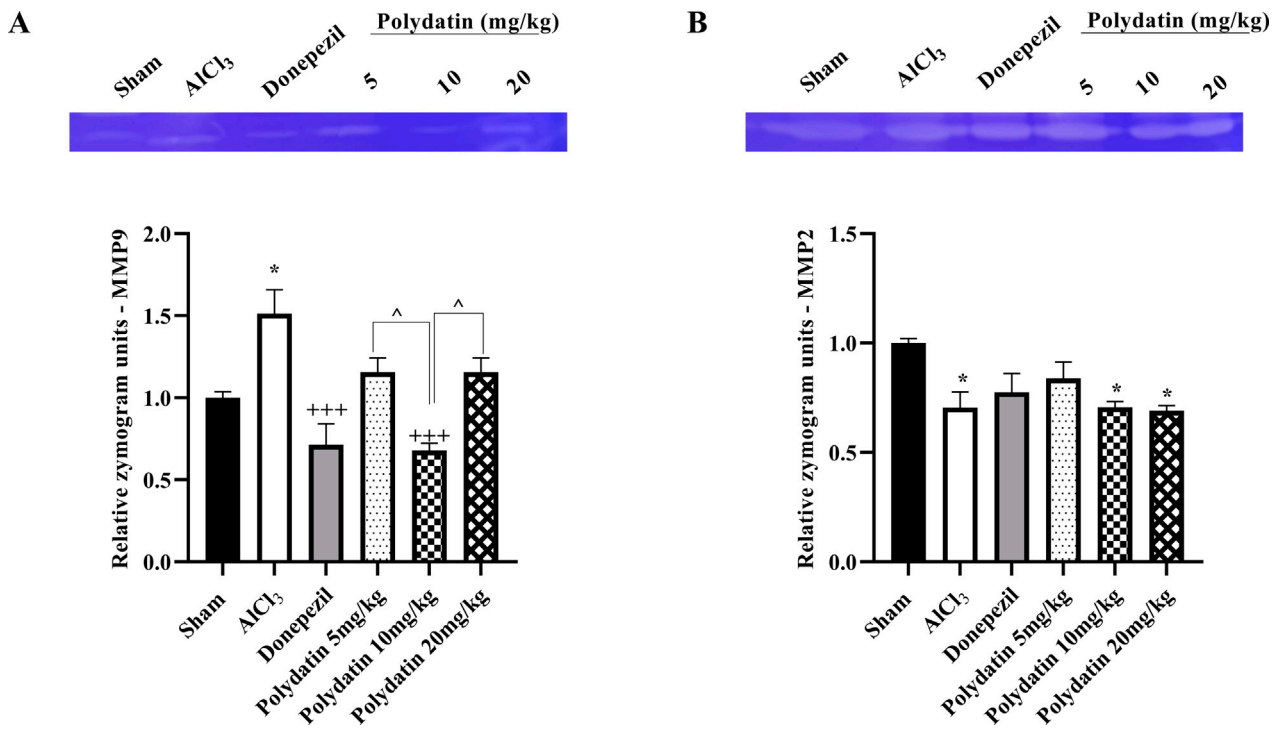


FIGURE 6
Effects of polydatin on MMPs levels in a rat model of AD induced by $AlCl_3$. **(A)** MMP-9 and **(B)** MMP-2. The data passed the Shapiro-Wilk test for normality. The one-way ANOVA with Tukey *post hoc* analysis was employed, and data were presented as mean ± SEM. *$P < 0.05$ vs. Sham group; $^{+++}P < 0.001$ vs. $AlCl_3$ group; ^$P < 0.05$ vs. Donepezil group.

hippocampus neurons compared to the $AlCl_3$ group ($P < 0.05$ and $P < 0.01$). There were no significant changes in neuronal health or structure between groups treated with donepezil and those receiving PD at dosages of 10 and 20 mg/kg ($P < 0.001$).

4 Discussion

In the present study, we found that rats with AD had reduced rates of learning, recognition memory, and short- and long-term memory. Over 14 days, there was a noticeable drop in motor function along with a decrease in mobility and exploratory behaviors in rats with AD. AD rats showed reduced MMP-2, GSH, and CAT activity, together with increased MMP-9 and nitrite levels. Besides, PD demonstrated anti-inflammatory benefits by increasing the activity of MMP-2, lowering serum nitrite, and enhancing CAT/GSH while inhibiting inflammatory MMP-9. Both behavioral and biochemical indicators showed varied degrees of improvement after PD therapy. Finally, via improving neuronal survival, PD demonstrated neuroprotective benefits in the rat hippocampal CA2, CA4, and DG.

Oxidative stress and inflammation are elevated in the brains of AD patients, and these elevations have been widely confirmed in a variety of model animals. Suppression of the start and/or advancement of AD is a major concern in modern society, and studies have shown that improvements in these pathological circumstances may be advantageous for preserving or enhancing the neuronal functioning of Alzheimer's patients (Uruno et al., 2020). According to reports, Al is one of the factors that contributes to AD and is deposited in the brain. Important symptoms of Al neurotoxicity, which contributes to the etiology of AD, include Aβ deposition, hyperphosphorylation of tau protein, increased activity of acetylcholinesterase, imbalance in neurotransmitter levels, inflammatory cytokines, mitochondrial damage, oxidative stress, and deficits in memory and learning (Wojtunik-Kulesza et al., 2016; Auti and Kulkarni, 2019). As a result, in this investigation, we employed an animal model of AD caused by $AlCl_3$. The current study found that the treatment of $AlCl_3$ causes a progressive loss of short-term, spatial, and diagnostic memory in rats. Previous reports have shown the antioxidative potentials of medicinal herbs (Selamoglu-Talas et al., 2015), plant-derived active compounds (e.g., polyphenols and iridoids), and related diets (Vasile et al., 2024) in combating neurodegenerative diseases (Daglia et al., 2014; Sureda et al., 2023; Ishak et al., 2024).

Resveratrol has sparked attention in health studies owing to its therapeutic potential for some neuroprotective properties associated with cognitive impairment. As a polyphenol, resveratrol's positive benefits have been connected to its action as an antioxidant and anti-inflammatory agent (Yang et al., 2021). Even though the mechanistic insights of resveratrol in AD have been widely examined (Ahmed et al., 2017; Kou and Chen, 2017; Sawda et al., 2017; Drygalski et al., 2018; Gomes et al., 2018), the comparative literature on its role with other stilbenes, particularly PD, is still sparse. PD is a naturally occurring resveratrol glucoside, also known as resveratrol-3-β-mono-D-glucoside derived from the roots of *Reynoutria japonica* Houtt [Polygonaceae]. It is additionally found in grapes, red wines,

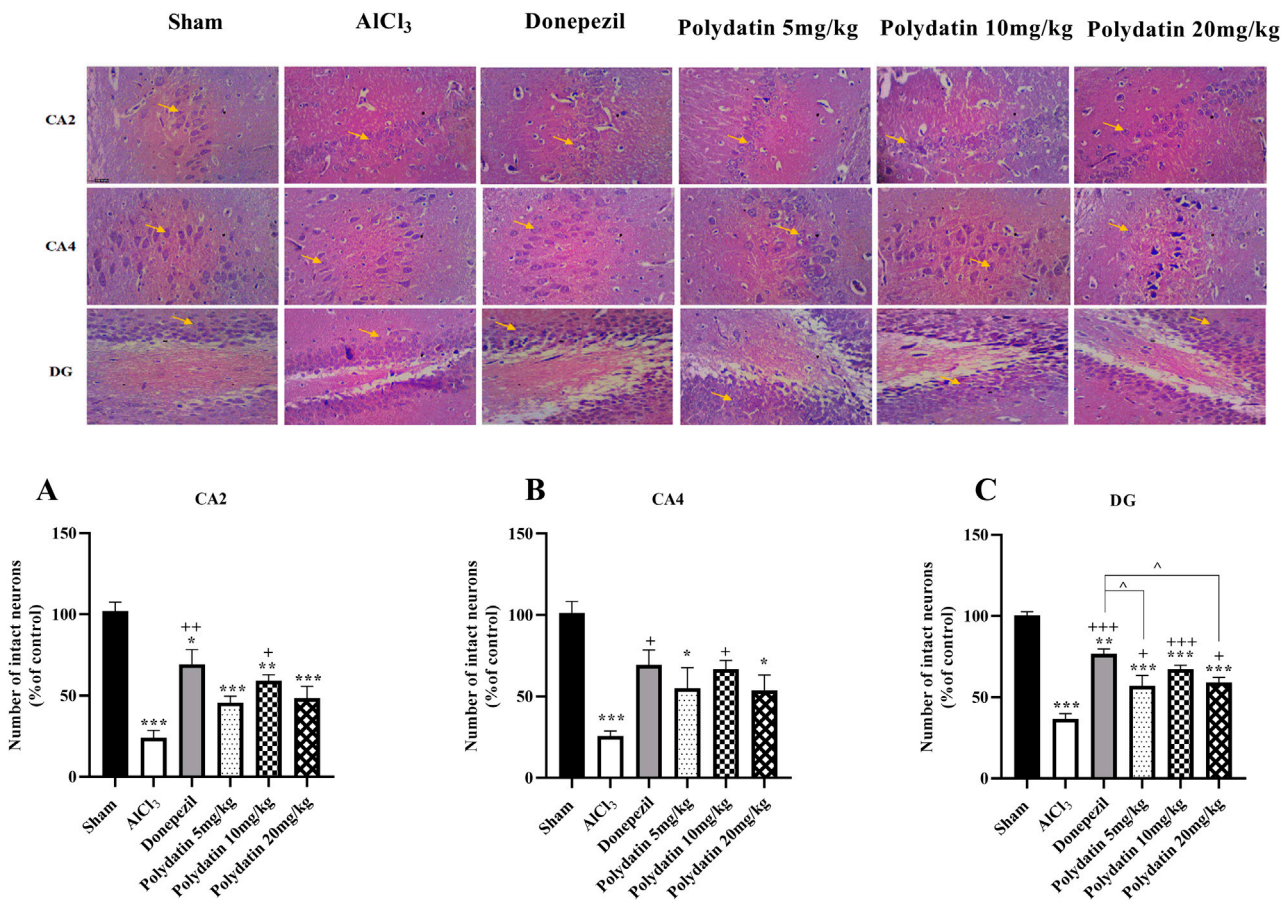


FIGURE 7
Polydatin impaired histological changes in an $AlCl_3$-induced rat model of AD. **(A)** CA2, **(B)** CA4, and **(C)** DG. The data passed the Shapiro-Wilk test for normality. The one-way ANOVA with Tukey *post hoc* analysis was employed, and data were presented as mean ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. Sham group; $^{+}P < 0.05$, $^{++}P < 0.01$, $^{+++}P < 0.001$ vs. $AlCl_3$ group; $^{\wedge}P < 0.05$ vs. Donepezil group.

hop cones, peanuts, cocoa/chocolate goods, and a variety of other foods (Du et al., 2013). It has been observed that PD protects against neurodegenerative disorders such as AD (Tang, 2021), Parkinson's disease (Huang et al., 2018), cerebral ischemia (Ji et al., 2012; Huang et al., 2018; Shah et al., 2019), traumatic brain injury (Li et al., 2019), and spinal cord injury (Lv et al., 2019b; 2019a).

To examine if PD could have an impact on learning and memory impairment, as well as anxiety-like behaviors, behavioral tests were conducted in AD rats (Webster et al., 2014; Lombardero et al., 2020). The open-field test is used to evaluate the mobility and exploratory behaviors of rats (Prut and Belzung, 2003). Comparing the $AlCl_3$ group to the control group, there was a notable drop in the number of crossing lines, rearing, and grooming. Rats receiving PD showed a substantial increase in the number of crossing lines, rearing, and grooming behaviors as compared to the $AlCl_3$ group. The passive avoidance test was used to evaluate short-term and long-term memory by recording ITL and STL (Ghafarimoghadam et al., 2022). A noteworthy increase in ITL in rats treated with PD was observed compared to the group treated with $AlCl_3$. In this experiment, the increased STL showed improvement in the recognition memory of rats. In addition, the Y-maze test evaluates the spatial memory of the animal based on the percentage of non-repetitive frequency of the total number of entering arms. Healthy animals explore the new arm, whereas animals with peripheral destruction re-enter the old arm that is already been examined. Therefore, compared to healthy animals, a lower number of non-repetitive intervals is registered. The SAP% in the $AlCl_3$ group compared to the healthy and treatment groups intensity reduced, indicating a lower rate of learning and development of short-term working memory. This is important because the increased SAP% of rats in the group receiving the PD can be related.

The elevated plus maze test was used to determine learning and memory by measuring the ITL and transfer latency. The reduction in transfer delay time after determining the ITL time indicates the improvement of the rats' diagnostic memory (Pentkowski et al., 2021). In this test, a significant difference was observed in the learning time between the group receiving $AlCl_3$ and the control group. The use of PD improved the ITL (learning) time compared to the $AlCl_3$ group.

Oxidative stress acts as a connecting factor between different AD hypotheses and processes. A multifaceted process causes adverse effects on neurons. In AD, oxidative stress is significant and may play a major role in the etiology of the disease (Bai et al., 2022). Because of the crucial roles of oxidative stress in mediating AD difficulties, as well as to obtain further insights into the presumed mechanisms of action of PD, researchers assessed its effects on multiple oxidative stress indicators (GSH, CAT, and NO). This

investigation revealed that the group receiving $AlCl_3$ had meaningfully higher levels of NO and significantly lower levels of the antioxidant enzymes GSH and CAT as a measure of oxidative stress than the control group. The levels of CAT, GSH, and nitrite were enhanced in this study by PD ingestion.

Neuronal degeneration can be brought on by a variety of mediators released by inflammatory cells. Neuritis may directly cause AD or coexist with other causes of AD to contribute to the disease's development (Twarowski and Herbet, 2023). Specifically, several neuroinflammatory and neurodegenerative pathways can be triggered by MMP-2 and MMP-9 (Ciccone et al., 2021). Our findings showed that therapy with $AlCl_3$ increased inflammation, which eventually led to nerve injury. In this study, administering PD significantly increased MMP-2 levels while reducing MMP-9 levels.

Dementia is linked to the buildup of NFTs in the hippocampus area. The loss of synapses was caused by the buildup of NFT in the stratum lacunosum, dentate fasciculus, CA2, CA3, and CA4 regions. Oxidative damage can impair hippocampal and cortical cognitive function. As an information gateway, the DG plays a major role in the hippocampus's development of episodic memory (Rao et al., 2022). New evidence indicates that changes in CA4 may be connected with memory processing problems. Integrating information from both the DG and CA4 into larger hippocampal circuits is crucial for efficient memory formation (Aumont et al., 2023). Rats receiving PD at several dosages showed improvements in the histological characteristics of the hippocampus regions CA2, CA4, and DG at therapeutic levels; this was especially true for the group given a high dose of the treatment. Compared with the $AlCl_3$ groups, rats receiving PD doses of 10 and 20 mg/kg had the most therapeutic effect, as did the donepezil group.

Despite the effectiveness of resveratrol, an aglycone derivative of PD, it often suffers from low solubility, poor bioavailability, rapid metabolism/clearance, and chemical degradation, thereby limiting associated clinical applications (Tang, 2021). It urges the need for providing PD (as a glycoside resveratrol) and novel delivery formulations. Employing nanotechnology has auspiciously overcome these pharmacokinetic limitations by improving cellular uptake, bioavailability, targeted therapy, and increasing efficacy. Consequently, solid-lipid nanoparticles, liposomes, micelles, and polymeric/metallic nanoparticles have played critical roles in improving the neuroprotective effects of PD. We previously showed the therapeutic effects of PD amphiphilic chitosan nanocarriers against an $AlCl_3$-induced rat model of AD (Zarneshan et al., 2025), and the present report confirmed that naïve PD could also play such beneficial effects.

5 Conclusion

Using an $AlCl_3$-induced AD rat model, the current study examined the therapeutic potential and mechanisms of action of PD against AD. PD dramatically reduced learning and memory impairment as well as anxiety-like behaviors in AD rats, through antioxidant, anti-inflammatory, and neuroprotective mechanisms. Further investigation to establish PD as a novel therapy for AD is needed. To elucidate the more mechanistic pathways and evaluate the long-term safety and efficacy of PD in clinical applications, more studies are required.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was approved by the Dr. Leila Hosseinzadeh, Committee Director, Laboratory Animals - Kermanshah University of Medical Sciences Dr. Keivan Moradi, Committee Secretary, Laboratory Animals - Kermanshah University of Medical Sciences. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

SZ: Writing – original draft, Investigation, Data curation. SF: Writing – original draft, Investigation, Writing – review and editing. AK: Writing – original draft, Writing – review and editing. FA: Writing – original draft, Investigation. SH: Writing – original draft. EM-N: Writing – original draft, Investigation. JE: Writing – original draft, Writing – review and editing.

Funding

The author(s) declare that financial support was received for the research and/or publication of this article. The authors thank the Pharmaceutical Sciences Research Center, Kermanshah University of Medical Sciences, for the special Grant No. 4020128.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

Generative AI statement

The author(s) declare that no Generative AI was used in the creation of this manuscript.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

Aebi, H. E. (1980). Enzymes 1: oxidoreductases, transferases. *Methods enzym. Anal.* 3, 273–282.

Ahmed, T., Javed, S., Javed, S., Tariq, A., Šamec, D., Tejada, S., et al. (2017). Resveratrol and Alzheimer's disease: mechanistic insights. *Mol. Neurobiol.* 54, 2622–2635. doi:10.1007/s12035-016-9839-9

Anwar, H. M., Georgy, G. S., Hamad, S. R., Badr, W. K., El Raey, M. A., Abdelfattah, M. A. O., et al. (2021). A leaf extract of harrisonia abyssinica ameliorates neurobehavioral, histological and biochemical changes in the Hippocampus of rats with aluminum chloride-induced Alzheimer's disease. *Antioxidants Basel, Switz.* 10, 947. doi:10.3390/antiox10060947

Arifin, W. N., and Zahiruddin, W. M. (2017). Sample size calculation in animal studies using resource equation approach. *Malays. J. Med. Sci.* 24, 101–105. doi:10.21315/mjms2017.24.5.11

Athar, T., Al Balushi, K., and Khan, S. A. (2021). Recent advances on drug development and emerging therapeutic agents for Alzheimer's disease. *Mol. Biol. Rep.* 48, 5629–5645. doi:10.1007/s11033-021-06512-9

Aumont, E., Bussy, A., Bedard, M.-A., Bezgin, G., Therriault, J., Savard, M., et al. (2023). Hippocampal subfield associations with memory depend on stimulus modality and retrieval mode. *Brain Commun.* 5, fcad309. doi:10.1093/braincomms/fcad309

Auti, S. T., and Kulkarni, Y. A. (2019). Neuroprotective effect of cardamom oil against aluminum induced neurotoxicity in rats. *Front. Neurol.* 10, 399. doi:10.3389/fneur.2019.00399

Bai, R., Guo, J., Ye, X.-Y., Xie, Y., and Xie, T. (2022). Oxidative stress: the core pathogenesis and mechanism of Alzheimer's disease. *Ageing Res. Rev.* 77, 101619. doi:10.1016/j.arr.2022.101619

Bhattacharjee, S., Zhao, Y., Hill, J. M., Percy, M. E., and Lukiw, W. J. (2014). Aluminum and its potential contribution to Alzheimer's disease (AD). *Front. Aging Neurosci.* 6, 62. doi:10.3389/fnagi.2014.00062

Broderick, T. L., Rasool, S., Li, R., Zhang, Y., Anderson, M., Al-Nakkash, L., et al. (2020). Neuroprotective effects of chronic resveratrol treatment and exercise training in the 3xTg-AD mouse model of Alzheimer's disease. *Int. J. Mol. Sci.* 21, 7337. doi:10.3390/ijms21197337

Ciccone, L., Vandooren, J., Nencetti, S., and Orlandini, E. (2021). Natural marine and terrestrial compounds as modulators of matrix metalloproteinases-2 (MMP-2) and MMP-9 in Alzheimer's disease. *Pharmaceuticals* 14, 86. doi:10.3390/ph14020086

Cicero, A. F. G., Ruscica, M., and Banach, M. (2019). Resveratrol and cognitive decline: a clinician perspective. *Arch. Med. Sci.* 15, 936–943. doi:10.5114/aoms.2019.85463

Crous-Bou, M., Minguillón, C., Gramunt, N., and Molinuevo, J. L. (2017). Alzheimer's disease prevention: from risk factors to early intervention. *Alzheimers. Res. Ther.* 9, 71. doi:10.1186/s13195-017-0297-z

Daglia, M., Di Lorenzo, A., Nabavi, S. F., Talas, Z. S., and Nabavi, S. M. (2014). Polyphenols: well beyond the antioxidant capacity: gallic acid and related compounds as neuroprotective agents: you are what you eat. *Curr. Pharm. Biotechnol.* 15, 362–372. doi:10.2174/1389201015041408251207 37

Drygalski, K., Fereniec, E., Koryciński, K., Chomentowski, A., Kiełczewska, A., Odrzygóźdź, C., et al. (2018). Resveratrol and Alzheimer's disease. From molecular pathophysiology to clinical trials. *Exp. Gerontol.* 113, 36–47. doi:10.1016/j.exger.2018.09.019

Du, Q.-H., Peng, C., and Zhang, H. (2013). Polydatin: a review of pharmacology and pharmacokinetics. *Pharm. Biol.* 51, 1347–1354. doi:10.3109/13880209.2013.792849

Ellman, G. L. (1959). Tissue sulfhydryl groups. *Arch. Biochem. Biophys.* 82, 70–77. doi:10.1016/0003-9861(59)90090-6

Eratne, D., Loi, S. M., Farrand, S., Kelso, W., Velakoulis, D., and Looi, J. C. (2018). Alzheimer's disease: clinical update on epidemiology, pathophysiology and diagnosis. *Australas. Psychiatry* 26, 347–357. doi:10.1177/1039856218762308

Fakhri, S., Gravandi, M. M., Abdian, S., Akkol, E. K., Farzaei, M. H., and Sobarzo-Sánchez, E. (2021a). The neuroprotective role of polydatin: neuropharmacological mechanisms, molecular targets, therapeutic potentials, and clinical perspective. *Molecules* 26, 5985. doi:10.3390/molecules26195985

Fakhri, S., Kiani, A., Jalili, C., Abbaszadeh, F., Piri, S., Farzaei, M. H., et al. (2021b). Intrathecal administration of melatonin ameliorates the neuroinflammation- mediated sensory and motor dysfunction in A rat model of compression spinal cord injury. *Curr. Mol. Pharmacol.* 14, 646–657. doi:10.2174/1874467213666201230101811

Freyssin, A., Page, G., Fauconneau, B., and Rioux Bilan, A. (2020). Natural stilbenes effects in animal models of Alzheimer's disease. *Neural Regen. Res.* 15, 843–849. doi:10.4103/1673-5374.268970

Gambini, J., Inglés, M., Olaso, G., Lopez-Grueso, R., Bonet-Costa, V., Gimeno-Mallench, L., et al. (2015). Properties of resveratrol: *in vitro* and *in vivo* studies about metabolism, bioavailability, and biological effects in animal models and humans. *Oxid. Med. Cell. Longev.* 2015, 837042. doi:10.1155/2015/837042

Ghafarimoghadam, M., Mashayekh, R., Gholami, M., Fereydani, P., Shelley-Tremblay, J., Kandezi, N., et al. (2022). A review of behavioral methods for the evaluation of cognitive performance in animal models: current techniques and links to human cognition. *Physiol. Behav.* 244, 113652. doi:10.1016/j.physbeh.2021.113652

Giménez De Béjar, V., Caballero Bleda, M., Popović, N., and Popović, M. (2017). Verapamil blocks scopolamine enhancement effect on memory consolidation in passive avoidance task in rats. *Front. Pharmacol.* 8, 566. doi:10.3389/fphar.2017.00566

Goedert, M. (2015). NEURODEGENERATION. Alzheimer's and Parkinson's diseases: the prion concept in relation to assembled Aβ, tau, and α-synuclein. *Science* 349, 1255555. doi:10.1126/science.1255555

Gomes, B. A. Q., Silva, J. P. B., Romeiro, C. F. R., Dos Santos, S. M., Rodrigues, C. A., Gonçalves, P. R., et al. (2018). Neuroprotective mechanisms of resveratrol in Alzheimer's disease: role of SIRT1. *Oxid. Med. Cell. Longev.* 2018, 8152373. doi:10.1155/2018/8152373

Granger, D. L., Taintor, R. R., Boockvar, K. S., and Hibbs, J. B. (1996). Measurement of nitrate and nitrite in biological samples using nitrate reductase and Griess reaction. *Methods Enzymol.* 268, 142–151. doi:10.1016/s0076-6879(96)68016-1

Granger, D. L., Taintor, R. R., Boockvar, K. S., and Hibbs, J. B., Jr. (1995). Determination of nitrate and nitrite in biological samples using bacterial nitrate reductase coupled with the griess reaction. *Methods* 7, 78–83. doi:10.1006/meth.1995.1011

Hashemi, B., Fakhri, S., Kiani, A., Abbaszadeh, F., Miraghaee, S., Mohammadi, M., et al. (2024). Anti-neuropathic effects of astaxanthin in a rat model of chronic constriction injury: passing through opioid/benzodiazepine receptors and relevance to its antioxidant and anti-inflammatory effects. *Front. Pharmacol.* 15, 1467788. doi:10.3389/fphar.2024.1467788

Huang, B., Liu, J., Meng, T., Li, Y., He, D., Ran, X., et al. (2018). Polydatin prevents lipopolysaccharide (LPS)-Induced Parkinson's disease via regulation of the AKT/GSK3β-Nrf2/NF-κB signaling Axis. *Front. Immunol.* 9, 2527. doi:10.3389/fimmu.2018.02527

Iranpanah, A., Fakhri, S., Bahrami, G., Majnooni, M. B., Gravandi, M. M., Taghavi, S., et al. (2024). Protective effect of a hydromethanolic extract from Fraxinus excelsior L. bark against a rat model of aluminum chloride-induced Alzheimer's disease: relevance to its anti-inflammatory and antioxidant effects. *J. Ethnopharmacol.* 323, 117708. doi:10.1016/j.jep.2024.117708

Ishak, S., Allouche, M., Alotaibi, G. S., Alwthery, N. S., Al-Subaie, R. A., Al-Hoshani, N., et al. (2024). Experimental and computational assessment of antiparkinson medication effects on meiofauna: case study of benserazide and trihexyphenidyl. *Mar. Pollut. Bull.* 205, 116668. doi:10.1016/j.marpolbul.2024.116668

Ji, H., Zhang, X., Du, Y., Liu, H., Li, S., and Li, L. (2012). Polydatin modulates inflammation by decreasing NF-κB activation and oxidative stress by increasing Gli1, Ptch1, SOD1 expression and ameliorates blood-brain barrier permeability for its neuroprotective effect in pMCAO rat brain. *Brain Res. Bull.* 87, 50–59. doi:10.1016/j.brainresbull.2011.09.021

Kou, X., and Chen, N. (2017). Resveratrol as a natural autophagy regulator for prevention and treatment of Alzheimer's disease. *Nutrients* 9, 927. doi:10.3390/nu9090927

Kraeuter, A.-K., Guest, P. C., and Sarnyai, Z. (2019). The Y-maze for assessment of spatial working and reference memory in mice. *Methods Mol. Biol.* 1916, 105–111. doi:10.1007/978-1-4939-8994-2_10

Li, L., Tan, H.-P., Liu, C.-Y., Yu, L.-T., Wei, D.-N., Zhang, Z.-C., et al. (2019). Polydatin prevents the induction of secondary brain injury after traumatic brain injury by protecting neuronal mitochondria. *Neural Regen. Res.* 14, 1573–1582. doi:10.4103/1673-5374.255972

Lombardero, L., Llorente-Ovejero, A., Manuel, I., and Rodríguez-Puertas, R. (2020). *Genetics, neurology, behavior, and diet in dementia*. Elsevier. doi:10.1016/C2017-0-03834-X

Lu, Y., Sareddy, G. R., Wang, J., Wang, R., Li, Y., Dong, Y., et al. (2019). Neuron-derived estrogen regulates synaptic plasticity and memory. *J. Neurosci.* 39, 2792–2809. doi:10.1523/JNEUROSCI.1970-18.2019

Lv, R., Du, L., Liu, X., Zhou, F., Zhang, Z., and Zhang, L. (2019a). Polydatin alleviates traumatic spinal cord injury by reducing microglial inflammation via regulation of iNOS and NLRP3 inflammasome pathway. *Int. Immunopharmacol.* 70, 28–36. doi:10.1016/j.intimp.2019.02.006

Lv, R., Du, L., Zhang, L., and Zhang, Z. (2019b). Polydatin attenuates spinal cord injury in rats by inhibiting oxidative stress and microglia apoptosis via Nrf2/HO-1 pathway. *Life Sci.* 217, 119–127. doi:10.1016/j.lfs.2018.11.053

Ma, X., Sun, Z., Han, X., Li, S., Jiang, X., Chen, S., et al. (2019). Neuroprotective effect of resveratrol via activation of Sirt1 signaling in a rat model of combined diabetes and Alzheimer's disease. *Front. Neurosci.* 13, 1400. doi:10.3389/fnins.2019.01400

MacLellan, C. L., Langdon, K. D., Churchill, K. P., Granter-Button, S., and Corbett, D. (2009). Assessing cognitive function after intracerebral hemorrhage in rats. *Behav. Brain Res.* 198, 321–328. doi:10.1016/j.bbr.2008.11.004

Mossello, E., and Ballini, E. (2012). Management of patients with Alzheimer's disease: pharmacological treatment and quality of life. *Ther. Adv. Chronic Dis.* 3, 183–193. doi:10.1177/2040622312452387

Moussa, C., Hebron, M., Huang, X., Ahn, J., Rissman, R. A., Aisen, P. S., et al. (2017). Resveratrol regulates neuro-inflammation and induces adaptive immunity in Alzheimer's disease. *J. Neuroinflammation* 14, 1. doi:10.1186/s12974-016-0779-0

Pan, B., Ren, Y., and Liu, L. (2018). Uncovering the action mechanism of polydatin via network pharmacological target prediction. *RSC Adv.* 8, 18851–18858. doi:10.1039/c8ra03124j

Pentkowski, N. S., Rogge-Obando, K. K., Donaldson, T. N., Bouquin, S. J., and Clark, B. J. (2021). Anxiety and Alzheimer's disease: behavioral analysis and neural basis in rodent models of Alzheimer's-related neuropathology. *Neurosci. Biobehav. Rev.* 127, 647–658. doi:10.1016/j.neubiorev.2021.05.005

Prut, L., and Belzung, C. (2003). The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: a review. *Eur. J. Pharmacol.* 463, 3–33. doi:10.1016/s0014-2999(03)01272-x

Querfurth, H. W., and LaFerla, F. M. (2010). Alzheimer's disease. *N. Engl. J. Med.* 362, 329–344. doi:10.1056/NEJMra0909142

Rao, Y. L., Ganaraja, B., Murlimanju, B. V., Joy, T., Krishnamurthy, A., and Agrawal, A. (2022). Hippocampus and its involvement in Alzheimer's disease: a review. *3 Biotech.* 12, 55. doi:10.1007/s13205-022-03123-4

Sawda, C., Moussa, C., and Turner, R. S. (2017). Resveratrol for Alzheimer's disease. *Ann. N. Y. Acad. Sci.* 1403, 142–149. doi:10.1111/nyas.13431

Scheltens, P., De Strooper, B., Kivipelto, M., Holstege, H., Chételat, G., Teunissen, C. E., et al. (2021). Alzheimer's disease. *Lancet London, Engl.* 397, 1577–1590. doi:10.1016/S0140-6736(20)32205-4

Selamoglu-Talas, Z., Ozdemir, I., Ciftci, O., and Cakir, O. (2015). Propolis attenuates oxidative injury in brain and lung of nitric oxide synthase inhibited rats. *J. Pharm. Care* 1. Available online at: https://jpc.tums.ac.ir/index.php/jpc/article/view/11.

Shah, F. A., Kury, L.Al, Li, T., Zeb, A., Koh, P. O., Liu, F., et al. (2019). Polydatin attenuates neuronal loss via reducing neuroinflammation and oxidative stress in rat MCAO models. *Front. Pharmacol.* 10, 663. doi:10.3389/fphar.2019.00663

Shi, X., Bai, H., Wang, J., Wang, J., Huang, L., He, M., et al. (2021). Behavioral assessment of sensory, motor, emotion, and cognition in rodent models of intracerebral hemorrhage. *Front. Neurol.* 12, 667511. doi:10.3389/fneur.2021.667511

Singh, B., Day, C. M., Abdella, S., and Garg, S. (2024). Alzheimer's disease current therapies, novel drug delivery systems and future directions for better disease management. *J. Control. Release* 367, 402–424. doi:10.1016/j.jconrel.2024.01.047

Sureda, A., Tejada, S., Mir Khan, U., and Selamoglu, Z. (2023). An overview of the biological function of curcumin in the processes of oxidative stress, inflammation, nervous system, and lipid levels. *Cent. Asian J. Med. Pharm. Sci. Innov.* 3, 1–11. doi:10.22034/CAJMPSI.2023.01.01

Tang, K. S. (2021). Protective effects of polydatin against dementia-related disorders. *Curr. Neuropharmacol.* 19, 127–135. doi:10.2174/1570159X18666200611144825

Tang, K. S., and Tan, J. S. (2019). The protective mechanisms of polydatin in cerebral ischemia. *Eur. J. Pharmacol.* 842, 133–138. doi:10.1016/j.ejphar.2018.10.039

Twarowski, B., and Herbet, M. (2023). Inflammatory processes in Alzheimer's disease—pathomechanism, diagnosis and treatment: a review. *Int. J. Mol. Sci.* 24, 6518. doi:10.3390/ijms24076518

Uruno, A., Matsumaru, D., Ryoke, R., Saito, R., Kadoguchi, S., Saigusa, D., et al. (2020). Nrf2 suppresses oxidative stress and inflammation in app knock-in Alzheimer's disease model mice. *Mol. Cell. Biol.* 40. doi:10.1128/MCB.00467-19

Vasile, A., Ciobîcă, A., Kamal, F. Z., Mavroudis, I., Plăvan, G., Selamoglu, Z., et al. (2024). Latest developments on the connections between diet and most of the neuropsychiatric disorders. *Bull. Integr. Psychiatry* 101, 105–119. doi:10.36219/BPI.2024.2.11

Vuralli, D., Wattiez, A.-S., Russo, A. F., and Bolay, H. (2019). Behavioral and cognitive animal models in headache research. *J. Headache Pain* 20, 11. doi:10.1186/s10194-019-0963-6

Webster, S. J., Bachstetter, A. D., Nelson, P. T., Schmitt, F. A., and Van Eldik, L. J. (2014). Using mice to model Alzheimer's dementia: an overview of the clinical disease and the preclinical behavioral changes in 10 mouse models. *Front. Genet.* 5, 88. doi:10.3389/fgene.2014.00088

Witte, A. V., Kerti, L., Margulies, D. S., and Flöel, A. (2014). Effects of resveratrol on memory performance, hippocampal functional connectivity, and glucose metabolism in healthy older adults. *J. Neurosci.* 34, 7862–7870. doi:10.1523/JNEUROSCI.0385-14.2014

Wojtunik-Kulesza, K. A., Oniszczuk, A., Oniszczuk, T., and Waksmundzka-Hajnos, M. (2016). The influence of common free radicals and antioxidants on development of Alzheimer's Disease. *Biomed. Pharmacother.* 78, 39–49. doi:10.1016/j.biopha.2015.12.024

Wu, C., Yang, L., Li, Y., Dong, Y., Yang, B., Tucker, L. D., et al. (2020). Effects of exercise training on anxious-depressive-like behavior in alzheimer rat. *Med. Sci. Sports Exerc.* 52, 1456–1469. doi:10.1249/MSS.0000000000002294

Yang, A. J. T., Bagit, A., and MacPherson, R. E. K. (2021). Resveratrol, metabolic dysregulation, and Alzheimer's disease: considerations for neurogenerative disease. *Int. J. Mol. Sci.* 22, 4628. doi:10.3390/ijms22094628

Zamani, K., Fakhri, S., Kiani, A., Abbaszadeh, F., and Farzaei, M. H. (2025). Rutin engages opioid/benzodiazepine receptors towards anti-neuropathic potential in a rat model of chronic constriction injury: relevance to its antioxidant and anti-inflammatory effects. *Naunyn. Schmiedeb. Arch. Pharmacol.* doi:10.1007/s00210-025-03842-4

Zarneshan, S. N., Arkan, E., Kiani, A., Hosseini, S. Z., Abbaszadeh, F., and Fakhri, S. (2025). Protective effects of polydatin amphiphilic chitosan nanocarriers against an aluminum chloride-induced model of Alzheimer's disease in rats: relevance to its anti-inflammatory and antioxidant effects. *Naunyn. Schmiedeb. Arch. Pharmacol.* doi:10.1007/s00210-024-03696-2

frontiers | Frontiers in Pharmacology

TYPE Original Research
PUBLISHED 25 April 2025
DOI 10.3389/fphar.2025.1546256

Check for updates

OPEN ACCESS

EDITED BY
Huazheng Liang,
Monash University - Southeast University Joint Research Institute (Suzhou), China

REVIEWED BY
Ankul Singh S,
ACS Medical College and Hospital, India
Prabhash Nath Tripathi,
University of Arkansas for Medical Sciences, United States

*CORRESPONDENCE
Hong-He Xiao,
xiaohh89@163.com
De-Qiang Dou,
deqiangdou@126.com
Zhi-Li Xu,
xu_zhili@126.com

†These authors have contributed equally to this work

RECEIVED 16 December 2024
ACCEPTED 10 April 2025
PUBLISHED 25 April 2025

CITATION
Bai J-M, Li T, Di X, Yang J-X, Cui Z-Q, Min D-Y, Shen Y-F, Shan S-Y, Zhang Y-X, Shi Y-J, Xu Z-L, Dou D-Q and Xiao H-H (2025) The active metabolite of Epimedii Folium promotes hippocampal neurogenesis in APP/PS1 mice by alleviating mitochondrial dysfunction. *Front. Pharmacol.* 16:1546256. doi: 10.3389/fphar.2025.1546256

COPYRIGHT
© 2025 Bai, Li, Di, Yang, Cui, Min, Shen, Shan, Zhang, Shi, Xu, Dou and Xiao. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

The active metabolite of Epimedii Folium promotes hippocampal neurogenesis in APP/PS1 mice by alleviating mitochondrial dysfunction

Jia-Ming Bai[1†], Tong Li[1†], Xue Di[1†], Jing-Xian Yang[1], Zhao-Qi Cui[1], Dong-Yu Min[2], Yu-Feng Shen[1], Si-Yu Shan[1], Ye-Xin Zhang[1], Yi-Jun Shi[1], Zhi-Li Xu[1]*, De-Qiang Dou[1]* and Hong-He Xiao[1]*

[1]School of Pharmacy, Liaoning University of Traditional Chinese Medicine, Dalian, China, [2]Traditional Chinese Medicine Experimental Center, Affiliated Hospital of Liaoning University of Traditional Chinese Medicine, Shenyang, China

Introduction: Alzheimer's disease (AD), the most common form of dementia, currently has no effective cure. Epimedii Folium (EF), a traditional Chinese medicine known as Yin-yang-huo, has demonstrated significant neuroprotective properties.

Methods: In this study, neural stem cells overexpressing the APPswe gene (APP-NSCs) were used as an *in vitro* AD model. The CCK-8, LDH, neurosphere formation, and BrdU incorporation assays were employed to identify the most effective bioactive metabolite of EF in promoting NSC proliferation. Subsequently, JC-1 staining, ATP quantification, and ROS assays were conducted to evaluate the protective effects of Icariside II (ICS II)-identified as the most effective metabolite-on mitochondrial function. APP/PS1 transgenic mice received an oral administration of 10 mg/kg ICS II for 7 weeks. Cognitive function was assessed using the Morris water maze and nest-building tests, while H&E and Nissl staining were used to evaluate brain tissue pathology. Transmission electron microscopy (TEM) examined the ultrastructural integrity of hippocampal neurons, immunofluorescence assessed hippocampal neurogenesis, and Western blotting quantified proteins involved in mitochondrial dynamics. Additionally, Rotenone (Rot), a mitochondrial respiratory chain inhibitor, was applied to disrupt mitochondrial function, allowing an evaluation of whether the neurogenesis-promoting effect of ICS II depends on maintaining mitochondrial structure and function.

Results and discussion: The results demonstrated that ICS II exhibited the strongest capacity to promote APP-NSC proliferation ($P < 0.01$, $\eta^2 = 0.845$), followed by Icariin and Icaritin. ICS II treatment significantly ameliorated cognitive deficits ($P < 0.01$, $\eta^2 = 0.883$), neuronal damage, and impairments in neurogenesis in adult APP/PS1 mice. Moreover, ICS II rescued mitochondrial damage by upregulating fusion proteins (Mfn1 and Mfn2) and downregulating fission proteins (p-Drp1/Drp1 and Mff); however, these protective effects were negated by Rot administration. In conclusion, this study identifies ICS II as one

of the most effective metabolites of EF, promoting hippocampal neurogenesis and alleviating mitochondrial dysfunction in APP/PS1 mice, thereby offering promising therapeutic potential for AD.

KEYWORDS

Alzheimer's disease, Epimedii folium, neural stem cell, hippocampal neurogenesis, mitochondria

1 Introduction

Alzheimer's disease (AD), also referred to as senile dementia, is the most prevalent form of dementia and significantly impacts the physical health and quality of life of elderly individuals. The pathogenesis of AD is complex and remains incompletely understood. Key hypotheses include the classic mechanisms of amyloid-beta (Aβ) deposition and tau protein hyperphosphorylation. As research progresses, additional contributing factors have been identified, such as neuroinflammation, oxidative stress, cholinergic deficits, excitotoxicity, mitochondrial dysfunction, genetic mutations, prion infections, gut microbiota dysbiosis, and impaired neurogenesis (Golde et al., 2018; Jia et al., 2020). Notably, neuronal degeneration and loss are common outcomes of these pathogenic factors, underscoring the critical importance of repairing damaged neurons as a therapeutic strategy for AD.

Under physiological conditions, neural stem cells (NSCs) located in the subgranular zone (SGZ) of the hippocampal dentate gyrus (DG) proliferate and differentiate to generate new neurons. These new neurons establish connections with pre-existing neural networks by projecting axons and dendrites to the CA1 and CA3 regions of the hippocampus, thereby replenishing and repairing damaged neurons and synaptic connections. This process, known as hippocampal neurogenesis, is crucial for maintaining synaptic plasticity and the structural and functional integrity of the hippocampus (Doetsch et al., 1999). Unfortunately, pathological factors such as Aβ plaques, neuroinflammation, and oxidative stress impair the neurogenesis mechanism in the brain of AD patient. As a result, the proliferation and differentiation of NSCs are significantly reduced, and newly generated intermediate neurons struggle to mature into fully functional neurons (Christie and Turnley, 2013; Mao et al., 2015; MS and JWinpenny, 2009; Waldau and Shetty, 2008). Hippocampal neurogenesis is impaired in AD patient, with the number of newly generated neurons being less than one-third of the normal level. Moreover, the number of new neurons continues to decline as the disease progresses (Choi and Tanzi, 2019; Moreno-Jiménez et al., 2019). In AD model mice, such as APP/PS1 and 3xTg mice, the number of NSCs and newly generated neurons in the hippocampus is significantly lower compared to age-matched wild-type mice. Conditional ablation of hippocampal neurogenesis further exacerbates cognitive impairments (Hollands et al., 2017; Mireille et al., 2019). Fortunately, small molecule compounds such as metformin (Fatt et al., 2015) and ethosuximide (Shashi Kant et al., 2015) have been shown to promote hippocampal neurogenesis, increase the number of hippocampal neurons, and restore cognitive function in AD model animals. Thus, restoring neurogenesis in the brain represents a promising strategy for treating AD. Promoting endogenous neuro-regeneration may offer an effective therapeutic approach for AD and other neurodegenerative diseases.

Contemporary ethnopharmacological investigations have identified multiple phytocompounds from ancestral pharmacopeias that demonstrate multimodal neuroprotection in AD pathology (Dey et al., 2017; Sharma et al., 2024; Siddiqui et al., 2024; Tripathi et al., 2024). Epimedii Folium (EF) is a widely used kidney-tonifying traditional Chinese medicine known for its effects in tonifying kidney-yang, strengthening bones and tendons, and dispelling wind-dampness. EF primarily contains flavonol glycosides with an eight-isopentenyl structure, including Icariin (ICA), Epimedin A, Epimedin B, Epimedin C, Icariside I (ICS I), Icariside II (ICS II), Icaritin, and Anhydroicaritin. According to the 2020 edition of the Pharmacopoeia of the People's Republic of China, Epimedin A, Epimedin B, Epimedin C, and ICA are quality control markers for EF, while Epimedin A, Epimedin B, Epimedin C, ICA, and ICS II are quality control markers for processed EF (Committee, 2020). Numerous studies have demonstrated that EF and its active metabolites can improve neural damages, enhance cognitive function in animal models, and exhibit multiple neuroprotective effects by modulating neuroinflammation, reducing oxidative stress, and increasing neurotrophic factors (Chen et al., 2016; Cho et al., 2017; Li et al., 2015; Wang et al., 2023). For example, ICA and Icaritin have been reported to protect against neuroinflammation in depressive mice through regulation of high mobility group protein (HMGB1) and NF-κB pathways (Liu et al., 2019). Epimedin B has been reported to protect against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridin (MPTP)-induced PD mice through regulation of apoptosis and endoplasmic reticulum stress (Zhang M. et al., 2022). In our previous study, we found that ICS II could promote hippocampal neurogenesis *via* activation of Wnt/β-catenin signaling pathway to improve the cognitive function of APP/PS1 mice (Xiao et al., 2022; Xiao et al., 2021). However, the comparative efficacy of these metabolites in promoting NSC proliferation and enhancing hippocampal neurogenesis

Abbreviations: AD, Alzheimer's disease; NSCs, neural stem cells; SGZ, subgranular zone; EF, Epimedii Folium; ICA, Icariin; ICS I, Icariside I, ICS II, Icariside II; NeuN, neuron-specific nuclear protein; APP-NSCs, neural stem cells overexpressing the APPswe mutant amyloid precursor protein; CCK-8, Cell Counting Kit-8; EGF, epidermal growth factor; bFGF, basic fibroblast growth factor, NEAA, non-essential amino acids; H&E, Hematoxylin-Eosin; DG, dentate gyrus; BSA, bovine serum albumin; SD, standard deviation; ANOVA, analysis of variance; TEM, transmission electron microscopy; BrdU, 5-bromo-2′-deoxyuridine; EdU, 5-ethynyl-2′-deoxyuridine; LDH, lactate dehydrogenase; Sox-2, sex-determining region; Mfn 1, mitofusin 1; Mfn 2, mitofusin 2; Mff, mitochondrial fission factor; Drp 1, dynaminrelated protein 1; Rot, rotenone; MWM, Morris water maze; mtDNA, mitochondrial DNA; ROS, reactive oxygen species; ATP, adenosine 5′-triphosphate.

specifically in AD models remains to be systematically investigated. Particularly, the identification of the most potent metabolite for neurogenic enhancement in AD pathogenesis has not been established. Furthermore, while emerging evidence implicates mitochondrial dynamics as a critical regulator of NSC fate determination, the potential mitochondrial-mediated mechanisms underlying EF metabolite-induced neurogenesis remain unexplored.

In this study, by using neural stem cells overexpressing the APPswe mutant amyloid precursor protein (APP-NSCs) as an AD-mimetic cellular model, we first screened the eight key metabolites of EF for their capacity to enhance NSC survival and proliferation under pathological Aβ stress. Building on these findings, we subsequently validated the therapeutic efficacy of the lead metabolite ICS II through *in vivo* assessment of hippocampal neurogenesis in adult APP/PS1 transgenic mice, with mechanistic investigations focusing on mitochondrial dynamics regulation.

2 Materials and methods

2.1 Materials

ICS II (Lot No. wkq19050410, purity ≥98%), ICA (Lot No. wkq21042608, purity ≥98%), Epimedin A (Lot No. wkq21060111, purity ≥98%), Epimedin B (Lot No. wkq21071613, purity ≥98%), Epimedin C (Lot No. wkq21041304, purity ≥98%), Icaritin (Lot No. wkq21042304, purity ≥98%), Anhydroicaritin (Lot No. wkq210430006, purity ≥98%) and ICS I (Lot No. wkq21071908, purity ≥98%) were obtained from the Weikeqi Biotech (Chengdu, China), and the structures of the eight metabolites are shown in Supplementary Figure S1 in the supplementary materials. B27 supplement (17,054-044), 0.25% Trypsin (25,200,056) and Dulbecco's Modified Eagle's Medium/F12 (DMEM/F12) were purchased from Gibco (Rockville, MD). The epidermal growth factor (EGF, 315-09) and basic fibroblast growth factor (bFGF, 100-18B) were purchased from Pepro Tech (Suzhou, China). The P/S (J19007) was purchased from Hyclone (United States). The 5-bromo-2′-deoxyuridine (BrdU, MB3126-2) was purchased from the Meilun Biotech (Dalian, China). The Cell count kit-8 (CCK-8) and the lactate dehydrogenase (LDH) kit were purchased from Nanjing Jiancheng Biotechnology, Co. (Nanjing, China). The JC-1 kit was purchased from Biosynthesis Biotech (Beijing China). 5-ethynyl-2′-deoxyuridine (EdU, 61,135-33–9) was obtained from Sigma-Aldrich (Shanghai, China). DAPI (C1005) was purchased from Beyotime Biotechnology (Shanghai, China). The Rabbit anti Sex-determining region y-box 2 (Sox-2, 20118-1-AP) and rabbit anti-NeuN (26975-1-AP) were obtained from proteintech (Wuhan, China). The rabbit anti-Mitofusin 1 (Mfn 1, bs-0557R), rabbit anti-Mitofusin 2 (Mfn 2, bs-2988R), rabbit anti-Mitochondrial fission factor (Mff, bs-7628R), rabbit anti-Dynaminrelated protein 1 (Drp 1, bs-4100R), and rabbit anti-p-Ser616-Drp 1 (bs-12702R) were purchased from Biosynthesis Biotech (Beijing, China). Rabbit anti-GAPDH (WL01114) were purchased from Wanlei Biotech (Shenyang, China). The Goat anti-Rabbit IgG antibody conjugated with Alexa Fluor® 488 (159082) was obtained from Jackson ImmunoResearch Inc. (West Grove, PA, United States).

2.2 Culture of NSCs and transfection with APPswe gene

Hippocampal NSCs were isolated from the hippocampus of neonatal C57BL/6 mice and cultured in proliferation medium consisting of DMEM/F12 supplemented with 20 ng/mL EGF, 20 ng/mL bFGF, 1% B27, 1% non-essential amino acids (NEAA), 1% GlutaMAX, and 0.5% penicillin/streptomycin, as previously described (Duan et al., 2015). To establish an AD cell model, hippocampal NSCs were transfected with either the APP-GFP lentiviral vector or a GFP lentiviral vector (negative control), as detailed in our prior studies (Xiao et al., 2020). The third passage of NSCs was used for subsequent experiments.

2.3 Cell viability and LDH assays

Cell viability was assessed using the CCK-8 assay. Briefly, a single-cell suspension was seeded into 96-well plates at a density of approximately 1×10^6 cells/mL and cultured in proliferation medium for 24 h. Subsequently, the cells were treated with ICS II, ICA, Epimedin A, Epimedin B, Epimedin C, Icaritin, Anhydroicaritin, or ICS I at concentrations of 0, 0.25, 0.5, and 1 μM for an additional 24 h. After treatment, 10 μL of CCK-8 solution was added to each well, and the plates were incubated at 37°C for 4 h. Absorbance was measured at 450 nm using a microplate reader (MR-96, Mindray, Shenzhen, China).

The LDH assay was conducted to evaluate the cytoprotective effects of the aforementioned metabolites (ICS II, ICA, Epimedin A, Epimedin B, Epimedin C, Icaritin, Anhydroicaritin, ICS I) on APP-NSCs, following the manufacturer's instructions. Absorbance at 450 nm was measured using the same microplate reader.

2.4 Neurosphere formation assay

Following dissociation of the neurospheres containing NSCs, cells were seeded at a density of 1×10^5 cells/mL in a 24-well plate and treated with the selected active metabolite from Section 2.4, which demonstrated the most effective anti-cell damage properties. The cells were cultured under proliferative conditions, with media changes every 2 days. On day 7, neurosphere morphology and quantity were assessed using a microscope, and images were captured. The number of neurospheres was quantified using ImageJ software, and their diameters were measured.

2.5 BrdU incorporation assay

Single-cell suspension of NSCs was plated into a 96-well plate at a density of 1×10^6 cells/mL and cultured in proliferation medium, with or without ICS II (1 μM), for 24 h. After 24 h, all cells were labeled with BrdU (20 μM) for 12 h, followed by fixation with 4% paraformaldehyde. The cells were then processed for immunofluorescent analysis as described by Shashi Kant (Shashi Kant et al., 2015).

2.6 JC-1 staining

GFP-NSCs and APP-NSCs cells in the logarithmic growth phase were divided into four groups: GFP-NSCs + PBS, GFP-NSCs + ICS II, APP-NSCs + PBS, and APP-NSCs + ICS II. Cells were seeded at a density of 5×10^3 cells/well in a 96-well plate and cultured for 24 h. After incubation, the culture medium was removed, and equal volumes of H-DMEM and JC-1 staining solution were added. The plate was gently mixed and incubated in the dark at 37°C for 20 min. During the incubation, the 5× JC-1 staining buffer was diluted with four volumes of ddH_2O and kept on ice. After the incubation period, the staining solution was discarded, and the cells were washed twice with freshly prepared 1× JC-1 staining buffer. Finally, 100 μL of H-DMEM was added, and the cells were observed under a microscope.

2.7 Reactive oxygen species (ROS) assay

The levels of ROS in NSCs and brain tissues were measured using the DCFH-DA fluorescent probe (E004-one to one, Nanjing Jiancheng Bioengineering Institute, Nanjing, China), as described in a previous study (Wen et al., 2021). NSCs from different experimental groups were treated with DCFH-DA solution for 30 min, and then collected, washed three times with sterile PBS. Fluorescence intensity was measured using an automated microplate reader.

2.8 Adenosine 5′-triphosphate (ATP) assay

The ATP levels in NSCs and brain tissues were measured using a firefly luciferase-based assay (S0027, Beyotime Biotechnology, Shanghai, China), as described in the previous study (Liu et al., 2021). NSCs cultured *in vitro* or brain tissues collected from mice across different groups were lysed, and the resulting supernatant was used to detect ATP levels according to the manufacturer's instructions.

2.9 Animals and drug administration

Male APP/PS1 mice were purchased from Jiangsu Huachuang Sino Pharmaceutical Technology Co., Ltd. (SCXK [Su] 2020-0000), and male C57BL/6J mice were obtained from Liaoning Changsheng Biotechnology Co., Ltd. (SCXK [Liao] 2020-0001). All mice were housed in transparent brown plastic cages within a controlled environment (temperature: 25°C ± 1°C, humidity: 65% ± 5%, and a 12-hour light/dark cycle) with *ad libitum* access to food and water. All experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of Liaoning University of Traditional Chinese Medicine (Approval No. SYXK [Liao] 2021-0001) and were conducted in accordance with the ARRIVE guidelines.

ICS II was prepared in a 0.5% sodium carboxymethyl cellulose (CMC-Na) aqueous solution. EdU (CAS 61135-33–9, Sigma-Aldrich, Shanghai, China) was dissolved in normal saline, while Rotenone (Rot, Aladdin, China, F2122218) was dissolved in a minimal volume of dimethyl sulfoxide (DMSO) and then diluted with sunflower seed oil. Six-month-old male APP/PS1 mice, weighing approximately 30 g, were used as the AD model, with wild-type C57BL/6J littermates serving as normal controls. APP/PS1 mice were randomly divided into five groups: Model, ICS II, Rotenone (Rot), ICS II + Rot, and Normal Control. The ICS II group received 10 mg/kg ICS II via oral gavage (i.g.) according to our previous study (Xiao et al., 2022), the Rot group was administered 2.5 mg/kg Rot via intraperitoneal injection (i.p.), and the ICS II + Rot group received both treatments (10 mg/kg ICS II via i.g. and 2.5 mg/kg Rot via i.p.). An additional ten C57BL/6J mice were included as normal controls (n = 10 per group). Mice in the Model, Control, and Rot groups were administered an equivalent volume of CMC-Na daily via oral gavage for 49 consecutive days.

After 4 weeks of treatment, the animals were intraperitoneally injected with EdU (100 mg/kg) to label proliferating cells in the hippocampus (Fatt et al., 2015). Following this, treatment continued for another 3 weeks with their respective drug regimens. At the end of the 49-day treatment period, the mice were euthanized, and brains were collected for immunofluorescence (IF) staining and Western blot analysis.

2.10 Nest-building test

The nest-building test was used to evaluate the self-care capacity of the mice, as previously described (Torres-Lista and Giménez-Llort, 2013; Xiao et al., 2022). The test was conducted on day 21 of drug administration, with assessments performed once daily for three consecutive days. Mice were housed individually in cages with corn cob bedding and provided with two 5 cm × 5 cm square sheets of sanitary paper (10 sheets per stack) at the feeding port. Nest-building behavior was scored at 0 h, 24 h, 36 h, and 72 h using a five-point scale. The scoring criteria were as follows: one point for no noticeable interaction or tearing of the paper; two points for partial tearing; three points for most of the paper torn, but not forming a distinct nest shape; four points for the paper torn into pieces and arranged into a relatively flat nest-like structure; and five points for the paper torn into pieces and built into a complete or nearly complete nest-like structure.

2.11 Morris water maze (MWM) test

The MWM test was used to evaluate the learning and memory abilities of the mice, as previously described. After completing the nest-building test, the MWM was conducted with daily assessments over six consecutive days. The test consisted of two phases: training trials and a probe trial. During the training phase, a platform was fixed in a specific location within the pool, and mice underwent two trials per day for five consecutive days. The time spent and the swimming distance to reach the escape platform were recorded to assess spatial learning ability. In the probe trial, the platform was removed, and the time spent in the target quadrant, as well as the number of crossings over the platform's original location, were measured to evaluate spatial memory (n = 10).

2.12 H&E staining and Nissl staining

H&E Staining: Three mice per group were deeply anesthetized, and their brains were harvested and fixed in 4% formaldehyde

solution for 24 h. After dehydration and clarification, the brains were embedded in paraffin, sectioned, and deparaffinized in xylene. The sections were then dehydrated through a graded ethanol series (100%, 95%, 80%, and 70%). Following this, the sections were stained with hematoxylin for 7 min, differentiated in 1% hydrochloric acid-alcohol for 10 s, and stained with eosin for 2 min. After dehydration, the sections were cleared in xylene and mounted with neutral resin. Pathological changes in the cortex and hippocampus were observed under a microscope.

Nissl Staining: Paraffin-embedded brain sections were deparaffinized and incubated in toluidine blue staining solution at 50°C in the dark for 7 min. The reaction was stopped when Nissl bodies became clearly visible. The sections were then rinsed twice in distilled water for 20 s each, dehydrated, cleared in xylene, and mounted with neutral resin. Alterations in the cortex and the number of Nissl bodies were observed under a microscope.

2.13 Immunofluorescence staining (IF) and EdU staining

Brain tissue sections from each mouse group were deparaffinized through a graded ethanol series and subjected to antigen retrieval in sodium citrate buffer. The sections were incubated with 2 mg/mL glycine for 10 min, followed by permeabilization with 0.5% Triton X-100 solution at room temperature for 10 min. Apollo staining solution was applied and incubated at room temperature for 30 min. After discarding the staining solution, sections were further permeabilized twice with 0.5% Triton X-100 for 10 min each. The sections were then washed with methanol for 5 min and blocked with 5% BSA solution at room temperature for 30 min. Primary antibodies (Sox-2: 1:150, NeuN: 1:200) were added and incubated overnight at 4°C. The following day, after washing with PBS three times, the sections were incubated with 488-labeled goat anti-rabbit secondary antibodies (1:100) at room temperature in the dark for 2 h. After three PBS washes, the sections were counterstained with DAPI-containing fluorescence quenching mounting medium, cover-slipped, and observed and photographed under an inverted fluorescence microscope. EdU staining was performed using the Cell-Light Apollo 567 Stain Kit (#C10371-1, Guangzhou RiboBio, Guangzhou, China) according to the manufacturer's protocol.

2.14 Transmission electron microscopy (TEM) test

Three mice from each group were deeply anesthetized, and their brains were harvested to isolate hippocampal tissue from the ischemic side. The hippocampal CA1 region was dissected into 1 mm × 1 mm × 1 mm tissue blocks, which were fixed in electron microscopy fixative at 4°C for 24 h. After dehydration through a graded ethanol series and acetone, the samples were infiltrated, embedded, and sectioned into 70 nm ultrathin slices. The sections were double-stained with uranyl acetate and lead citrate before being examined under a TEM, and images were captured for analysis.

2.15 Western blot assays

Three mice from each group were deeply anesthetized, and the ischemic brain tissue was harvested and immediately frozen in liquid nitrogen. The tissue was then pulverized into a fine powder, and total protein was extracted using RIPA lysis buffer supplemented with 1% phosphatase inhibitor and 1% protease inhibitor. Protein concentrations were determined using the BCA method. Subsequently, proteins were separated by SDS-PAGE, transferred to PVDF membranes using wet transfer, and blocked at room temperature for 1.5 h. The membranes were incubated overnight at 4°C with primary antibodies against Mff (1:500), Drp1 (1:1,000), p-Drp1 (1:1,000), Mfn1 (1:500), Mfn2 (1:500), and GAPDH (1:1,000). The following day, the membranes were incubated with HRP-conjugated secondary antibodies for 2 h. Protein bands were visualized using a gel imaging system, and grayscale values were analyzed using ImageJ software.

2.16 Statistical analysis

Statistical analysis was performed using GraphPad Prism 9.4. The optical density in Western blot experiments was quantified with ImageJ software. For comparisons across multiple groups, one-way analysis of variance (ANOVA) followed by Dunnett's post-hoc test was applied. For the escape latency data in MWM test, two-way ANOVA followed by Bonferroni post-hoc test was conducted. Results are presented as mean ± standard deviation (SD), with significance set at $P < 0.05$, indicating statistical significance.

3 Results

3.1 Protective effects of eight active metabolites from Epimedium Folium on APP-NSCs

The CCK-8 assay revealed that APP-NSCs overexpressing the APP gene exhibited significantly reduced cell viability compared to GFP-NSCs (Figures 1A–H, $^{****}P < 0.0001$ vs GFP-NSCs, $\eta^2 = 0.819$). Treatment with the eight active metabolites for 24 h partially restored cell viability, with ICS II, ICA, and Icaritin showing the most pronounced effects. These three metabolites demonstrated statistically significant improvements compared to the untreated APP-NSCs group (Figures 1A–H, ICS II: $^{***}P < 0.001$, $\eta^2 = 0.804$; ICA: $^{**}P < 0.01$, $\eta^2 = 0.859$; Icaritin: $^{**}P < 0.01$, $\eta^2 = 0.853$ at 1 μM). Similarly, all eight metabolites reduced LDH release from APP-NSCs, with ICS II, ICA, and Icaritin showing the greatest reductions, which were also statistically significant compared to the APP-NSCs group (Figures 1I–P, ICS II: $^{****}P < 0.0001$, $\eta^2 = 0.827$; ICA: $^{**}P < 0.01$, $\eta^2 = 0.676$; Icaritin: $^{*}P < 0.05$, $\eta^2 = 0.623$ at 1 μM). These findings indicate that ICS II, ICA, and Icaritin provide substantial protective effects for APP-NSCs. Consequently, these three metabolites were selected for subsequent NSC proliferation experiments. Notably, at the concentration of 0.25 μM, only ICS II demonstrated significant neuroprotective effects (cell viability: $P < 0.05$, $\eta^2 = 0.804$; LDH release: $P < 0.05$, $\eta^2 = 0.827$), whereas neither ICA nor Icaritin exhibited neuroprotective activity at the same

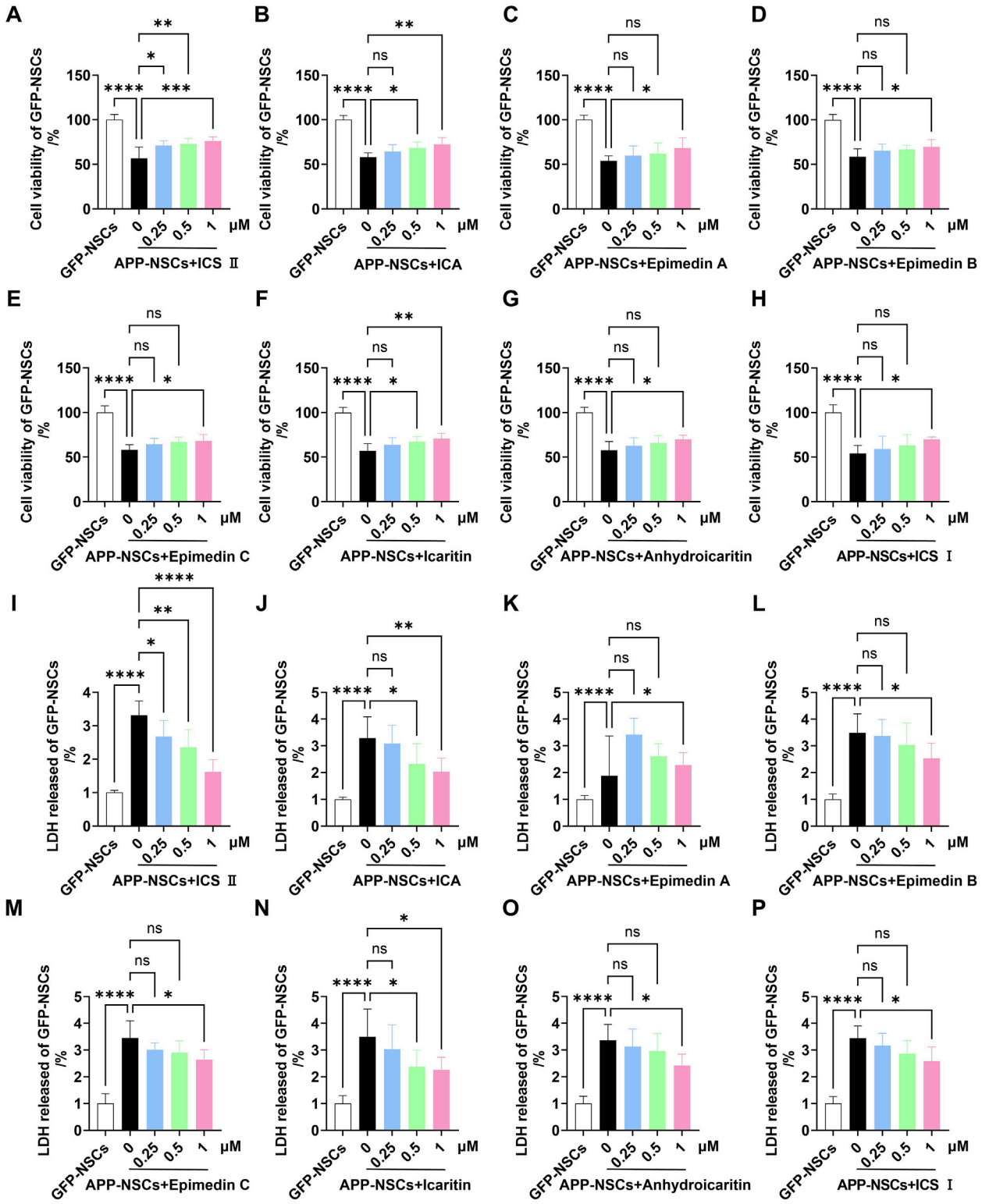


FIGURE 1
Protective effects of eight active metabolites from *Epimedium Folium* on APP-NSCs Monolayer APP-NSCs were cultured in proliferation medium for 24 h, followed by exposure to ICS II (0.25, 0.5, and 1 μM), ICA (0.25, 0.5, and 1 μM), Epimedin A (0.25, 0.5, and 1 μM), Epimedin B (0.25, 0.5, and 1 μM), Epimedin C (0.25, 0.5, and 1 μM), Icaritin (0.25, 0.5, and 1 μM), Anhydroicaritin (0.25, 0.5, and 1 μM), or ICS I (0.25, 0.5, and 1 μM) for another 24 h. Cell viability was assessed using the CCK-8 assay, and LDH leakage was measured by the LDH kit. **(A–H)** The viability of APP-NSCs exposed to the metabolites was measured by CCK-8 assay **(A)**: ICS II, **(B)** ICA, **(C)** Epimedin A, **(D)** Epimedin B, **(E)** Epimedin C, **(F)** Icaritin, **(G)** Anhydroicaritin, **(H)** ICS **(I)**. **(I–P)** LDH leakage in APP-NSCs exposed to the metabolites was assessed by LDH assay. Statistical significance: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; $^{****}P < 0.0001$; ns: no significant difference. Data are presented as mean ± SD (n = 6/group). Data were analyzed by one-way ANOVA.

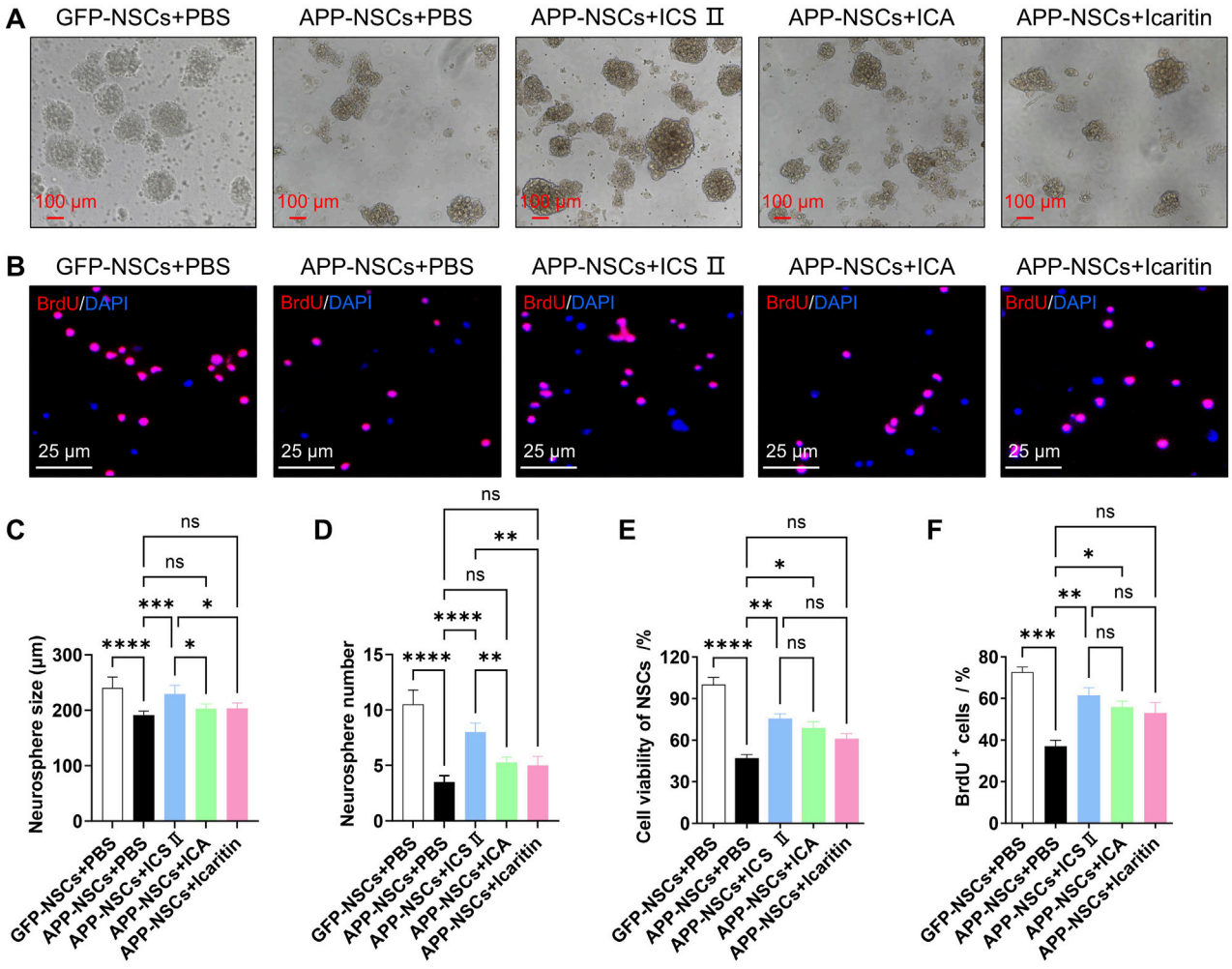


FIGURE 2
Effects of ICS II, ICA, and Icaritin on APP-NSCs proliferation APP-NSCs were cultured in proliferation medium and exposed to ICS II (1 μM), ICA (1 μM), or Icaritin (1 μM) for 7 days. Neurosphere images were captured, and their diameters were measured using ImageJ software. **(A)** Representative micrographs of neurospheres from each group. Scale bar = 100 μm. **(B)** Representative micrographs of BrdU$^+$ proliferating cells. Scale bar = 25 μm. **(C)** Quantitative analysis of neurosphere diameters. **(D)** Quantification of neurosphere numbers. **(E)** Viability of BrdU$^+$ proliferating cells. **(F)** Quantification of BrdU$^+$ proliferating cells. Statistical significance: $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; $^{****}P < 0.0001$; ns: no significant difference. Data are presented as mean ± SD (n = 6/group). Data were analyzed by one-way ANOVA.

concentration (cell viability: $P > 0.05$, $\eta^2 = 0.859$; LDH release: $P > 0.05$, $\eta^2 = 0.676$). These findings suggest that ICS II may represent the most potent neuroprotective metabolite in EF.

3.2 Effects of ICS II, ICA, and icaritin on the proliferation of APP-NSCs

As shown in Figure 2, the APP-NSCs + PBS group exhibited significantly smaller neurosphere diameters and fewer neurospheres compared to the GFP-NSCs + PBS group (Figures 2A,C,D, $^{****}P < 0.0001$, C: $\eta^2 = 0.696$, D: $\eta^2 = 0.920$). Treatment with 1 μM ICS II significantly increased both the neurosphere diameter and number in APP-NSCs (Figures 2A,C,D, $^{****}P < 0.0001$, $^{***}P < 0.001$, C: $\eta^2 = 0.696$, D: $\eta^2 = 0.920$ vs APP-NSCs + PBS), while ICA and Icaritin exhibited no improvement in the neurosphere diameter or number of APP-NSCs (Figures 2A,C,D, $P > 0.05$, C: $\eta^2 = 0.696$, D: $\eta^2 = 0.920$).

Similarly, the proportion of BrdU-positive cells in the APP-NSCs + PBS group was markedly reduced compared to the GFP-NSCs + PBS group (Figures 2B,E,F, $^{****}P < 0.0001$, $^{***}P < 0.001$, E: $\eta^2 = 0.907$, F: $\eta^2 = 0.845$). Treatment with 1 μM ICS II or ICA significantly increased the proportion of BrdU-positive cells (Figures 2B,E,F, ICS II: $^{**}P < 0.01$ vs APP-NSCs + PBS, ICA: $^*P < 0.05$ vs APP-NSCs + PBS, E: $\eta^2 = 0.907$, F: $\eta^2 = 0.845$). Combined with the results of CCK-8 and LDH leakage in the 3.1 section, ICS II was determined as the most potential agent for subsequent experimental studies.

3.3 Protective effects of ICS II on mitochondria of APP-NSCs

As illustrated in Figure 3, the APP-NSCs group showed a significant reduction in mitochondrial membrane potential

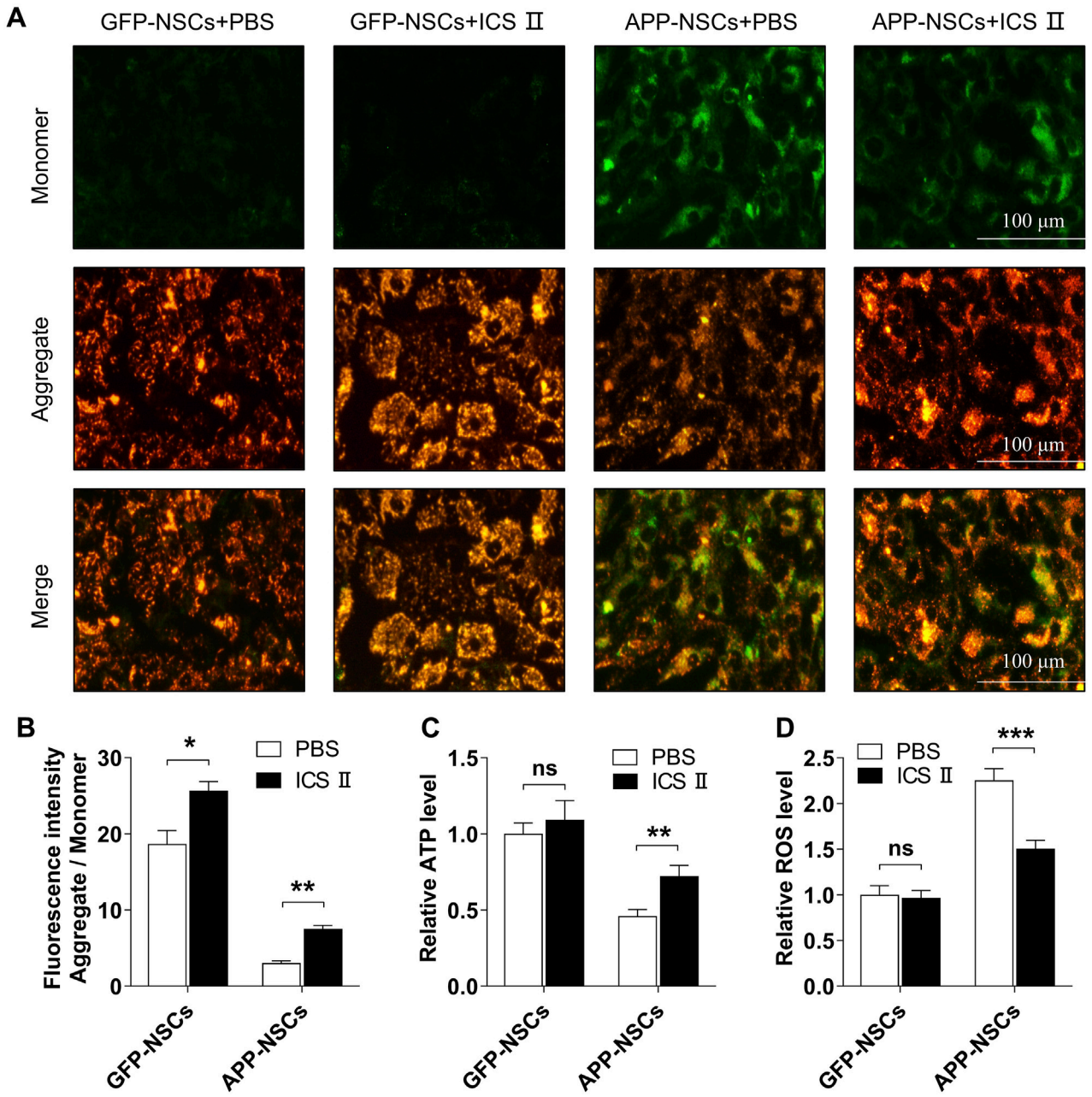


FIGURE 3
Effects of ICS II on mitochondrial function in APP-NSCs **(A)** Representative image of JC-1 staining; **(B)** Ratio of fluorescence intensity (Aggregate/Monomer); **(C)** ATP relative level. **(D)** ROS relative level. Statistical significance: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$; ns: no significant difference. Data are presented as mean ± SD (n = 6/group). Data were analyzed by T-test.

compared to the GFP-NSCs group, as evidenced by decreased JC-1 aggregates and increased JC-1 monomers (Figure 3A). Treatment with 1 μM ICS II restored mitochondrial membrane potential by increasing the JC-1 aggregate-to-monomer fluorescence intensity ratio (Figure 3B, GFP-NSCs + ICS II: $^{*}P < 0.05$ vs GFP-NSCs + PBS group, $\eta^2 = 0.729$; APP-NSCs + ICS II: $^{**}P < 0.01$ vs APP-NSCs + PBS group, $\eta^2 = 0.946$). Furthermore, treatment with 1 μM ICS II significantly improved the ATP level (Figure 3C $^{**}P < 0.01$, $\eta^2 = 0.509$), and suppressed the ROS level (Figure 3D $^{***}P < 0.001$, $\eta^2 = 0.696$) compared with that in the APP-NSCs + PBS group. These results demonstrate that 1 μM ICS II effectively alleviates mitochondrial damage.

3.4 ICS II enhances learning, memory, and self-care capacity in APP/PS1 mice

The MWM test was conducted to evaluate learning and memory abilities across different mouse groups. The typical swimming paths of each group are shown in Figure 4A. During training, escape latency

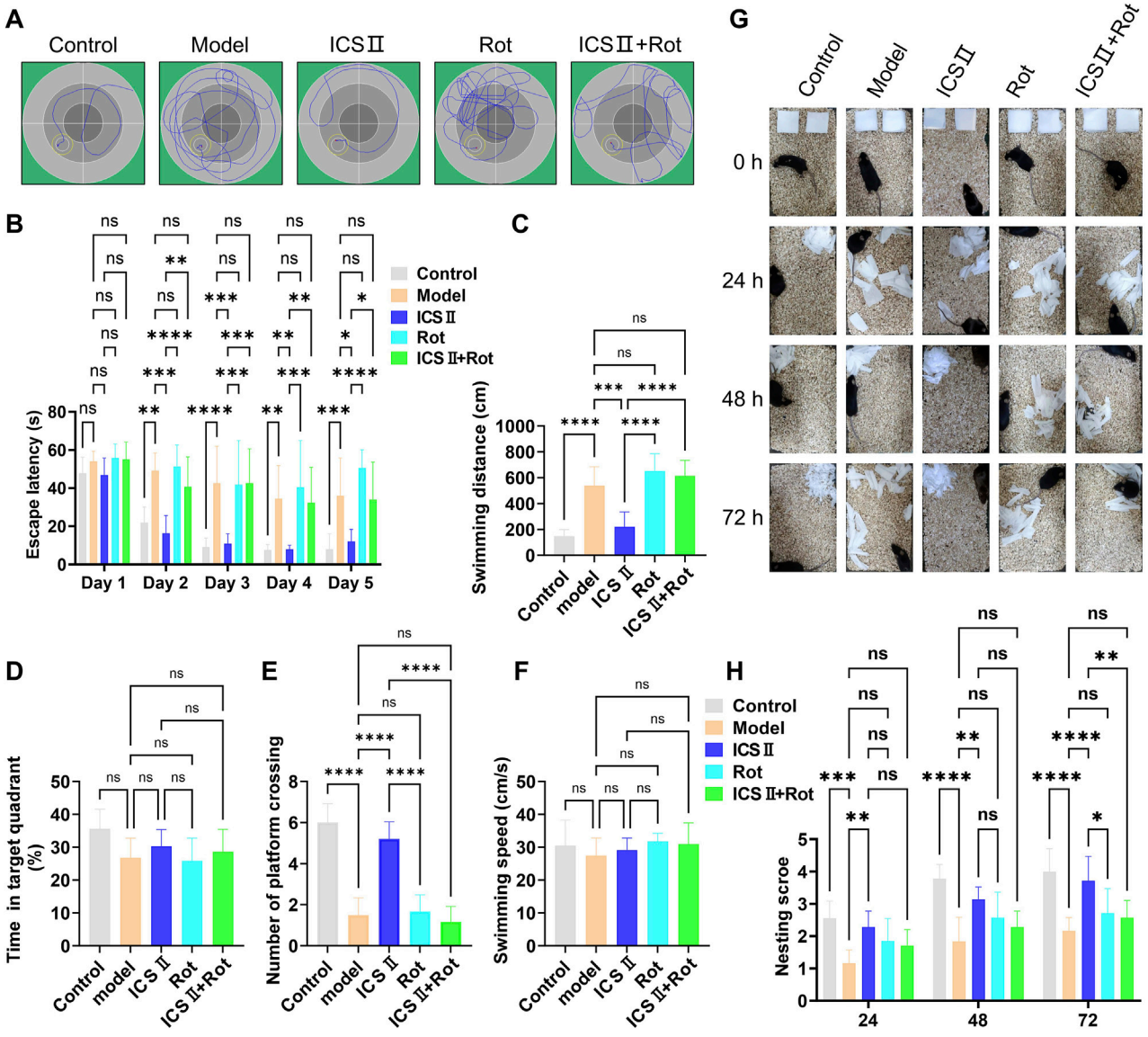


FIGURE 4
ICS II enhances learning, memory, and self-care capacity in APP/PS1 Mice **(A–F)** The Morris water maze (MWM) test was performed to assess cognitive function in each group of mice. **(A)** Typical swimming paths during the final training trial. **(B)** Escape latency during training trials. **(C)** Swimming distance before crossing the platform. **(D)** Percentage of time spent in the target quadrant. **(E)** Number of platform crossings. **(F)** Swimming speed during probe trials. **(G,H)** The nest building test was conducted following the MWM test to assess daily living activities. **(G)** Representative images of nests at 0, 24, 28, and 72 h. **(H)** Nesting score for each group. Data are presented as mean ± SD (n = 10 mice/group). Statistical significance: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; $^{****}P < 0.0001$. Data were analyzed by repeated-measures two-way ANOVA with Bonferroni post-hoc test.

gradually decreased in all groups, but the reduction was notably less pronounced in the Model group. From the second training day onward, the Model group demonstrated significantly prolonged escape latencies (Figure 4B, Day 2: $^{**}P < 0.01$, Day 3: $^{****}P < 0.0001$, Day 4: $^{**}P < 0.01$, Day 5: $^{***}P < 0.001$ vs Control group), increased swimming distances (Figure 4C, $^{****}P < 0.0001$ vs Control group, $\eta^2 = 0.797$), and fewer platform crossings (Figure 4E, $^{****}P < 0.0001$ vs Control group, $\eta^2 = 0.883$), indicating cognitive deficits in APP/PS1 mice. Treatment with ICS II significantly shortened escape latency (Figure 4B, Day 2: $^{***}P < 0.001$, Day 3: $^{***}P < 0.001$, Day 4: $^{**}P < 0.01$, Day 5: $^{*}P < 0.05$ vs Model group), reduced swimming distance (Figure 4C, $^{***}P < 0.001$ vs Model group, $\eta^2 = 0.797$), and enhanced platform crossings (Figure 4E, $^{****}P < 0.0001$ vs Model group, $\eta^2 = 0.883$), indicating that ICS II effectively improved learning and memory in APP/PS1 mice. However, these improvements were absent in the Rot and ICS II + Rot groups, with no significant differences compared to the Model group ($^{ns}P > 0.05$, Figure 4C: $\eta^2 = 0.797$; Figure 4D: $\eta^2 = 0.294$; Figure 4E: $\eta^2 = 0.883$; Figure 4F: $\eta^2 = 0.650$). This suggests that the cognitive benefits of ICS II depend on intact mitochondrial function.

The nesting experiment was used to assess the preserved executive function and self-care capacity. The Model group failed to complete nest-building within 72 h (Figures 4G,H, 24 h: $^{****}P < 0.0001$, 48 h: $^{***}P < 0.001$, 72 h: $^{***}P < 0.001$ vs Control group). In contrast, ICS II-treated mice exhibited nesting behavior within 24 h and formed relatively complete nests by 72 h, shredding paper into fragments to build the structure (Figure 4G). The nesting scores of the ICS II group were

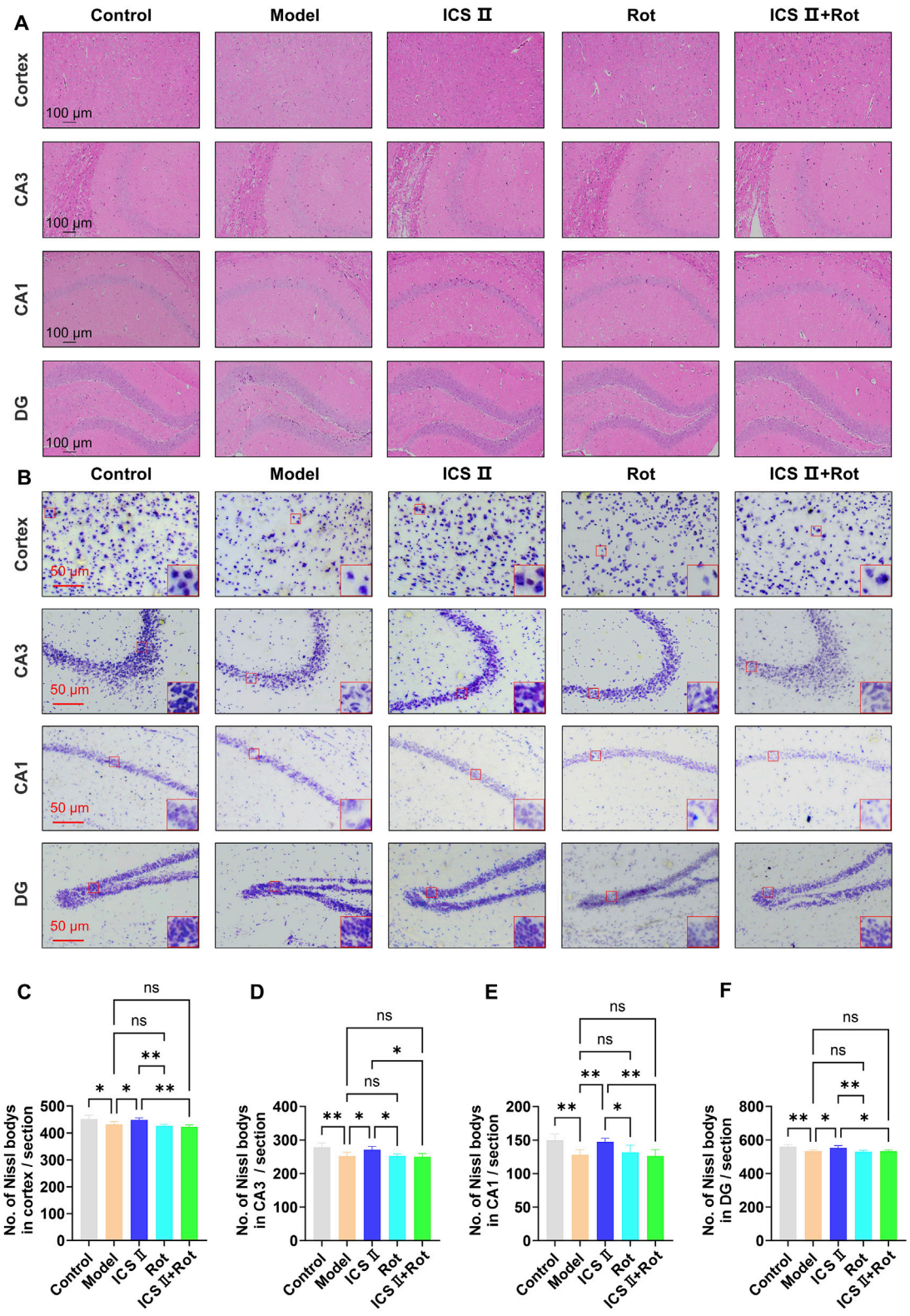


FIGURE 5
ICS II mitigates neuronal pathological damage in APP/PS1 Mice **(A)** Representative images of H&E staining (scale bar = 100 μm). **(B)** Representative images of Nissl staining in the cortex, hippocampal CA3, CA1, and DG regions (scale bar = 50 μm). **(C–F)** Quantitative analysis of Nissl bodies in the cortex **(C)**, hippocampal CA3 **(D)**, CA1 **(E)**, and DG **(F)** regions. Statistical significance: $^{*}P < 0.05$; $^{**}P < 0.01$; ns: no significant difference. Data are presented as mean ± SD (n = 3 mice/group). Data were analyzed by one-way ANOVA.

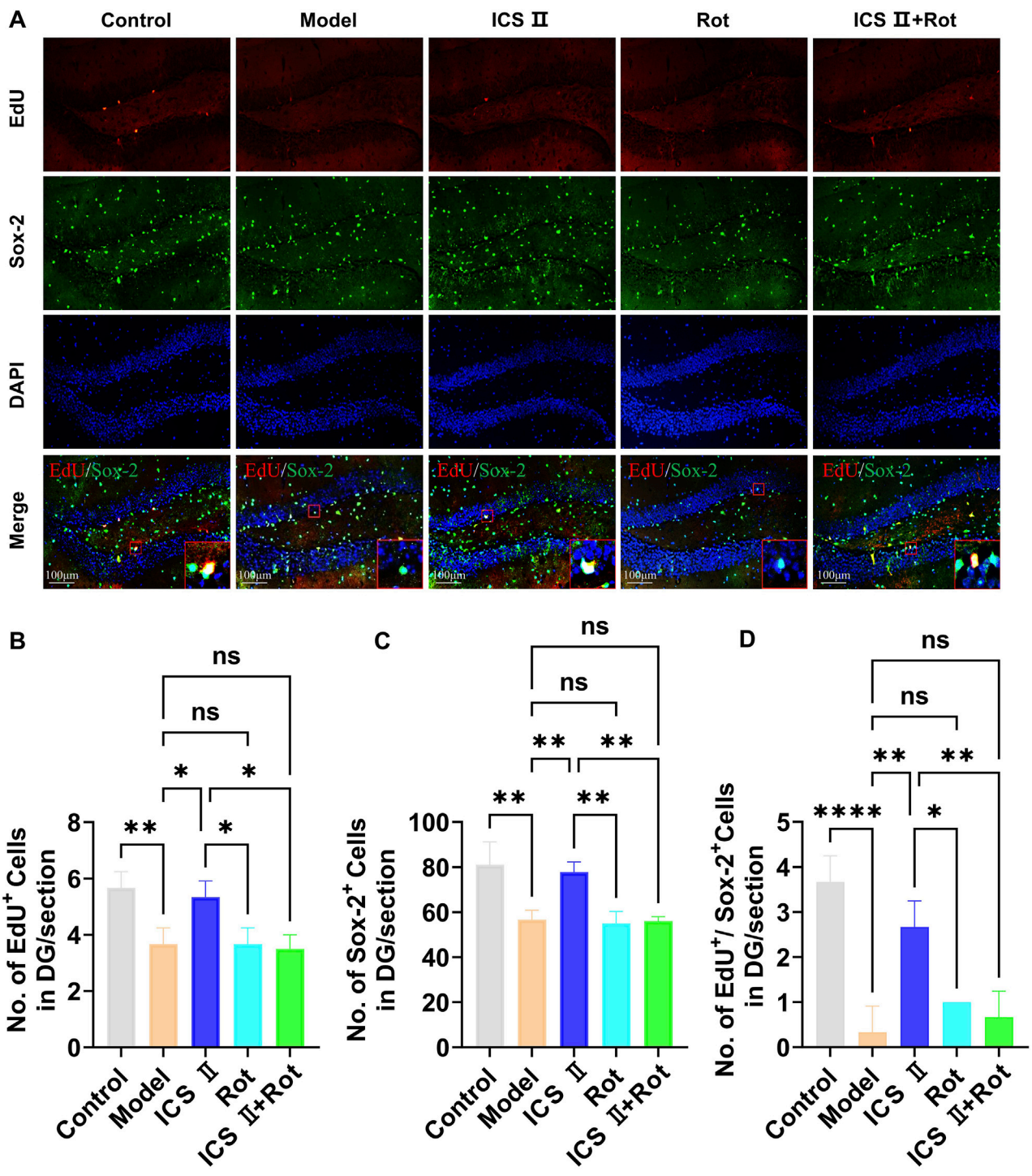


FIGURE 6
ICS II promotes hippocampal NSC proliferation in APP/PS1 Mice **(A)** Representative images of Sox-2 expression in the hippocampal DG region. Scale bar = 100 μm. **(B)** Quantification of EdU⁺ cells. **(C)** Quantification of Sox-2⁺ cells. **(D)** Quantification of EdU⁺/NeuN⁺ cells. Statistical significance: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{****}P < 0.0001$; ns: no significant difference. Data are presented as mean ± SD (n = 3 mice/group). Data were analyzed by one-way ANOVA.

significantly higher than those of the Model group (Figure 4H, 24 h: $^{**}P < 0.01$, 48 h: $^{**}P < 0.01$, 72 h: $^{****}P < 0.0001$). However, co-treatment with Rot significantly reduced nesting scores (Figure 4H, 72 h: $^{**}P < 0.01$ ICS II + Rot vs ICS II group). These results indicate that ICS II enhances the preserved executive function and self-care capacity of APP/PS1 mice, but this effect is hindered by Rot-induced mitochondrial dysfunction.

3.5 ICS II mitigates neuronal pathological damage in APP/PS1 mice

H&E staining revealed that neurons in the cortex and hippocampus of the Model group were loosely arranged, displayed irregular morphology, and exhibited hyperchromatic,

shrunken nuclei (Figure 5A). In contrast, ICS II treatment improved neuronal organization, resulting in clearer layering, uniform cytoplasmic and nuclear staining, and a significant reduction in nuclear condensation (Figure 5A). Consistently, Nissl staining showed a marked reduction in the number of Nissl bodies in the cortex (Figures 5B,C, $^{*}P < 0.05$ vs Control group, $\eta^2 = 0.638$), hippocampal CA3 (Figures 5B,D, $^{**}P < 0.01$ vs Control group, $\eta^2 = 0.611$), CA1 (Figures 5B,E, $^{**}P < 0.01$ vs Control group, $\eta^2 = 0.605$) and dentate gyrus (DG) regions of the Model group (Figures 5B,F, $^{**}P < 0.01$ vs Control group, $\eta^2 = 0.628$). Treatment with ICS II significantly increased the number of Nissl bodies compared to the Model group, partially ameliorating neuronal damage (Figures 5B–F, Cortex: $^{*}P < 0.05$, $\eta^2 = 0.638$; CA3: $^{*}P < 0.05$, $\eta^2 = 0.611$; CA1: $^{**}P < 0.01$, $\eta^2 = 0.605$; DG: $^{*}P < 0.05$, $\eta^2 = 0.628$). However, neuronal damage remained unaltered in the Rot and Rot + ICS II groups compared to the Model group (Figures 5B–F, $^{ns}P<0.05$). These results indicate that ICS II effectively alleviates hippocampal neuronal pathological damage in APP/PS1 mice, but its neuroprotective effects are blocked by Rot-induced mitochondrial dysfunction.

3.6 ICS II promotes hippocampal NSCs proliferation in APP/PS1 mice

EdU is a thymidine analog that can integrate into replicating DNA molecules during cell proliferation, thereby marking proliferating cells. Sox-2 is a specific marker for NSCs. In this study, we employed $EdU^+/Sox\text{-}2^+$ immunofluorescence double staining to evaluate NSCs proliferation. As shown in Figure 6, the number of EdU^+ positive cells (Figures 6A,B, $^{**}P < 0.01$ vs Control group, $\eta^2 = 0.805$), $Sox\text{-}2^+$ positive cells (Figures 6A,C, $^{**}P < 0.01$ vs Control group, $\eta^2 = 0.852$), and $EdU^+/Sox\text{-}2^+$ double-positive cells (Figures 6A,D, $^{****}P < 0.0001$ vs Control group, $\eta^2 = 0.902$) in the hippocampal DG region were significantly reduced in the Model group, indicating impaired hippocampal NSCs proliferation in APP/PS1 mice. However, following 4 weeks of ICS II treatment, the numbers of EdU^+ positive cells ($^{*}P < 0.05$, $\eta^2 = 0.805$), Sox-2 positive cells ($^{**}P < 0.01$, $\eta^2 = 0.852$), and $EdU^+/Sox\text{-}2^+$ double-positive cells ($^{**}P < 0.01$, $\eta^2 = 0.902$) were significantly increased compared to Model group. Importantly, Rot aggravated the proliferation impairment of hippocampal neural stem cells in APP/PS1 mice and attenuated the proliferative effects of ICS II ($EdU^+/Sox\text{-}2^+$: $^{**}P < 0.01$, ICS II + Rot vs ICS II; $^{ns}P > 0.05$, ICS II + Rot vs Model group, $\eta^2 = 0.902$). These findings suggest that ICS II promotes hippocampal neural stem cell proliferation in APP/PS1 mice, and this effect is dependent on mitochondrial function.

3.7 ICS II promotes hippocampal NSCs differentiation in APP/PS1 mice

The differentiation of NSCs was assessed using EdU/NeuN immunofluorescence double staining. As shown in Figure 7, compared to the Control group, the number of NeuN-positive cells (CA1: $^{**}P < 0.01$, $\eta^2 = 0.903$; DG: $^{***}P < 0.001$, $\eta^2 = 0.928$) and $EdU^+/NeuN^+$ double-positive cells in the hippocampal CA1($^{*}P < 0.05$, $\eta^2 = 0.813$) and DG ($^{***}P < 0.001$, $\eta^2 = 0.931$) regions was significantly reduced in the Model group, indicating impaired differentiation of hippocampal NSCs in APP/PS1 mice. After 7 weeks of ICS II treatment, the numbers of NeuN positive cells (CA1: $^{**}P < 0.01$, $\eta^2 = 0.903$; DG: $^{***}P < 0.001$, $\eta^2 = 0.928$ vs. Model group) and $EdU^+/NeuN^+$ double-positive cells (CA1: $^{*}P < 0.05$, $\eta^2 = 0.813$; DG: $^{***}P < 0.001$, $\eta^2 = 0.931$ vs. Model group) were significantly increased, demonstrating that ICS II promotes the differentiation of hippocampal NSCs. Similar to the proliferation results, Rot further inhibited hippocampal neurogenesis in APP/PS1 mice and negated the effect of ICS II on increasing the number of $EdU^+/NeuN^+$ double-positive cells (CA1: $^{*}P < 0.05$, ICS II + Rot vs ICS II; $^{ns}P > 0.05$, ICS II + Rot vs Model group, $\eta^2 = 0.813$; DG: $^{***}P < 0.001$, ICS II + Rot vs ICS II; $^{ns}P > 0.05$, ICS II + Rot vs Model group, $\eta^2 = 0.931$). These findings suggest that ICS II promotes the differentiation of hippocampal NSCs, and this effect is dependent on the normal function of mitochondria.

3.8 ICS II inhibits mitochondrial hyper-division in APP/PS1 mice

As shown in Figures 8A–G, neurons in the Model group displayed folded and ruptured nuclear membranes (Figure 8A, yellow arrow), increased number of damaged mitochondria (Figures 8A,D, blue arrow, $^{***}P < 0.001$, $\eta^2 = 0.616$), decreased mitochondrial area (Figures 8A,E, $^{**}P < 0.01$, $\eta^2 = 0.674$) and cristae density (Figures 8A,F, $^{**}P < 0.01$, $\eta^2 = 0.656$), as well as reduced number of synapses (Figures 8B,G, $^{****}P < 0.0001$, $\eta^2 = 0.781$) compared with Control group, indicating severe ultrastructural damage of neurons and mitochondria. Moreover, the ATP production was significantly decreased in Model group ($^{***}P < 0.001$, $\eta^2 = 0.703$). In contrast, ICS II treatment resulted in significant restoration of these mitochondrial damages, as evidenced by decreased number of damaged mitochondria (Figures 8A,D, $^{**}P < 0.01$, $\eta^2 = 0.616$), increased mitochondrial area (Figures 8A,E, $^{**}P < 0.01$, $\eta^2 = 0.674$) and cristae density (Figures 8A,F, $^{**}P < 0.01$, $\eta^2 = 0.656$), increased number of synapses (Figures 8B,G, $^{**}P < 0.01$, $\eta^2 = 0.781$), and higher level of ATP ($^{*}P < 0.001$, $\eta^2 = 0.703$) compared with Model group. Rot blocked the protective effects of ICS II on neuronal ultrastructure and mitochondria.

As shown in Figures 8F–J, the Model group exhibited significantly reduced expression of mitochondrial fusion proteins Mfn1 (Figures 8I,J, $^{**}P < 0.01$, $\eta^2 = 0.898$) and Mfn2 (Figures 8I,K, $^{*}P < 0.05$, $\eta^2 = 0.756$), while the expression of fission proteins p-Drp1/Drp1(Figures 8I,L, $^{****}P < 0.0001$, $\eta^2 = 0.833$) and Mff (Figures 8I,M, $^{**}P < 0.01$, $\eta^2 = 0.788$) was significantly elevated compared to the Control group, suggesting abnormal mitochondrial dynamics in the hippocampus of APP/PS1 mice. After 7 weeks of ICS II treatment, the expression of mitochondrial fusion proteins Mfn1(Figures 8I,J, $^{*}P < 0.05$ vs Model group, $\eta^2 = 0.898$) and Mfn2 (Figures 8I,K, $^{*}P < 0.05$ vs Model group, $\eta^2 = 0.756$) was significantly increased, while the expression of mitochondrial fission proteins p-Drp1/Drp1(Figures 8I,L, $^{****}P < 0.0001$ vs Model group, $\eta^2 = 0.833$) and Mff (Figures 8I,M, $^{**}P < 0.01$ vs Model group, $\eta^2 = 0.788$) was reduced. Simultaneous administration of Rot blocked ICS II's effect in correcting the abnormal protein expression associated with

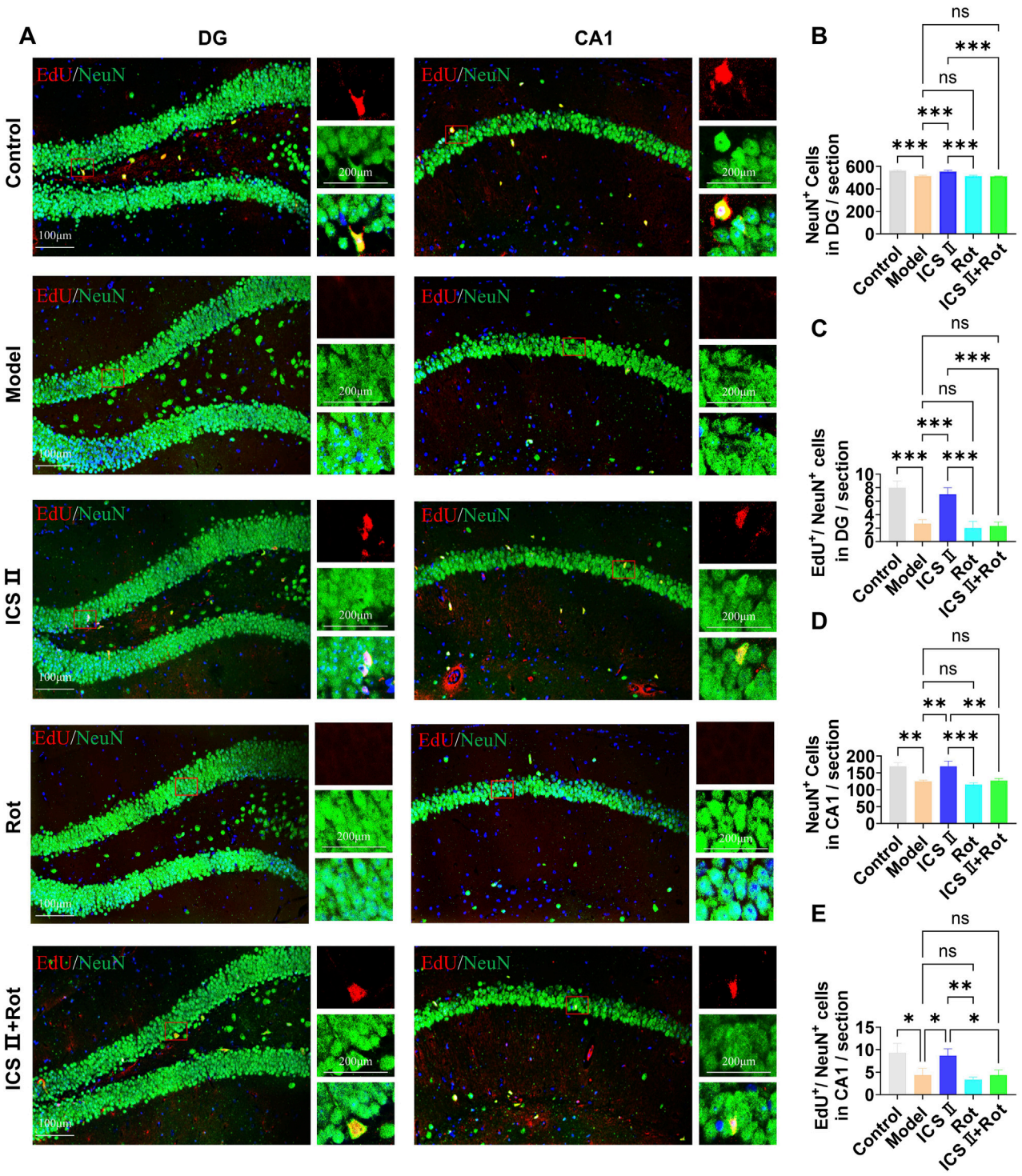


FIGURE 7
ICS II promotes hippocampal NSC differentiation in APP/PS1 Mice **(A)** Representative images of NeuN expression in the hippocampal DG and CA1 regions. Scale bar = 100 μm (original images), 200 μm (magnified images). **(B,D)** Quantification of NeuN$^+$ cells in the hippocampal DG **(B)** and CA1 **(D)** regions. **(C,E)** Quantification of EdU$^+$/NeuN$^+$ cells in the hippocampal DG **(C)** and CA1 **(E)** regions. Statistical significance: $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; $^{****}P < 0.0001$; ns: no significant difference. Data are presented as mean ± SD (n = 3 mice/group). Data were analyzed by one-way ANOVA.

mitochondrial dynamics. These results indicate that ICS II enhances mitochondrial fusion protein expression and reduces mitochondrial fission protein expression. The mitochondrial dysfunction induced by Rot blocks these effects.

4 Discussion

Neurogenesis is the process by which NSCs generate new neurons to restore existing neural circuits and repair damaged

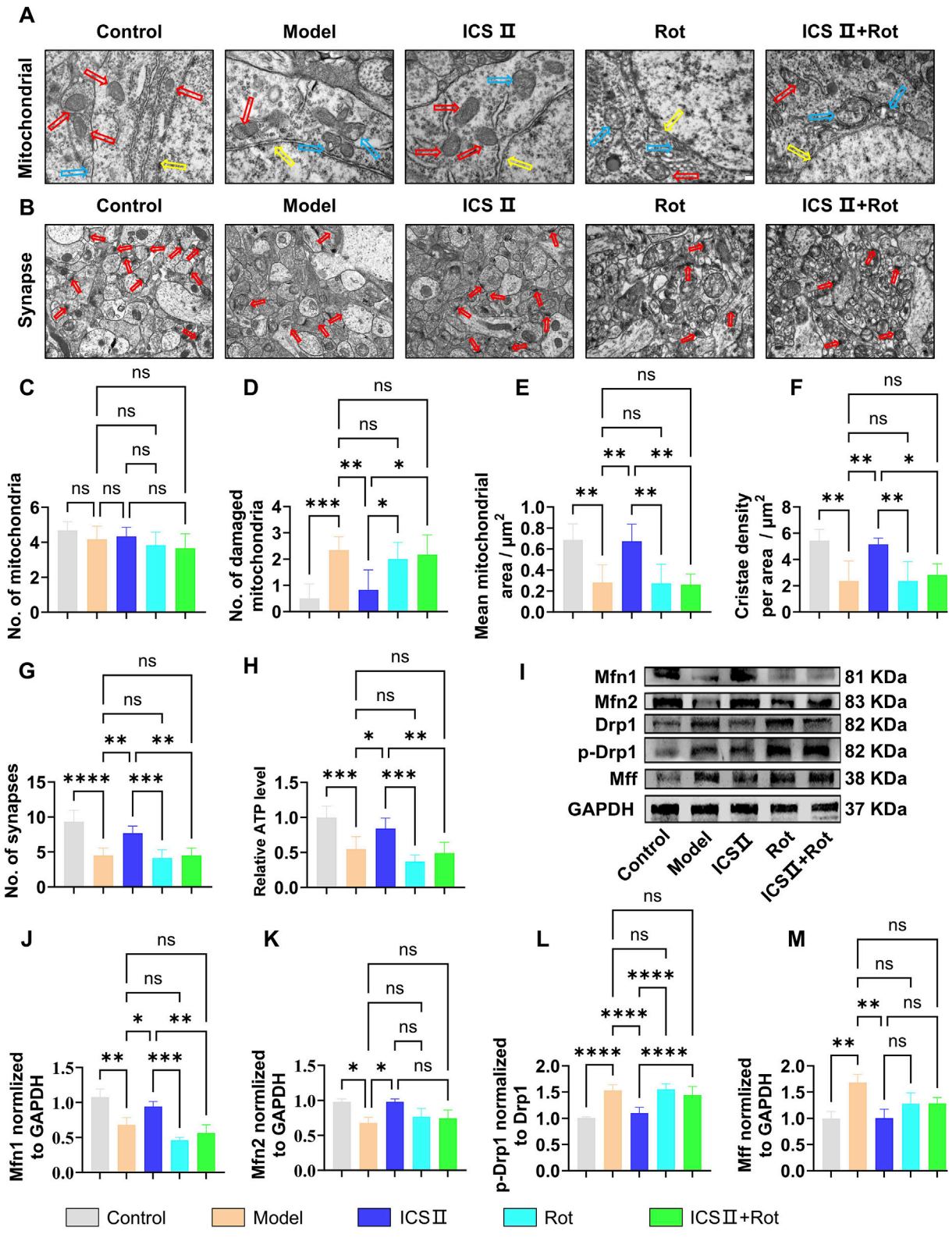


FIGURE 8
ICS II inhibits mitochondrial hyper-division in APP/PS1 Mice **(A)** Ultrastructural images of mitochondria (red arrow: healthy mitochondria, blue arrow: damaged mitochondria, yellow arrow: nuclear membrane) in neurons detected by TEM. Scale bar = 500 nm. **(B)** Ultrastructural images of synapses (red arrow: synapse) in neurons detected by TEM, Scale bar = 1 μm, (n = 3 mice/group). **(C)** Total number of mitochondria. **(D)** Number of damaged mitochondria. **(E)** Mean mitochondrial area as in **(A)**. **(F)** Mitochondria cristae density as in **(A)**. **(G)** Number of synapses as in **(A)**. **(H)** ATP relative level (n = 10 mice/group). **(I–M)** Changes in the expression levels of mitochondrial dynamics-related proteins (n = 3 mice/group). **(I)** Representative Western blot bands. **(J–M)** Quantitative analysis of Mfn1 **(J)**, Mfn2 **(K)**, p-Drp1/Drp1 **(L)**, and Mff **(M)** protein expression. Statistical significance: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; $^{****}P < 0.0001$; ns: no significant difference. Data are presented as mean ± SD. Data were analyzed by one-way ANOVA followed by Bonferroni post-hoc test.

brain tissue. This process is essential for maintaining synaptic plasticity and ensuring proper central nervous system function. Extensive research has established that impairments in neurogenesis contribute to the pathogenesis and progression of AD (Disouky and Lazarov, 2021; Scopa et al., 2020; Young, 2020). In this study, we initially conducted *in vitro* experiments to assess the effects of eight active metabolites of EF on the survival and proliferation of APP-NSCs, identifying ICS II as the most effective protective metabolite. Subsequent animal experiments confirmed that ICS II promotes hippocampal neurogenesis and enhances cognitive function in AD mice. We demonstrated that ICS II stimulates hippocampal neurogenesis by regulating mitochondrial dynamics, thereby improving cognitive function in APP/PS1 mice.

EF is a traditional Chinese medicine used to tonify the kidneys, with multiple protective effects on the central nervous system. It contains various chemical metabolites, including flavonoids, polysaccharides, lignans, and alkaloids, among which flavonoids are the primary pharmacologically active metabolites. These include Epimedin A, Epimedin B, Epimedin C, ICA, ICS II, Icaritin, Anhydroicaritin, and ICS I (Li et al., 2020; Wang et al., 2016; Zhang L. B. et al., 2022; Zhuang et al., 2023). Epimedin A and Epimedin C have primarily been studied in the context of osteoporosis (Li et al., 2024; Xu et al., 2022). Epimedin B has been reported to counteract MPTP-induced dopaminergic neuronal damage and improve behavioral function in a Parkinson's mouse model (Zhang M. et al., 2022). Icaritin exerts neuroprotective effects through estrogen-like receptor pathways, mitigating Aβ-induced neuronal damage (Wang et al., 2007), promoting the proliferation and differentiation of oligodendrocyte precursor cells, and improving cuprizone-induced demyelination (Yang et al., 2023). ICS II has been found to mediate signaling pathways, including BDNF/TrkB/CREB and Wnt/β-catenin, thereby improving cognitive function in various brain disease models (Xiao et al., 2022; Yin et al., 2018).

Mitochondria are double-membraned organelles that not only provide energy for cells but also play a crucial role in regulating biological processes such as signal transduction, nuclear gene transcription, and epigenetic modifications (Seo et al., 2018; Zhang et al., 2018). A 2019 review in Nature Reviews Neuroscience emphasized the critical role of mitochondria in regulating the proliferation and differentiation of NSCs, positioning them as key regulators of neurogenesis (Mireille et al., 2019). The activation, proliferation, and differentiation of NSCs require substantial energy and metabolic support. In resting NSCs, the mitochondrial network is fragmented, the cristae are underdeveloped, and mitochondrial function is immature, relying primarily on glycolysis for ATP production. Upon mitochondrial fusion and elongation, along with cristae maturation, mitochondrial DNA (mtDNA) copy numbers increase, and the energy supply shifts to oxidative phosphorylation, promoting the proliferation and differentiation of NSCs (Seo et al., 2018; Zhang et al., 2018). Studies have shown that knockout of mitochondrial fusion genes in NSCs impairs hippocampal NSC proliferation and differentiation, resulting in cognitive deficits in mice (Khacho et al., 2016). In AD, mitochondrial dynamics are disrupted, leading to excessive fragmentation of the mitochondrial network, reduced respiratory chain enzyme activity, decreased ATP production, increased mtDNA mutations, and elevated ROS levels, all of which exacerbate neurogenesis impairment (Monzio Compagnoni et al., 2020; Shevtsova et al., 2021; Wang et al., 2020). Additionally, studies have shown that inhibiting mitochondrial fission with Mdivi-1 promotes mitochondrial fusion, increases ATP levels, and enhances hippocampal neurogenesis in a Down syndrome mouse model (Valenti et al., 2017).

In the present study, we found that ICS II effectively enhanced mitochondrial membrane potential, increased ATP production, and reduced ROS levels in APP-NSCs (Figure 3), indicating promising mitochondrial protection. *In vivo* experiments demonstrated that continuous administration of 10 mg/kg ICS II for 4 weeks promoted the proliferation of hippocampal NSCs ($EdU^+/SOX\text{-}2^+$, Figure 6), while a 7-week treatment further enhanced their differentiation ($EdU^+/NeuN^+$, Figure 7), confirming the neurogenic effects of ICS II. Notably, ICS II improved ATP production, promoted the expression of mitochondrial fusion proteins Mfn1 and Mfn2, inhibited the expression of mitochondrial fission proteins p-Drp1/Drp1 and Mff, and alleviated mitochondrial ultrastructural damage in the brain of APP/PS1 mice (Figure 8).

To further investigate whether the neurogenesis-promoting effect of ICS II depends on the protection of mitochondrial structure and function, we administered Rot to disrupt the mitochondrial respiratory chain, thereby impairing mitochondrial structure and function, and assessed neurogenesis. The results showed that in the presence of Rot, the neurogenesis-promoting effect of ICS II was significantly diminished, with a marked difference compared to ICS II alone. These findings suggest that the neurogenesis-enhancing effect of ICS II is indeed dependent on the preservation of mitochondrial structure and function.

Recent research has identified several natural metabolites capable of modulating mitochondrial function. For instance, huperzine A enhances the activity of respiratory chain complexes, thereby reducing the accumulation of subcellular amyloid-beta (Aβ) (Yang et al., 2012). Ginsenoside Rg1 improves mitochondrial membrane potential and interconnectivity (Kwan et al., 2021). Salvianolic acid B mitigates mitochondrial stress and preserves mitochondrial bioenergetics against Aβ toxicity (He et al., 2018). In our study, ICS II treatment resulted in the suppression of Drp1 hyperactivation and Mff overexpression, alongside an increase in Mfn1 and Mfn2 levels (Figure 8), providing the first evidence that ICS II restores mitochondrial homeostasis by balancing fusion and fission proteins, a process crucial for NSC proliferation and differentiation. Unlike previous reports, we link the restoration of mitochondrial dynamics to enhanced neurogenesis in APP/PS1 mice, addressing a critical gap in understanding how ICS II alleviates the bioenergetic deficits underlying AD pathogenesis. Considering the complexity of AD-related mitochondrial dysfunction, ICS II's potential to promote neurogenesis and protect mitochondrial function may offer synergistic benefits.

This study primarily focused on the pharmacodynamics and mechanisms of action of ICS II, and does not include pharmacokinetic data. Therefore, the findings from animal models may not fully reflect human responses, particularly regarding metabolic pathways and BBB permeability. Clinical trials are needed to validate ICS II's safety and efficacy in humans. Additionally, long-term toxicity and off-target effects were not explored in this study, highlighting the need for further preclinical and clinical research.

In conclusion, integrated *in vitro* screening identified ICS II as one of the most effective metabolites of EF for promoting NSC

proliferation. Subsequent *in vivo* studies demonstrated its ability to enhance hippocampal neurogenesis, primarily through the regulation of mitochondrial dynamics, highlighting its therapeutic potential for AD.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding authors.

Ethics statement

The animal study was approved by the Institutional Animal Care and Use Committee (IACUC) of Liaoning University of Traditional Chinese Medicine. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

J-MB: Methodology, Validation, Writing – original draft. TL: Data curation, Methodology, Writing – original draft. XD: Data curation, Methodology, Writing – original draft. J–XY: Data curation, Writing – review and editing. Z-QC: Methodology, Writing – original draft. D-YM: Data curation, Writing – original draft. Y-FS: Software, Writing – original draft. S-YS: Methodology, Writing – original draft. Y-XZ: Data curation, Writing – review and editing. Y-JS: Validation, Writing – review and editing. Z–LX: Data curation, Writing – original draft, Writing – review and editing. D-QD: Investigation, Supervision, Writing – review and editing. H-HX: Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Writing – original draft, Writing – review and editing.

Funding

The author(s) declare that financial support was received for the research and/or publication of this article. This work was financially supported by the National Natural Science Foundation of China (No. 82204648), Natural Science Foundation of Liaoning Province (No. 2021-MS-250), Key Research and Development Project of Liaoning Province (2024JH2/102500064), Project funded by China Postdoctoral Science Foundation (No. 2022M710706), National Natural Science Foundation of Liaoning University of Traditional Chinese Medicine (2021LZY046), and the Natural Science Foundation of Liaoning Provincial Department of Education (LJ222410162067).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

Generative AI statement

The authors declare that no Generative AI was used in the creation of this manuscript.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2025.1546256/full#supplementary-material

References

Chen, M., Wu, J., Luo, Q., Mo, S., Lyu, Y., Wei, Y., et al. (2016). The anticancer properties of herba epimedii and its main bioactive components Icariin and Icariside II. *Nutrients* 8 (9), 563. doi:10.3390/nu8090563

Cho, J. H., Jung, J. Y., Lee, B. J., Lee, K., Park, J. W., and Bu, Y. (2017). Epimedii herba: a promising herbal medicine for neuroplasticity. *Phytother. Res.* 31, 838–848. doi:10.1002/ptr.5807

Choi, S. H., and Tanzi, R. E. (2019). Is Alzheimer's disease a neurogenesis disorder? *Cell Stem Cell* 25, 7–8. doi:10.1016/j.stem.2019.06.001

Christie, K. J., and Turnley, A. M. (2013). Regulation of endogenous neural stem/progenitor cells for neural repair-factors that promote neurogenesis and gliogenesis in the normal and damaged brain. *Front. Cell Neurosci.* 6, 70. doi:10.3389/fncel.2012.00070

Committee, N. P. (2020). *Chinese Pharmacopoeia*. Beijing: China Medical Science and Technology Press, 340–341.

Dey, A., Bhattacharya, R., Mukherjee, A., and Pandey, D. K. (2017). Natural products against Alzheimer's disease: pharmaco-therapeutics and biotechnological interventions. *Biotechnol. Adv.* 35 (2), 178–216. doi:10.1016/j.biotechadv.2016.12.005

Dey, A., Bhattacharya, R., Mukherjee, A., and Pandey, D. K. (2017). Natural products against Alzheimer's disease: pharmaco-therapeutics and biotechnological interventions. *Biotechnol. Adv.* 35 (2), 178–216. doi:10.1016/j.biotechadv.2016.12.005

Doetsch, F., Caillé, I., Lim, D. A., Garcíaverdugo, J. M., and Alvarezbuylla, A. (1999). Subventricular zone astrocytes are neural stem cells in the adult mammalian brain. *Cell* 97, 703–716. doi:10.1016/s0092-8674(00)80783-7

Duan, C.L., Liu, C.W., Shen, S.W., Yu, Z., Mo, J.L., Chen, X.H., et al. (2015). Striatal astrocytes transdifferentiate into functional mature neurons following ischemic brain. *injury. Glia.* 63 (2), 1660–1670.

Fatt, M., Hsu, K., He, L., Wondisford, F., Miller, F. D., Kaplan, D. R., et al. (2015). Metformin acts on two different molecular pathways to enhance adult neural precursor proliferation/self-renewal and differentiation. *Stem Cell Rep.* 5 (6), 988–995. doi:10.1016/j.stemcr.2015.10.014

Golde, T. E., DeKosky, S. T., and Galasko, D. (2018). Alzheimer's disease: the right drug, the right time. *Science* 362 (6420), 1250–1251. doi:10.1126/science.aau0437

He, Y., Jia, K., Li, L., Wang, Q., Zhang, S., Du, J., et al. (2018). Salvianolic acid B attenuates mitochondrial stress against Aβ toxicity in primary cultured mouse neurons. *Biochem. Biophys. Res. Commun.* 498 (4), 1066–1072. doi:10.1016/j.bbrc.2018.03.119

Hollands, C., Tobin, M. K., Hsu, M., Musaraca, K., Yu, T.-S., Mishra, R., et al. (2017). Depletion of adult neurogenesis exacerbates cognitive deficits in Alzheimer's disease by compromising hippocampal inhibition. *Mol. Neurodegener.* 12 (1), 64. doi:10.1186/s13024-017-0207-7

Jia, L., Du, Y., Chu, L., Zhang, Z., Li, F., Lyu, D., et al. (2020). Prevalence, risk factors, and management of dementia and mild cognitive impairment in adults aged 60 years or older in China: a cross-sectional study. *Lancet Public Health* 5 (12), e661–e671. doi:10.1016/S2468-2667(20)30185-7

Khacho, M., Clark, A., Svoboda, D. S., Azzi, J., MacLaurin, J. G., Meghaizel, C., et al. (2016). Mitochondrial dynamics impacts stem cell identity and fate decisions by regulating a nuclear transcriptional program. *Cell Stem Cell* 19 (2), 232–247. doi:10.1016/j.stem.2016.04.015

Kwan, K. K. L., Yun, H., Dong, T. T. X., and Tsim, K. W. K. (2021). Ginsenosides attenuate bioenergetics and morphology of mitochondria in cultured PC12 cells under the insult of amyloid beta-peptide. *J. Ginseng Res.* 45 (4), 473–481. doi:10.1016/j.jgr.2020.09.005

Li, C., Li, Q., Mei, Q., and Lu, T. (2015). Pharmacological effects and pharmacokinetic properties of icariin, the major bioactive component in Herba Epimedii. *Life Sci.* 126, 57–68. doi:10.1016/j.lfs.2015.01.006

Li, J., Wei, J. J., Wu, C. H., Zou, T., Zhao, H., Huo, T. Q., et al. (2024). Epimedin A inhibits the PI3K/AKT/NF-κB signalling axis and osteoclast differentiation by negatively regulating TRAF6 expression. *Mol. Med.* 30 (1), 125. doi:10.1186/s10020-024-00893-w

Li, Y., Yang, Q., and Yu, Y. (2020). A network pharmacological approach to investigate the mechanism of action of active ingredients of epimedii herba and their potential targets in treatment of Alzheimer's disease. *Med. Sci. Monit.* 26, e926295. doi:10.12659/MSM.926295

Liu, L., Zhao, Z., Lu, L., Liu, J., Sun, J., and Dong, J. (2019). Icariin and icaritin ameliorated hippocampus neuroinflammation via mediating HMGB1 expression in social defeat model in mice. *Int. Immunopharmacol.* 75, 105799. doi:10.1016/j.intimp.2019.105799

Liu, X., Huang, L., Rensing, C., Ye, J., Nealson, K. H., and Zhou, S. (2021). Syntrophic interspecies electron transfer drives carbon fixation and growth by rhodopseudomonas palustris under dark, anoxic conditions. *Sci. Adv.* 7 (27), eabh1852. doi:10.1126/sciadv.abh1852

Mao, J., Huang, S., Liu, S., Feng, X. L., Yu, M., Liu, J., et al. (2015). A herbal medicine for Alzheimer's disease and its active constituents promote neural progenitor proliferation. *Aging Cell* 14 (5), 784–796. doi:10.1111/acel.12356

Mireille, K., Richard, H., and Slack, R. S. (2019). Mitochondria as central regulators of neural stem cell fate and cognitive function. *Nat. Rev. Neurosci.* 20 (1), 34–48. doi:10.1038/s41583-018-0091-3

Monzio Compagnoni, G., Di Fonzo, A., Corti, S., Comi, G. P., Bresolin, N., and Masliah, E. (2020). The role of mitochondria in neurodegenerative diseases: the lesson from Alzheimer's disease and Parkinson's disease. *Mol. Neurobiol.* 57 (7), 2959–2980. doi:10.1007/s12035-020-01926-1

Moreno-Jiménez, E. P., Flor-García, M., Terreros-Roncal, J., Rábano, A., Cafini, F., Pallas-Bazarra, N., et al. (2019). Adult hippocampal neurogenesis is abundant in neurologically healthy subjects and drops sharply in patients with Alzheimer's disease. *Nat. Med.* 25 (4), 554–560. doi:10.1038/s41591-019-0375-9

Ms, B., Jwinpenny, N., Winpenny, E., Hodge, R. D., Ozen, I., Yang, R., et al. (2009). Adult generation of glutamatergic olfactory bulb interneurons. *Nat. Neurosci.* 12 (12), 1524–1533. doi:10.1038/nn.2416

Scopa, C., Marrocco, F., Latina, V., Ruggeri, F., Corvaglia, V., La Regina, F., et al. (2020). Impaired adult neurogenesis is an early event in Alzheimer's disease neurodegeneration, mediated by intracellular Aβ oligomers. *Cell Death Differ.* 27 (3), 934–948. doi:10.1038/s41418-019-0409-3

Seo, B. J., Yoon, S. H., and Do, J. T. (2018). Mitochondrial dynamics in stem cells and differentiation. *Int. J. Mol. Sci.* 19 (12), 3893. doi:10.3390/ijms19123893

Sharma, P., Giri, A., and Tripathi, P. N. (2024). Emerging trends: neurofilament biomarkers in precision neurology. *Neurochem. Res.* 49 (12), 3208–3225. doi:10.1007/s11064-024-04244-3

Shashi Kant, T., Brashket, S., Swati, A., Anuradha, Y., Madhumita, K., Shailendra Kumar, G., et al. (2015). Ethosuximide induces hippocampal neurogenesis and reverses cognitive deficits in an amyloid-β toxin-induced Alzheimer rat model via the phosphatidylinositol 3-Kinase (PI3K)/Akt/Wnt/β-Catenin pathway. *J. Biol. Chem.* 290 (47), 28540–28558. doi:10.1074/jbc.M115.652586

Shevtsova, E. F., Maltsev, A. V., Vinogradova, D. V., Shevtsov, P. N., and Bachurin, S. O. (2021). Mitochondria as a promising target for developing novel agents for treating Alzheimer's disease. *Med. Res. Rev.* 41 (2), 803–827. doi:10.1002/med.21715

Siddiqui, N., Saifi, A., Chaudhary, A., Tripathi, P. N., Chaudhary, A., and Sharma, A. (2024). Multifaceted neuroprotective role of punicalagin: a review. *Neurochem. Res.* 49 (6), 1427–1436. doi:10.1007/s11064-023-04081-w

Torres-Lista, V., and Giménez-Llort, L. (2013). Impairment of nesting behaviour in 3xTg-AD mice. *Behav. Brain Res.* 247, 153–157. doi:10.1016/j.bbr.2013.03.021

Tripathi, P. N., Lodhi, A., Rai, S. N., Nandi, N. K., Dumoga, S., Yadav, P., et al. (2024). Review of pharmacotherapeutic targets in Alzheimer's disease and its management using traditional medicinal plants. *Degener. Neurol. Neuromuscul. Dis.* 14, 47–74. doi:10.2147/DNND.S452009

Valenti, D., Rossi, L., Marzulli, D., Bellomo, F., De Rasmo, D., Signorile, A., et al. (2017). Inhibition of Drp1-mediated mitochondrial fission improves mitochondrial dynamics and bioenergetics stimulating neurogenesis in hippocampal progenitor cells from a Down syndrome mouse model. *Biochim. Biophys. Acta Mol. Basis Dis.* 1863 (12), 3117–3127. doi:10.1016/j.bbadis.2017.09.014

Waldau, B., and Shetty, A. K. (2008). Behavior of neural stem cells in the Alzheimer brain. *Cell Mol. Life Sci.* 15, 2372–2384. doi:10.1007/s00018-008-8053-y

Wang, L., Li, Y., Guo, Y., Ma, R., Fu, M., Niu, J., et al. (2016). Herba epimedii: an ancient Chinese herbal medicine in the prevention and treatment of osteoporosis. *Curr. Pharm. Des.* 22 (3), 328–349. doi:10.2174/1381612822666151112145907

Wang, W., Zhao, F., Ma, X., Perry, G., and Zhu, X. (2020). Mitochondria dysfunction in the pathogenesis of Alzheimer's disease: recent advances. *Mol. Neurodegener.* 15 (1), 30. doi:10.1186/s13024-020-00376-6

Wang, Y., Shang, C., Zhang, Y., Xin, L., Jiao, L., Xiang, M., et al. (2023). Regulatory mechanism of icariin in cardiovascular and neurological diseases. *Biomed. Pharmacother.* 158, 114156. doi:10.1016/j.biopha.2022.114156

Wang, Z., Zhang, X., Wang, H., Qi, L., and Lou, Y. (2007). Neuroprotective effects of icaritin against beta amyloid-induced neurotoxicity in primary cultured rat neuronal cells via estrogen-dependent pathway. *Neuroscience* 145 (3), 911–922. doi:10.1016/j.neuroscience.2006.12.059

Wen, L., Zhou, T., Jiang, Y., Gong, L., and Yang, B. (2021). Identification of prenylated phenolics in mulberry leaf and their neuroprotective activity. *Phytomedicine* 90, 153641. doi:10.1016/j.phymed.2021.153641

Xiao, H., LI, H., Song, H., Kong, L., Yan, X., Li, Y., et al. (2020). Shenzao jiannao oral liquid, an herbal formula, ameliorates cognitive impairments by rescuing neuronal death and triggering endogenous neurogenesis in AD-like mice induced by a combination of Aβ42 and scopolamine. *J. Ethnopharmacol.* 259, 112957

Xiao, H. H., Chen, J. C., Li, H., Li, R. H., Wang, H. B., Song, H. P., et al. (2022). Icarisid II rescues cognitive dysfunction via activation of Wnt/β-catenin signaling pathway promoting hippocampal neurogenesis in APP/PS1 transgenic mice. *Phytother. Res.* 36 (5), 2095–2108. doi:10.1002/ptr.7430

Xiao, H. H., Zhang, M. B., Xu, J. T., Deng, Y., Li, N., Gao, P., et al. (2021). Icarisid II promotes proliferation and neuronal differentiation of neural stem cells via activating Wnt/β-catenin signaling pathway. *Phytother. Res.* 35 (5), 2773–2784. doi:10.1002/ptr.7022

Xu, Y., Chen, S., Huang, L., Han, W., Shao, Y., Chen, M., et al. (2022). Epimedin C alleviates glucocorticoid-induced suppression of osteogenic differentiation by modulating PI3K/AKT/RUNX2 signaling pathway. *Front. Pharmacol.* 13, 894832. doi:10.3389/fphar.2022.894832

Yang, F., Wen, H., Ma, S., Chang, Q., Pan, R., Liu, X., et al. (2023). Icaritin promotes myelination by simultaneously enhancing the proliferation and differentiation of oligodendrocyte precursor cells. *Molecules* 28 (15), 5837. doi:10.3390/molecules28155837

Yang, L., Ye, C. Y., Huang, X. T., Tang, X. C., and Zhang, H. Y. (2012). Decreased accumulation of subcellular Amyloid-β with improved mitochondrial function mediates the neuroprotective effect of Huperzine A. *J. Alzheimers Dis.* 31 (1), 131–142. doi:10.3233/JAD-2012-120274

Yin, C., Deng, Y., Liu, Y., Gao, J., Yan, L., and Gong, Q. (2018). Icariside II ameliorates cognitive impairments induced by chronic cerebral hypoperfusion by inhibiting the amyloidogenic pathway: involvement of BDNF/TrkB/CREB signaling and Up-Regulation of PPARα and PPARγ in rats. *Front. Pharmacol.* 9, 1211. doi:10.3389/fphar.2018.01211

Young, J. K. (2020). Neurogenesis makes a crucial contribution to the neuropathology of Alzheimer's disease. *J. Alzheimers Dis. Rep.* 4 (1), 365–371. doi:10.3233/ADR-200218

Zhang, H., Menzies, K. J., and Auwerx, J. (2018). The role of mitochondria in stem cell fate and aging. *Development* 145 (8), dev143420. doi:10.1242/dev.143420

Zhang, L. B., Yan, Y., He, J., Wang, P. P., Chen, X., Lan, T. Y., et al. (2022a). Epimedii Herba: an ancient Chinese herbal medicine in the prevention and treatment of rheumatoid arthritis. *Front. Chem.* 10, 1023779. doi:10.3389/fchem.2022.1023779

Zhang, M., Hu, Z. F., Dong, X. L., and Chen, W. F. (2022b). Epimedin B exerts neuroprotective effect against MPTP-induced mouse model of Parkinson's disease: GPER as a potential target. *Biomed. Pharmacother.* 156, 113955. doi:10.1016/j.biopha.2022.113955

Zhuang, W., Sun, N., Gu, C., Liu, S., Zheng, Y., Wang, H., et al. (2023). A literature review on Epimedium, a medicinal plant with promising slow aging properties. *Heliyon* 9 (11), e21226. doi:10.1016/j.heliyon.2023.e21226

frontiers | Frontiers in Pharmacology

TYPE Original Research
PUBLISHED 01 May 2025
DOI 10.3389/fphar.2025.1552493

Check for updates

OPEN ACCESS

EDITED BY
Huazheng Liang,
Monash University - Southeast University Joint Research Institute (Suzhou), China

REVIEWED BY
Shimaa K Mohamed,
Helwan University, Egypt
Yulin Dai,
Changchun University of Chinese Medicine, China
Wenqiao Fu,
Chongqing Medical University, China

*CORRESPONDENCE
Le Wang,
13661172704@139.com

RECEIVED 28 December 2024
ACCEPTED 15 April 2025
PUBLISHED 01 May 2025

CITATION
Wu J, Li Z, Dong X, Liu J and Wang L (2025) Shenmai Injection enhances short-term outcomes in ischemic stroke patients after thrombolysis via AMPKα1.
Front. Pharmacol. 16:1552493.
doi: 10.3389/fphar.2025.1552493

COPYRIGHT
© 2025 Wu, Li, Dong, Liu and Wang. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

Shenmai Injection enhances short-term outcomes in ischemic stroke patients after thrombolysis via AMPKα1

Jing Wu[1], Zhonghao Li[2], Xiaoke Dong[3], Jinmin Liu[4] and Le Wang[5]*

[1]Department of Rehibition, Dongfang Hospital Beijing University of Chinese Medicine, Beijing, China, [2]Department of Neurosurgery, Dongfang Hospital Beijing University of Chinese Medicine, Beijing, China, [3]Department of Neurology, Beijing Daxing District Hospital of Integrated Chinese and Western Medicine, Beijing, China, [4]Department of Neurology, Dongzhimen Hospital Beijing University of Chinese Medicine, Beijing, China, [5]Department of Neurology, Dongfang Hospital Beijing University of Chinese Medicine, Beijing, China

Background: Shenmai Injection (SMI), a traditional Chinese medicine with nourishing properties, has been explored for its therapeutic effects in ischemic stroke (IS). This study aimed to evaluate the protective effects of SMI in patients with IS who received intravenous thrombolysis and to elucidate its potential molecular mechanisms through laboratory investigations.

Methods: Patients with IS were randomized to receive either SMI or a placebo for 10 days within 12 h post-intravenous thrombolysis. Clinical efficacy and safety were assessed. An IS cell model was induced using H2O2, followed by treatment with SMI to explore its therapeutic effects and underlying mechanisms.

Results: The modified Rankin Scale (mRS) score at 30 days was significantly lower in the SMI group (n = 35) compared to the placebo group (n = 35), indicating improved functional outcomes. No significant difference was observed in NIHSS scores between the groups. Adverse events and biochemical indices showed no significant differences, confirming the safety of SMI. In the H2O2-induced cell model, SMI enhanced cell viability, reduced apoptosis, and decreased the levels of malondialdehyde (MDA) and reactive oxygen species (ROS). It also improved ATP content and mitochondrial membrane potential. Mechanistic studies revealed that these protective effects were partially mediated through the AMPKα1.

Conclusion: SMI significantly improves short-term outcomes in IS patients treated with rt-PA thrombolysis. Its protective effects are likely mediated through the AMPKα1, highlighting its potential as an adjunctive therapy for IS.

KEYWORDS

Acute ischemic stroke, shenmai injection, AMPK, modified rankin scale, randomized controlled trial

1 Introduction

Ischemic stroke (IS) is an important contributor to mortality and long-term disability, significantly impacting global health (Tang et al., 2021); (Powers, 2020). While timely restoration of blood supply via thrombolytic therapy is considered the main therapy for IS (Cai et al., 2021), the subsequent reperfusion process following ischemic attack may further

aggravate brain injury, a phenomenon known as ischemia/reperfusion injury (Dai et al., 2020); (Pérez-Mato et al., 2019). It is increasingly recognized that a variety of pathophysiologic processes, including apoptosis, oxidative stress, and mitochondrial dysfunction play a key role in the occurrence and development of this injury (Jiao et al., 2020; Hu et al., 2020). Therefore, treatments based on the above mechanisms are considered as a promising strategy for alleviating the outcome of IS.

Shenmai injection (SMI), a traditional Chinese medicine injection approved by the China Food and Drug Administration in 1995, has gained recognition for its organ-protective properties (Hu et al., 2020; Yu et al., 2019; Wang et al., 2019). Comprising aqueous extracts from red ginseng (Hong Shen) and Ophiopogonis Radix (Mai Dong), SMI has demonstrated potential in improving clinical outcomes for IS patients. Previous studies have shown that SMI can reduce neurological deficits, lower rates of deterioration, and enhance quality of life (Liu et al., 2017; Xu et al., 2019). However, limitations in study design, including inadequate control groups and unclear treatment protocols, have hindered the comprehensive understanding of SMI's clinical benefits and mechanisms of action.

Emerging evidence suggests that the AMPKα1 plays a pivotal role in energy metabolism and cellular protection, making it a promising target in mitigating oxidative stress and mitochondrial dysfunction in IS (Chen et al., 2018; Li et al., 2020). Despite its potential, the mechanism through which SMI exerts its protective effects in IS remains inadequately understood.

Taken together, this study aimed to address these gaps by employing a rigorous randomized controlled clinical trial to evaluate the clinical efficacy and safety of SMI in IS patients receiving intravenous thrombolysis. Additionally, the study explored the role of SMI in modulating oxidative stress, mitochondrial dysfunction, and apoptosis, with a focus on the AMPKα1. By integrating clinical and mechanistic insights, this research seeks to provide robust evidence for the therapeutic potential of SMI in IS and contribute to the development of novel treatment strategies.

2 Methods

2.1 High performance liquid chromatography (HPLC) analysis of SMI

SMI is a commercial injection prepared from the extracts of Panax ginseng C.A.Mey [Araliaceae; Ginseng radix et rhizome rubra] and Ophiopogon japonicus (Thunb.) Ker Gawl [Asparagaceae, Ophiopogonis radix] (Zhang et al., 2023). It was purchased from Chiatai Qingchunbao Pharmaceutical Co., Ltd (Hangzhou, China). The active ingredients present in the SMI include ginsenoside, amino acids, and small molecule organic acids (Li et al., 2022). The quality of the SMI was found to be stable and uniform, exhibiting minimal between-batch variations (Yang et al., 2023).

The composition of SMI was analyzed using HPLC. The HPLC conditions were as follows: Column: Waters Symmetry Shield™ RP18 (4.6 mm × 250 mm, 5.0 μm). Column temperature: 30°C. Mobile phase: Acetonitrile (A) and water (B) with gradient elution (0–30 min: 0% A→10% A, 30–40 min: 10% A→23% A, 40–50 min: 23% A, 50–85 min: 23% A→60% A, 85–95 min: 60% A→100% A). Flow rate: 1 mL/min. Detection wavelength: 203 nm. Injection volume: 10 μL. The fingerprint of SMI is available in Supplementary Figure S1.

2.2 Study design

This clinical investigation was designed as a randomized, double-blind, placebo-controlled, parallel-group trial. The study protocol was reviewed and approved by the Ethics Committee of Dongfang Hospital (approval number: JDF-IRB-2020032802). The trial was conducted in accordance with the Declaration of Helsinki and adhered to Good Clinical Practice (GCP) guidelines. The trial was registered with the Chinese Clinical Trial Registry (registration number: ChiCTR2000040106).

2.3 Participants

Between January 2021 and January 2022, 72 patients diagnosed with IS who received intravenous rt-PA thrombolysis within 12 h of symptom onset at Dongfang Hospital Beijing University of Traditional Chinese Medicine, were enrolled in this study. Informed consent was obtained from all participants or their legal guardians. Patients with significant comorbidities affecting other organ systems or those with known allergies to any component of the study medication (SMI) were excluded from the study.

2.4 Interventions and comparisons

Each participant received an intravenous infusion of either 100 mL of SMI or 0.9% saline solution within 12 h following the rt-PA administration, with treatment continuing for 10 consecutive days. Both treatment groups received standardized supportive care, which included blood pressure management, intracranial pressure reduction, maintenance of fluid and electrolyte balance, and other symptomatic treatments, as per established clinical guidelines.

SMI (batch number: 6901990180090, 100 mL/bottle), which contains 10 g each of Red Ginseng and Ophiopogonis Radix, was produced by Zhengda Qingchunbao Pharmaceutical Co., Ltd. SMI is listed in the Chinese Pharmacopoeia and approved by the China Food and Drug Administration (CFDA) (National Pharmaceutical Approval number: Z33020018). The 0.9% saline solution (batch number: H13023202, 100 mL/bottle) was produced by Shijiazhuang No. 4 Pharmaceutical Co., Ltd.

2.5 Randomization, allocation concealment and blinding

Randomization was performed using SPSS version 22.0 software, which generated random numbers that were then equally allocated into two groups. The allocations were transcribed onto distribution cards, which were placed in opaque, sealed envelopes to ensure concealment. Upon enrollment of eligible

participants, the envelopes were sequentially opened in accordance with the order of participant inclusion. The randomization procedure was conducted by individuals who were not involved in the study itself.

To maintain blinding, the SMI and 0.9% saline bottles were wrapped in black opaque bags, and an opaque infusion device was used, ensuring that both patients and researchers were unaware of the treatment allocation. During the study, the personnel responsible for data collection and outcome assessment were also blinded to the group assignments, minimizing the potential for bias.

2.6 Outcome measurements

The primary outcome measure was the modified Rankin Scale (mRS) score at 30 days, which was used to evaluate short-term prognosis in patients.

The secondary outcome measure was the National Institutes of Health Stroke Scale (NIHSS) score on day 10, which assesses neurological impairment.

Additional outcome measures included biochemical indices assessed on day 10, as well as the monitoring of adverse events and any abnormal changes in biochemical parameters during treatment, to evaluate the safety profile of SMI.

2.7 Cell cultures and treatment

The neuron-like rat pheochromocytoma cell line (PC12) was cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) in a humidified incubator set to 5% CO_2 and 37°C. Oxidative stress was induced by adding hydrogen peroxide (H_2O_2) to glucose-free medium, followed by 24 h of incubation. Subsequently, SMI was added to the culture medium, and cells were incubated for an additional 24 h.

PC12 cells were seeded in 6-well plates and transfected with either si-AMPKα1 (Santa Cruz Biotechnology, Inc., United States). or a negative control small interfering RNA (si-NC) using Lipofectamine 2000 (ThermoFisher Scientific, United States) in accordance with the manufacturer's protocol. The transfection process was carried out over 24 h, after which cells were harvested for subsequent experiments.

2.8 CCK-8 assay

To determine the optimal concentrations of H_2O_2 and SMI for further experiments, cell viability was assessed using the Cell Counting Kit-8 (CCK-8) assay (CK04, Dojindo, Japan). In brief, 10 μL of CCK-8 reagent was added to each well, followed by incubation for 2 h. Absorbance was measured at 450 nm using an automated microplate reader (ELX-800, BioTek Instruments, United States).

2.9 TUNEL staining

PC12 cells were seeded in 6-well culture plates. After treatment, cells were fixed in 4% paraformaldehyde for 30 min. Subsequently, cells were permeabilized with 0.3% Triton X-100 in phosphate-buffered saline (PBS) for 5 min and incubated with TUNEL detection solution at 37°C for 1 h (C1089, Beyotime, China). Fluorescent images were captured using a BX71 fluorescence microscope (Olympus, Japan).

2.10 Oxidative stress assessment

Intracellular reactive oxygen species (ROS) levels were measured using the fluorescent probe 2′,7′-dichlorofluorescein diacetate (S0033S, Beyotiem, China). Fluorescence images were captured using a fluorescence microscope, as described previously. The levels of malondialdehyde (MDA), and superoxide dismutase (SOD) in PC12 cells were quantified using specific commercial assay kits, following the manufacturers' protocols (Cat. No.: S0131S, S0101S, respectively, Beyotime, China). Absorbance values were measured with a microplate reader, and the results were normalized to total cellular protein content, determined using a bicinchoninic acid (BCA) assay (P1511, Solarbio, China).

2.11 Mitochondrial function assessment

Mitochondrial membrane potential was evaluated using JC-1 dye, in conjunction with commercial assay kits, as per the manufacturers' instructions (C2006, Beyotime, China). Fluorescence images were captured using a fluorescence microscope, as described previously. Adenosine triphosphate (ATP) levels were measured using specific commercial kits (S0026, Beyotime, China), with absorbance values recorded by a microplate reader. ATP levels were normalized to total cellular protein content, as determined by the BCA assay.

2.12 Immunofluorescence staining

PC12 cells were fixed with 4% paraformaldehyde for 30 min and permeabilized with 0.3% Triton X-100. Following permeabilization, the cells were blocked with 5% bovine serum albumin blocking buffer and incubated with AMPK alpha 1 primary antibody (5,831, Cell Signaling Technology, United States) overnight at 4°C. Subsequently, cells were incubated with a CoraLite488-conjugated goat anti-rabbit IgG secondary antibody (SA00013-2, proteintech, China) at 37°C for 1 h, followed by staining with DAPI for 5 min. Fluorescent images were acquired using a fluorescence microscope.

2.13 Statistical analysis

Statistical analyses were performed using SPSS version 22.0. Categorical variables were described using frequencies and percentages, while continuous variables were summarized using means and standard deviations or medians and quartiles, depending on data distribution. Comparisons between groups for continuous variables were conducted using the t-test or Wilcoxon rank-sum test, and categorical variables were analyzed using the chi-square test or Fisher's exact test as appropriate. Logistic regression analysis was employed to examine associations between risk factors and outcome events. A significance level of 0.05 was applied to all statistical tests.

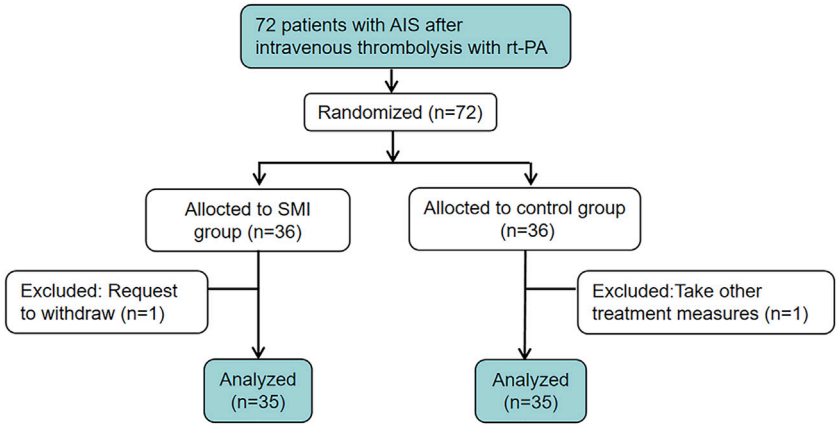


FIGURE 1
The clinical trial flowchart.

TABLE 1 Baseline characteristics of the participants.

Clinical values	Placebo group (*n* = 35)	SMI group (*n* = 35)	*P*
Age (year)	64.71 ± 9.84	67.91 ± 11.99	0.226
Gender (Male)	19 (54.3%)	17 (48.6%)	0.632
Systolic pressure (mmHg)	147.83 ± 14.71	146.89 ± 18.43	0.775
Diastolic pressure (mmHg)	86.06 ± 8.14	82.69 ± 9.93	0.125
NIHSS (quartiles)	8 (7,9)	8 (7,9)	0.766
mRS (quartiles)	3 (3,4)	3 (3,4)	0.680
Anterior circulation infarction	30 (85.7%)	29 (82.9%)	0.743
Past medical history			
Stroke	9 (25.7%)	10 (28.6%)	0.788
Hypertension	28 (80.0%)	21 (60.0%)	0.068
Hyperlipidemia	8 (22.9%)	3 (8.6%)	0.101
Diabetes	8 (22.9%)	14 (40.0%)	0.122
Atrial fibrillation	3 (8.6%)	2 (5.7%)	0.643
Living habit			
Smoker	12 (34.3%)	9 (25.7%)	0.434
Alcohol	8 (22.9%)	6 (17.1%)	0.550

3 Results

3.1 Patient recruitment and baseline characteristics

A total of 72 patients were enrolled in the study, with 36 participants assigned to the SMI group and 36 to the placebo group. During the trial, one participant withdrew from each group. (Figure 1). The baseline characteristics of the participants are summarized in Table 1. There was no significant difference in demographic and clinical characteristics between the two groups at baseline, indicating a well-balanced distribution.

3.2 SMI improved the 30-day outcome in patients with IS receiving intravenous rt-PA thrombolysis

The primary outcome measure, the mRS Scale at 30 days post-onset, was significantly lower in the SMI group compared to the

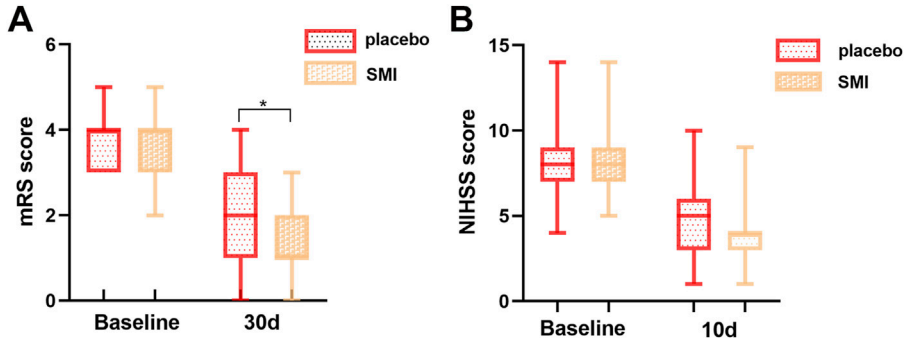


FIGURE 2
Comparison of scale scores of two groups of patients. **(A)** modified Rankin Scale (mRS) score. **(B)** National Institutes of Health Stroke Scale (NIHSS) score.

TABLE 2 Baseline characteristics of serum biochemical indices of participants.

Name	Placebo group	SMI group	*P*
Triglyceride	1.33 (0.89,1.90)	1.31 (1.09,1.71)	0.92
Total Cholesterol	3.94 (3.52,4.77)	4.18 (3.58,5.05)	0.449
LDL-cholesterol	2.65 ± 0.92	2.61 ± 0.66	0.851
HDL-cholesterol	1.20 ± 0.33	1.29 ± 0.35	0.25
Homocysteine	12.1 (10.2,14.8)	12.5 (10.7,15.3)	0.626
uric acid	281.25 ± 82.39	281.06 ± 98.09	0.993
urea	5.26 (4.31,6.56)	5.8 (4.71,7.20)	0.184
AST	14.4 (10.9,27.5)	20.0 (16.0,33.0)	0.053
ALT	20.0 (14.1,23.0)	22.0 (16.0,27.0)	0.109
TBIL	14.62 ± 5.06	14.30 ± 5.71	0.802
DBIL	5.33 (3.90,6.55)	5.10 (4.50,7.21)	0.685
Scr	66.8 (54.0,79.5)	62.0 (53.0,78.4)	0.431

TABLE 3 Serum biochemical indices of participants after 10-day treatment.

Name	Placebo group	SMI group	P
Triglyceride	1.17 (0.97,1.70)	1.26 (0.86,1.46)	0.394
Total Cholesterol	3.46 (2.85,3.86)	3.82 (3.01,4.39)	0.069
LDL-cholesterol	2.02 ± 0.69	2.21 ± 0.53	0.189
HDL-cholesterol	1.11 ± 0.24	1.17 ± 0.23	0.309
Homocysteine	11.1 (8.4,12.5)	11.2 (8.8,13.6)	0.449
uric acid	255.77 ± 81.02	242.68 ± 74.18	0.483
urea	4.55 (3.56,5.24)	4.65 (3.54,5.26)	0.916
AST	24.0 (14.1,30.4)	25.2 (18.2,32.1)	0.428
ALT	21.4 (15.4,28.7)	23.0 (19.5,26.2)	0.411
TBIL	11.72 ± 4.13	13.86 ± 5.16	0.06
DBIL	4.39 (3.49,6.18)	5.20 (4.50,6.41)	0.113
Scr	65.8 (53.9,79.3)	62.4 (52.1,73.3)	0.363

placebo group, indicating improved functional outcomes. However, no significant difference was observed between the two groups for the secondary outcome, the NIHSS score, assessed at day 10 post-treatment (Figure 2).

3.3 Safety of ten days of SMI injection

Serum biochemical indices, including liver and kidney function, were compared before and after treatment. The results indicated no statistically significant differences between the pre- and post-treatment values in either group (Tables 2, 3), suggesting that a 10-day course of SMI injection is safe.

3.4 Adverse events

Within 10 days of treatment, one patient in the placebo group experienced hemorrhagic transformation, two patients had gingival bleeding, and one patient experienced gastrointestinal bleeding. In the SMI group, two patients had hemorrhagic transformation, and two patients experienced a progressive stroke with deterioration following thrombolysis. The incidence of adverse events was not significantly different between the two groups.

3.5 SMI protects the cell viability of H2O2-stimulated PC12 cells

The CCK-8 assay was used to evaluate the effects of varying concentrations of H_2O_2 and SMI on PC12 cell viability. As shown in Figures 3A,B), increasing concentrations of H_2O_2 led to a progressive decrease in cell survival rates. Conversely, SMI at concentrations ranging from 0 to 100 μL/mL did not significantly affect cell viability under normal conditions. When cells were exposed to 100 μM H_2O_2, the survival rate decreased to 45.56%. Based on these results, 100 μM H_2O_2 was selected to induce oxidative stress as a pathological model for subsequent experiments.

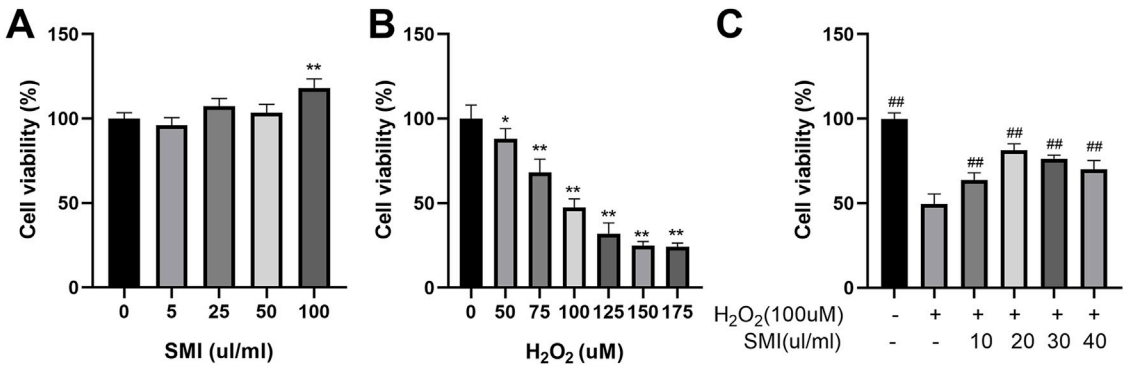


FIGURE 3
Shenmai Injection treatment enhances the viability of H2O2-induced PC12 cells. **(A,B)** Effects of different concentrations of H2O2 and Shenmai Injection on the viability of PC12 cells. **(C)** Effects of Shenmai Injection at different concentrations on the viability of PC12 cells exposed 10 μM H2O2. *P < 0.05 compared with the control group; **P < 0.01 compared with the control group; ##P < 0.01 compared with the H2O2-treated group. "+": indicates the presence of treatments in cells; "–": indicates the absence of treatments in cells.

After inducing oxidative stress with 100 μM H_2O_2, cells were treated with different concentrations of SMI. Among the concentrations tested, 20 μL/mL SMI resulted in the highest cell survival rate (Figure 3C). Therefore, 20 μL/mL SMI was used in subsequent experiments.

3.6 SMI ameliorated cells injury by enhancing AMPKα1 level

To clarify the mechanism of SMI, the expression of AMPKα1 was measured by immunofluorescence method, and to further investigate whether AMPKα1 plays the key role in SMI treatment, we used AMPKα1-specific siRNA to significantly decrease AMPKα1. As shown in Figure 4, SMI significantly enhanced the decreased fluorescence intensity due to H2O2 stimulation, and this effect was reversed by Si-AMPKα1. SiRNA-NC had no obvious effect on cells.

3.7 SMI attenuates H_2O_2-Induced Apoptosis in PC12 cells via AMPKα1

The TUNEL assay was employed to detect apoptosis in PC12 cells. As shown in Figure 5, exposure to H2O2 significantly increased the proportion of TUNEL-positive cells (red fluorescence) compared to the control group. Treatment with SMI markedly reduced the apoptotic index relative to the H2O2 group.

Furthermore, the anti-apoptotic effect of SMI was negated by AMPKα1-specific siRNA (si-AMPKα1), indicating that AMPKα1 plays a critical role in the anti-apoptotic mechanism of SMI.

3.8 SMI attenuated H_2O_2-Induced Oxidative Stress in PC12 cells via AMPKα1

To investigate the potential mechanism of SMI's protective effects against H_2O_2-induced injury, key markers of oxidative stress, including MDA, ROS, and SOD activity, were measured. Compared to the control group, H_2O_2 exposure significantly increased MDA and ROS levels while reducing SOD activity (Figure 6).

SMI treatment markedly increased SOD activity and decreased MDA and ROS levels in H_2O_2-stimulated cells. However, these antioxidative effects were inhibited by AMPKα1-specific siRNA (si-AMPKα1), highlighting the essential role of AMPKα1 in mediating SMI's antioxidative properties.

3.9 SMI attenuated H_2O_2-Induced mitochondrial damage in PC12 cells via AMPKα1

Mitochondrial function was assessed by measuring mitochondrial membrane potential using JC-1 staining and ATP levels. SMI treatment significantly shifted JC-1 fluorescence from green to red, indicating improved mitochondrial membrane potential, and increased ATP levels compared to the H2O2 group (Figure 7). However, these effects were partially reversed by si-AMPKα1, highlighting the role of AMPKα1 in the protective mechanism of SMI.

4 Discussion

In this study, we found that SMI could significantly reduce the mRS score after 30 days, indicating improved functional outcomes for patients with ischemic stroke. But no significant difference was found in the NIHSS score between the patients treated with SMI for 10 days and those in the placebo group. Additionally, there was no statistical differences in adverse events or safety indicators between the two groups, suggesting that SMI is safe for use in this context.

SMI treatment obviously improved cell viability. Further experiments confirmed that SMI exerted antioxidation effect by regulating MDA, SOD, and ROS levels or activities. These effects were accompanied by improvements in mitochondrial membrane

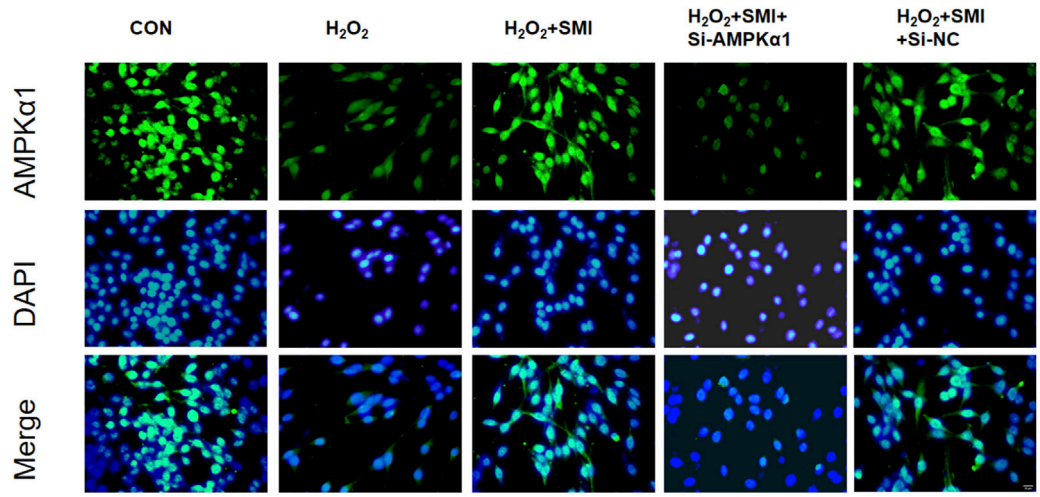


FIGURE 4
Immunofluorescence images showed the effect of SMI on AMPKα1 expression. Scale bar = 20 μM.

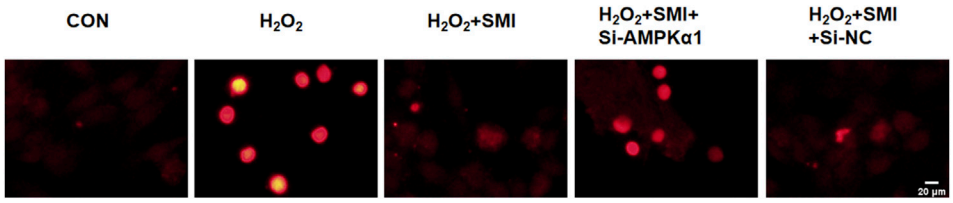


FIGURE 5
Shenmai Injection Attenuates H2O2-Induced Apoptosis in PC12 Cells via AMPKα1. Apoptosis detection using TUNEL assay, Scale bar = 20 μm.

potential and ATP content, ultimately preventing cell apoptosis, as evidenced by a decreased TUNEL-positive rate.

SMI is widely used in clinical treatment of coronary heart disease (Zhou and Jia, 2022), heart failure (Jia et al., 2022), myocardial infarction (Chen, 2019), cerebral infarction (Zhang et al., 2021). Numerous studies have shown that SMI can improve cellular tolerance to ischemia and hypoxia and enhances microcirculation (Tian, 2010; Zhuang et al., 2005). These findings align with our results, where SMI treatment improved the 30-day functional outcomes of IS patients. SMI is a Chinese patent medicine injection that has demonstrated significant neuroprotective effects in previous studies. Specifically, SMI and its main active components, ginsenosides Rb1 and Rg1, have been shown to reduce lactate dehydrogenase release and improve the survival of cultured neurons, vascular endothelial cells, and astrocytes under hypoxic/hypoglycemic/reoxygenation injury. (Zhang et al., 2005). Additionally, SMI helps maintain blood-brain barrier integrity during focal cerebral ischemic injury (Chen et al., 2018) and protects mitochondria from oxidative stress by increasing the level of pyruvate dehydrogenase (Zhao et al., 2018). These findings support the therapeutic potential of SMI in ischemic stroke and other ischemic conditions.

The AMP-activated protein kinase (AMPK), known as an "energy sensor" or "gauge," is expressed across all types of cells and plays a crucial role in regulating cellular energy homeostasis. Previous studies have demonstrated that AMPK has a protective effect on global cerebral ischemia (Ashabi et al., 2014; Duan et al., 2019). AMPK is a heterotrimeric complex composed of α, β, and γ subunits, which respectively mediate enzymatic, scaffolding, and regulatory functions (Hardie, 2015; (Crozet et al., 2014). AMPKα1, an important subtype of AMPK, plays a pivotal role in arteriogenesis and collateral remodeling. Its activation has been shown to contribute to the recovery of occlusive vascular diseases (Zhu et al., 2016), and promotes the activity of antioxidant enzymes, such as SOD, which helps protect neurons from oxidative stress-induced damage and neuronal death (St-Pierre et al., 2006).

In this study, we observed that SMI could activate the AMPKα1 in PC12 cells exposed to oxidative stress. Furthermore, the protective effect of SMI on cell viability and apoptosis was partially inhibited following transfection with si-AMPKα1, supporting the notion that AMPKα1 plays a crucial role in mediating the neuroprotective effects of SMI. Our findings suggest that SMI may attenuate oxidative stress and apoptosis induced by H2O2 through the AMPKα1, highlighting a potential mechanism for its therapeutic effects in ischemic stroke.

However, our study has several limitations. First, the 10-day treatment period with SMI and the outcome evaluation conducted on the 10th day may not have been long enough to see significant differences in clinical outcomes. Previous studies suggest that a

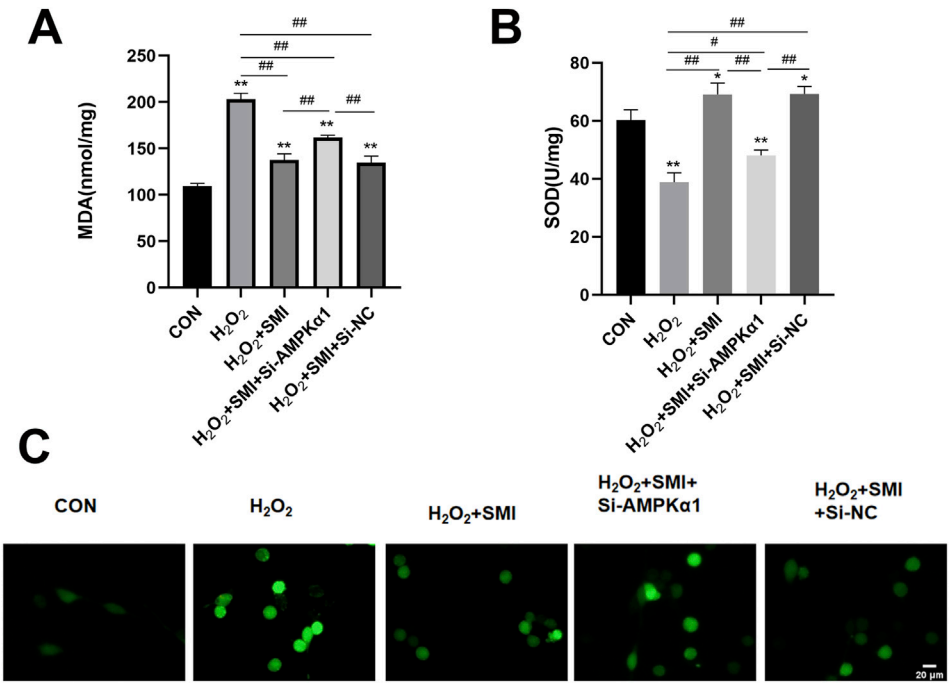


FIGURE 6
Shenmai Injection Attenuated H2O2-Induced Oxidative Stress in PC12 Cells via AMPKα1. **(A,B)** Levels of MDA and SOD in different group of PC12 cells. **(C)** Fluorescent image of ROS in different group of PC12 cells. Scale bar = 20 μm *P < 0.05 compared with the control group; **P < 0.01 compared with the control group; #P < 0.05; ##P < 0.01.

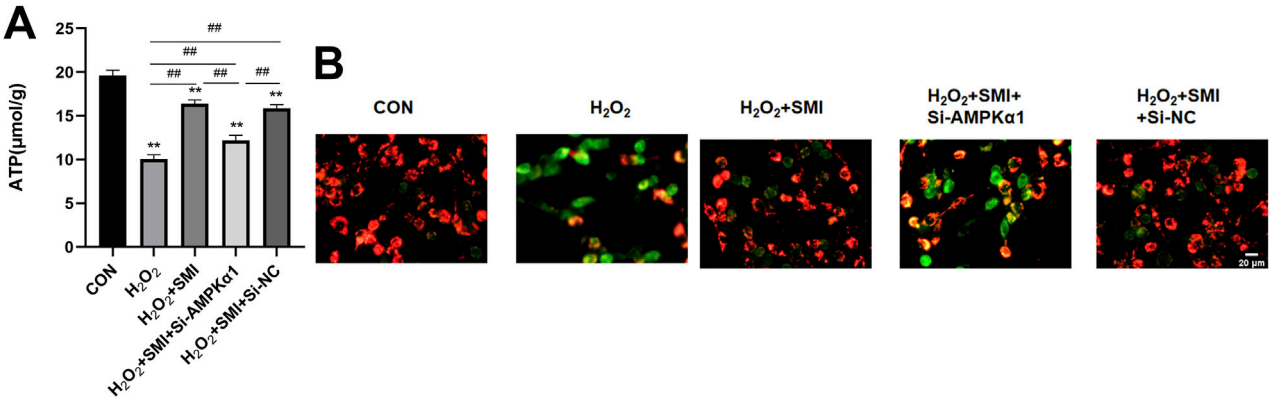


FIGURE 7
Shenmai Injection attenuates H2O2-induced mitochondrial damage in PC12 cells via AMPKα1. **(A)** Fluorescent images of mitochondrial membrane potential using JC-1 staining. Scale bar = 20 μm. **(B)** ATP levels in PC12 cells. **P < 0.01 compared with the control group; ##P < 0.01.

longer treatment duration, typically at least 14 days (Zhen, 2019), is required to observe more pronounced effects. Also, the relatively small sample size in our study could impact the statistical power of the results. A larger cohort in future studies would help to validate these findings. Another limitation is that our study only employed *in vitro* experiments to explore the potential mechanisms of SMI for IS, which provides a lower level of evidence. To better understand the pharmacological effects of SMI and confirm its clinical relevance, further *in vivo* studies and more comprehensive laboratory research are necessary. These additional studies should aim to elucidate the full scope of SMI's mechanisms and its therapeutic potential in ischemic stroke. What's more, according to traditional Chinese medicine theory, SMI is suitable for patients with Qi deficiency, but not for patients without Qi deficiency symptoms.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by the Ethics Committee of Dongfang Hospital. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

JW: Data curation, Formal Analysis, Resources, Validation, Writing – original draft. ZL: Conceptualization, Investigation, Methodology, Writing – original draft. XD: Formal Analysis, Investigation, Resources, Writing – original draft. JL: Conceptualization, Project administration, Writing – review and editing. LW: Funding acquisition, Project administration, Supervision, Writing – review and editing.

Funding

The author(s) declare that financial support was received for the research and/or publication of this article. This study was supported by the Fundamental Research Funds for the Central Universities (Serial number: 2020-JYB-ZDGG-131, 2025-JYB-XJSJJ039 and 2025-JYB-XJSJJ041).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declare that no Generative AI was used in the creation of this manuscript.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2025.1552493/full#supplementary-material

References

Ashabi, G., Khodagholi, F., Khalaj, L., Goudarzvand, M., and Nasiri, M. (2014). Activation of AMP-activated protein kinase by metformin protects against global cerebral ischemia in male rats: interference of AMPK/PGC-1α pathway. *Metab. brain Dis.* 29 (1), 47–58. doi:10.1007/s11011-013-9475-2

Cai, H., Bian, X., Chen, L., Zhang, N., Li, L., Tang, W., et al. (2021). Selective intra-arterial brain cooling induces cerebral protection against ischemia/reperfusion injury through SENP1-Sirt3 signaling. *Free Radic. Biol. Med.* 171, 272–283. doi:10.1016/j.freeradbiomed.2021.05.026

Chen, T., Dai, S. H., Li, X., Luo, P., Zhu, J., Wang, Y. H., et al. (2018). Sirt1-Sirt3 axis regulates human blood-brain barrier permeability in response to ischemia. *Redox Biol.* 14, 229–236. doi:10.1016/j.redox.2017.09.016

Chen, Y. B. (2019). Clinical observation of Shenmai injection combined with urokinase thrombolytic therapy in patients with acute myocardial infarction before treatment. Cardiovascular Disease Electronic Journal of Integrated Traditional Chinese and Western Medicine, 7 (34), 22–24. doi:10.16282/j.cnki.cn11-9336/r.2019.34.014

Crozet, P., Margalha, L., Confraria, A., Rodrigues, A., Martinho, C., Adamo, M., et al. (2014). Mechanisms of regulation of SNF1/AMPK/SnRK1 protein kinases. *Front. plant Sci.* 5 (Article ID 190), 190. doi:10.3389/fpls.2014.00190

Dai, M., Wu, L., Yu, K., Xu, R., Wei, Y., Chinnathambi, A., et al. (2020). D-Carvone inhibit cerebral ischemia/reperfusion induced inflammatory response TLR4/NLRP3 signaling pathway. *Biomed. Pharmacother.* 132, 110870. doi:10.1016/j.biopha.2020.110870

Duan, J. L., Cui, J., Yang, Z. F., Guo, C., Cao, J., Xi, M., et al. (2019). Neuroprotective effect of Apelin 13 on ischemic stroke by activating AMPK/GSK-3β/Nrf2 signaling. *J. neuroinflammation* 16 (24), 24. doi:10.1186/s12974-019-1406-7

Hardie, D. G. (2015). AMPK: positive and negative regulation, and its role in whole-body energy homeostasis. *Curr. Opin. cell Biol.* 33, 1–7. doi:10.1016/j.ceb.2014.09.004

Hu, W., Wang, H., Shu, Q., Chen, M., and Xie, L. (2020). Green tea polyphenols modulated cerebral SOD expression and endoplasmic reticulum stress in cardiac arrest/cardiopulmonary resuscitation rats. *BioMed Res. Int.* 2020, 5080832. doi:10.1155/2020/5080832

Jia, M., Zhu, M., Wang, Y., Li, B., and Yu, R. (2022). Efficacy and Safety of Traditional Chinese Medicine injection in the treatment of Coronary Heart Disease Heart Failure: a Network Meta-analysis. Chin. J. Integr. Med., 20 (02), 14–224.

Jiao, Y., Wang, J., Zhang, H., Cao, Y., Qu, Y., Huang, S., et al. (2020). Inhibition of microglial receptor-interacting protein kinase 1 ameliorates neuroinflammation following cerebral ischaemic stroke. *J. Cell. Mol. Med.* 24 (21), 12585–12598. doi:10.1111/jcmm.15820

Li, C. X., Wang, H. W., Jiang, W. J., Li, G. C., Zhang, Y. D., Luo, C. H., et al. (2020). The nhibition of aldose reductase accelerates liver regeneration through regulating energy metabolism. *Oxidative Med. Cell. Longev.* 2020, 3076131. doi:10.1155/2020/3076131

Li, W. Z., Yang, J. Y., Zhao, F., Pan, J. Y., and Qu, H. B. (2022). Fingerprint of shenmai injection based on ~1H-nmr technique. *Zhongguo Zhong Yao Za Zhi* 47 (3), 581–586. doi:10.19540/j.cnki.cjcmm.20211108.303

Liu, M., Zhang, Q., Chen, X., and Li, K. (2017). Efficacy and Safety of Shenmai Injection in Treatment of Acute Cerebral Infarction: a Meta Analysis. Liaoning J. Traditional Chin. Med. 44 (02), 228–232. doi:10.13192/j.issn.1000-1719.2017.02.002

Pérez-Mato, M., Iglesias-Rey, R., Vieites-Prado, A., Dopico-López, A., Argibay, B., Fernández-Susavila, H., et al. (2019). Blood glutamate EAAT2-cell grabbing therapy in cerebral ischemia. *EBioMedicine* 39, 118–131. doi:10.1016/j.ebiom.2018.11.024

Powers, W. J. (2020). Acute ischemic stroke. *N. Engl. J. o f Med.* 383 (3), 252–260. doi:10.1056/NEJMcp1917030

St-Pierre, J., Drori, S., Uldry, M., Silvaggi, J. M., Rhee, J., Jäger, S., et al. (2006). Suppression of reactive oxygen species and neurodegeneration by the PGC-1 transcriptional coactivators. *Cell* 127 (2), 397–408. doi:10.1016/j.cell.2006.09.024

Tang, C., Hong, J., Hu, C., Huang, C., Gao, J., Huang, J., et al. (2021). Palmatine protects against cerebral ischemia/reperfusion injury by activation of the AMPK/Nrf2 pathway. *Oxidative Med. Cell. Longev.* 2021, 6660193. doi:10.1155/2021/6660193

Tian, R. Z. (2010). Experimental study on the effect of Shenmai Injection on the proliferation of angiogenic nerve cells in cerebral ischemia reperfusion rats. [dissertation/doctor's thesis]. [Chicago (IL)]: Chengdu University of Traditional Chinese Medicine.

Wang, S., Ye, L., and Wang, L. (2019). Protective mechanism of shenmai on myocardial ischemia-reperfusion through the energy metabolism pathway. *Am. J. Transl. Res.* 11 (7), 4046–4062.

Xu, H. M., Liu, Y., Wang, D. S., and Zhang, Z. (2019). Shenmai injection maintains blood-brain barrier integrity following focal cerebral ischemia via modulating the

expression and trafficking of occludin in lipid rafts. *J. Ethnopharmacol.* 237, 55–63. doi:10.1016/j.jep.2019.03.034

Yang, G., Li, S., Sun, X., Lv, Y., and Huang, H. (2023). Quality monitoring of Shenmai injection by HPLC pharmacodynamic fingerprinting. *BMC Chem.* 17 (1), 28. doi:10.1186/s13065-023-00920-7

Yu, J., Li, Y., Liu, X., Ma, Z., Michael, S., Orgah, J. O., et al. (2019). Mitochondrial dynamics modulation as a critical contribution for Shenmai injection in attenuating hypoxia/reoxygenation injury. *J. Ethnopharmacol.* 237, 9–19. doi:10.1016/j.jep.2019.03.033

Zhang, C., Ouyang, L., Zhang, X., Wen, W., Xu, Y., Li, S., et al. (2023). Anaphylactoid reactions induced by Shuanghuanglian injection and Shenmai injection and metabolomics analysis. *Front. Pharmacol.* 14, 1200199. doi:10.3389/fphar.2023.1200199

Zhang, Z., Yan, Y. F., Wei, Y., Sun, S. L., Wang, S. R., Zhu, L. Q., et al. (2005). Protetcive effects of Shenmai injection and ginsenosides Rb1 and Rg1 on damage of neurons. VEC and astrocytes induced by hypoxia/hypoglycemia and reoxygenation. Journal of Beijing University of Traditional Chinese Medicine. 28 (03), 27–30.

Zhao, Y., Zhang, F., Zhao, X. P., Yuan, W., Zhang, J., and Wang, Y. (2018). Shenmai injection protects mitochondria from oxidative injury in myocardial cells and its mechanism. *J. Zhejiang Univ. Med. Ed.* 47 (05), 507–513. doi:10.3785/j.issn.1008-9292.2018.10.10

Zhen, H. X. (2019). Therapeutic effect of Shenmai Injection combined with Alteplase on patients with acute ischemic stroke. *Diabetes World* 12, 30.

Zhou, X., and Jia, W. (2022). Effects of Shenmai Injection combined with Atorvastatin calcium Tablets on vascular endothelial function and inflammatory factors in patients with coronary heart disease. *J. Pharm. Forum* 43, 33–36.

Zhu, H. P., Zhang, M., Liu, Z. Y., Xing, J., Moriasi, C., Dai, X., et al. (2016). AMP-activated protein kinase α1 in macrophages promotes collateral remodeling and arteriogenesis in mice *in vivo*. *Arteriosclerosis, thrombosis, Vasc. Biol.* 36 (9), 1868–1878. doi:10.1161/atvbaha.116.307743

Zhang, Z., Yang, B., Yang, J., Lyu, J., Zhang, H., and Ma, D. (2021). Clinical effect of Shenmai Injection in the treatment of acute cerebral infarction. China Medical Herald. 18 (20), 62–65. doi:10.20047/j.issn1673-7210.2021.20.015

frontiers | Frontiers in Pharmacology

TYPE Original Research
PUBLISHED 15 May 2025
DOI 10.3389/fphar.2025.1589751

Check for updates

OPEN ACCESS

EDITED BY
Huazheng Liang,
Monash University - Southeast University Joint Research Institute (Suzhou), China

REVIEWED BY
Zeliha Selamoglu,
Niğde Ömer Halisdemir University, Türkiye
Ankul Singh S,
ACS Medical College and Hospital, India

*CORRESPONDENCE
Sajad Fakhri,
pharmacy.sajad@yahoo.com
sajad.fakhri@kums.ac.ir
Javier Echeverría,
javier.echeverriam@usach.cl

RECEIVED 07 March 2025
ACCEPTED 28 April 2025
PUBLISHED 15 May 2025

CITATION
Rastinpour Z, Fakhri S, Abbaszadeh F, Ranjbari M, Kiani A, Namiq Amin M and Echeverría J (2025) Neuroprotective effects of astaxanthin in a scopolamine-induced rat model of Alzheimer's disease through antioxidant/anti-inflammatory pathways and opioid/benzodiazepine receptors: attenuation of Nrf2, NF-κB, and interconnected pathways. *Front. Pharmacol.* 16:1589751. doi: 10.3389/fphar.2025.1589751

COPYRIGHT
© 2025 Rastinpour, Fakhri, Abbaszadeh, Ranjbari, Kiani, Namiq Amin and Echeverría. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

Neuroprotective effects of astaxanthin in a scopolamine-induced rat model of Alzheimer's disease through antioxidant/anti-inflammatory pathways and opioid/benzodiazepine receptors: attenuation of Nrf2, NF-κB, and interconnected pathways

Zeinab Rastinpour[1], Sajad Fakhri[2]*, Fatemeh Abbaszadeh[3], Mohammad Ranjbari[1], Amir Kiani[2,4], Mohammed Namiq Amin[1] and Javier Echeverría[5]*

[1]Student Research Committee, Kermanshah University of Medical Sciences, Kermanshah, Iran, [2]Pharmaceutical Sciences Research Center, Health Institute, Kermanshah University of Medical Sciences, Kermanshah, Iran, [3]Neurobiology Research Center, Institute of Neuroscience and Cognition, Shahid Beheshti University of Medical Sciences, Tehran, Iran, [4]Regenerative Medicine Research Center, Health Technology Institute, Kermanshah University of Medical Sciences, Kermanshah, Iran, [5]Departamento de Ciencias del Ambiente, Facultad de Química y Biología, Universidad de Santiago de Chile, Santiago, Chile

Background: Given the complexity of pathological mechanisms behind Alzheimer's disease (AD), there is a pressing need for novel multi-targeting therapeutic agents. Astaxanthin, a natural compound with diverse biological effects, has emerged as a potential candidate in neuronal diseases.

Purpose: This study aimed to evaluate the neuroprotective effects of astaxanthin in a scopolamine-induced rat model of AD.

Materials and methods: In total, 36 male Wistar rats were divided into six groups, including a control group receiving normal saline, a negative control group treated with scopolamine (1 mg/kg), and two groups receiving astaxanthin at doses of 5 and 10 mg/kg. Additionally, two groups were pre-treated with naloxone (0.1 mg/kg) or flumazenil (0.5 mg/kg) to block opioid and benzodiazepine receptors, respectively, followed by receiving the most effective dose of astaxanthin (i.e., 10 mg/kg). Treatments were administered via intraperitoneal injection for 14 consecutive days and behavioral tests were done. Biochemical analyses, zymography, Western blotting, and histopathological examinations were also performed.

Results and discussion: Astaxanthin treatment significantly improved cognitive function, enhanced plasma antioxidant capacity by increasing catalase and glutathione levels, and reduced nitrite levels. It also increased serum activity of matrix metalloproteinase 2 (MMP-2), while decreasing MMP-9, increasing the

expression of nuclear factor erythroid 2–related factor 2 (Nrf-2) and decreasing nuclear factor kappa B (NF-κB) in hippocampal tissue. Histopathological findings indicated reduced hippocampal damage after astaxanthin administration. The aforementioned protective effects of astaxanthin were reversed by naloxone and flumazenil.

Conclusion: Astaxanthin demonstrates protective effects against scopolamine-induced AD through its neuroprotective, antioxidant, and anti-inflammatory properties, potentially involving interactions with opioid and benzodiazepine receptors.

KEYWORDS

astaxanthin, Alzheimer's disease, neuroinflammation, NF-κB, matrix metalloproteinase, oxidative stress, Nrf2

1 Introduction

Alzheimer's disease (AD) is the leading cause of dementia worldwide and presents a significant public health challenge due to its complex biology and the lack of effective treatments. Progressive cognitive decline, memory deficits, and behavioral changes primarily characterize the disease. AD is multifactorial, involving various pathways that lead to synaptic dysfunction and neuronal death (Scheltens et al., 2021). Key contributors to this pathology include the accumulation of amyloid-β (Aβ) plaques, hyperphosphorylation of tau protein, neuroinflammation (Smirnov and Galasko, 2022), and oxidative stress (Bai et al., 2022). These processes disrupt critical neuronal networks, particularly in the cerebral cortex and hippocampus, which are vital for memory and learning (Eichenbaum, 2004). Oxidative stress is an important factor in the pathophysiology of AD. The brain experiences an imbalance between pro-oxidants and antioxidants due to the excessive synthesis of reactive oxygen species (ROS) and reactive nitrogen species (RNS), which is often caused by Aβ aggregation (Zhao and Zhao, 2013). This imbalance contributes to the gradual neurodegeneration seen in AD by aggravating neuronal damage, promoting lipid peroxidation, and accelerating the creation of neurofibrillary tangles. The antioxidant defense mechanisms act through combating ROS and enzymes of stages I and II of biotransformation. Considerable increase in catalase activity further reveals the effects of drugs in combating oxidative stress (Ishak et al., 2024). Age is a significant risk factor for AD, and the reduced ability of the body to produce antioxidants makes neurons even more susceptible to oxidative damage (Moneim, 2015). Furthermore, research has shown that despite elevated levels of oxidative stress markers in the brains of individuals with AD, the nuclear translocation of nuclear factor erythroid 2-related factor 2 (Nrf2) is often impaired. This impairment contributes to decreased expression of antioxidant genes, further worsening oxidative damage (Khan et al., 2020; Davies et al., 2021).

Neuroinflammation is another critical factor in AD pathology. It involves the activation of nuclear factor kappa B (NF-κB) and the release of pro-inflammatory cytokines, which can exacerbate oxidative stress and neuronal injury (Sun et al., 2022; Thakur et al., 2023). Neuroinflammatory responses can disrupt the blood-brain barrier (BBB), allowing peripheral immune cells to infiltrate the central nervous system (CNS), thereby intensifying neuroinflammation. MMPs are involved in this process; they can degrade extracellular matrix components and facilitate inflammatory cell migration into the brain (Duits et al., 2015; Behl et al., 2021; Zarneshan et al., 2025).

On the other hand, opioid receptors (μ, δ, and κ) play a significant role in cognitive functions, including learning and memory. In individuals with AD, dysregulation of these receptors has been noted, especially in brain regions vital for cognition, such as the hippocampus and cortex (Klenowski et al., 2015; Tanguturi and Streicher, 2023). Studies have shown that signaling pathways that pass through opioid receptors can affect the expression of β-secretase and γ-secretase enzymes, which are essential for the production of Aβ peptides, as a key factor in the development of AD (Teng et al., 2010; Pellissier et al., 2018). This indicates that opioid receptors may influence amyloidogenic pathways and contribute to the neurodegenerative processes associated with AD. Besides, gamma-aminobutyric acid type A (GABA-A) receptors are integral to maintaining the balance between excitatory and inhibitory signals in the brain. These receptors are also involved in synaptic plasticity, which is essential for learning and memory (Sakimoto et al., 2021). Given these insights, enhancing brain antioxidant mechanisms and modulating neuroinflammatory processes, as well as opioid/benzodiazepine receptors appears to be a suitable therapeutic approach for AD. It urges the need for finding novel multi-targeting agents in combating AD.

Astaxanthin, a naturally occurring carotenoid pigment abundant in algae and shellfish, has garnered significant interest due to its potent antioxidant properties (Fakhri et al., 2021b). What distinguishes astaxanthin from many other antioxidants is its unique chemical structure, which allows it to effectively cross the blood-brain barrier and provide direct protection to the CNS (Galasso et al., 2018; Fakhri et al., 2021a; Medoro et al., 2023). Preclinical studies have demonstrated that astaxanthin can reduce oxidative stress, inhibit inflammatory responses, and prevent neuronal apoptosis in models of neurodegenerative diseases (Fakhri et al., 2019a; Fakhri et al., 2019b). Its ability to neutralize ROS and enhance mitochondrial function makes it a promising candidate for countering oxidative damage associated with AD (Taksima et al., 2019). Moreover, research in animal models of AD has shown that astaxanthin's neuroprotective and anti-inflammatory effects are correlated with improved cognitive performance, further highlighting its potential therapeutic benefits (Rahman et al., 2019; El Sayed and Ghoneum, 2020).

While the existing evidence supports the neuroprotective potential of astaxanthin, a detailed understanding of its major effects on oxidative stress and neuroinflammation, along with interactions with the GABA-A and opioid receptor systems, is crucial for developing effective treatment strategies for AD. The objective of this study was to explore the antioxidant and anti-inflammatory effects of astaxanthin in a rat model of AD induced by scopolamine, with a particular emphasis on its interactions with GABA-A and opioid receptors.

2 Materials and methods

2.1 Chemicals

Astaxanthin was purchased from Sigma-Aldrich Company (St. Louis, MO), and naloxone (Nal), an opioid antagonist, was sourced from Caspian Tamin Pharmaceutical Company (Gilan, Iran). Flumazenil (Flu), a benzodiazepine receptor antagonist obtained from Hameln (Hameln, Germany). Scopolamine, an anticholinergic agent, and astaxanthin were procured from Merck Company (Darmstadt, Germany).

2.2 Animals

Four-week-old male Wistar rats weighing 200–250 g were used in this research. The animals were kept in a controlled environment with a 12-h light/dark cycle, a steady temperature of 22°C ± 2°C, and a relative humidity of 65% ± 2%. To guarantee a constant intake of nutrients, the rats were given free access to water and a regular laboratory chow diet. In total, 36 rats were used in the investigation; they were divided into six experimental groups at random (n = 6/group). Under the ethical approval number IR.KUMS.REC.1400.546, the Institutional Animal Care and Use Committee (IACUC) of Kermanshah University of Medical Sciences authorized all animal handling, care, and experimental methods.

2.3 Experiment design

Male Wistar rats were divided into six groups and treated for 14 days. The control group (received only sodium chloride, i.p.), Scop (negative control group, received scopolamine 1 mg/kg/day, i.p.), two groups of AST 5 (receiving scopolamine 1 mg/kg/day + astaxanthin 5 mg/kg/day, i.p.) and AST 10 (receiving scopolamine 1 mg/kg/day + astaxanthin 10 mg/kg/day, i.p.). Groups V, Nal (receiving scopolamine 1 mg/kg/day + naloxone 0.1 mg/kg/day + astaxanthin 10 mg/kg/day, i.p.) and group VI Flu (receiving scopolamine 1 mg/kg/day + flumazenil 0.5 mg/kg/day + astaxanthin 10 mg/kg/day, i.p.). Astaxanthin was administered 30 min before scopolamine, while Flu and Nal were injected 15 min before the administration of astaxanthin. This treatment protocol was maintained for 14 days.

2.4 Behavioral tests

Behavioral evaluations were carried out on days 6, 7, 13, and 14.

2.4.1 Open field test

The open field test is a valuable behavioral assessment for evaluating locomotor activity and anxiety-like behaviors in rats. The open field box was divided into equal squares. During the test, each rat was placed in the center square of the apparatus. Several key behavioral metrics, including the total number of crossings, as an indicator of exploratory behavior, the frequency of rearing (standing on their hind legs, which reflects curiosity and exploration), and grooming (cleaning itself, which can indicate stress levels or comfort) are recorded for 5 min (Prut and Belzung, 2003). After each trial test, the apparatus was thoroughly cleaned with 70% ethanol to eliminate residual odors that could influence the rats' behavior. The study incorporated acclimation sessions on days 6 and 13 of the treatment protocol. These sessions were designed to familiarize the rats with the open field box and reduce potential anxiety or novelty effects during the evaluation sessions that occur on days 7 and 14 (Gould et al., 2009).

2.4.2 Passive avoidance test

One well-known technique for assessing memory and learning in mouse models of neurological diseases is the passive avoidance test (Ghafarimoghadam et al., 2022). The experimental apparatus consists of two equal-sized compartments: a light compartment and a dark compartment, separated by a gate. Both compartments have stainless steel floors that are electrically wired. On days 6 and 13, rats were placed in front of the open gate leading to the dark compartment. Upon entering the dark compartment, an electric shock (1.5 mA for 3 s) is administered. The time taken for the rat to move from the light compartment to the dark compartment is recorded as the initial transfer latency (ITL). On Days 7 and 14, the test was repeated to assess memory retention. The time taken for the rat to enter the dark compartment on these days was referred to as the step-through latency (STL). A longer delay in entering the dark compartment indicates better memory recall of the unpleasant experience associated with the electric shock (Zarneshan et al., 2025).

2.4.3 Elevated plus maze

The elevated plus maze is a commonly used experimental device for evaluating anxiety-related behaviors and cognitive abilities in animal models. Its structure consists of a cross-shaped platform elevated 50 cm above the ground, featuring two enclosed arms (50 cm × 10 cm × 40 cm) and two orthogonal open arms (10 cm × 40 cm), connected by a central platform (10 cm × 10 cm). During the learning session on day 6, rats were placed in an unprotected open arm, and the duration until they entered an enclosed arm was measured as the ITL. The experiment was conducted again on days 7 and 14 to assess memory retrieval, and the duration taken to transition from the open arm to the enclosed arm is documented as transfer latency (TL). A longer TL suggests improved memory recall and reduced anxiety (Danduga and Kola, 2024).

2.5 Biochemical analysis

2.5.1 Measurement of catalase level

The catalase activity was evaluated by the Aebi method, a recognized approach for quantifying the enzyme's action. For the experiment, 20 μL of serum was incorporated into a solution of 65 mM hydrogen peroxide. The reaction mixture was incubated at 25°C for 4 min. Subsequently, 100 μL of 4.32 mM ammonium molybdate was added to halt the process. The absorbance of the resultant solution was quantified at 405 nm with an ELISA reader. The percentage difference in optical absorbance between the experimental groups and the control group was determined to evaluate the levels of catalase (Aebi, 1984).

2.5.2 Measurement of glutathione level

The glutathione level was measured using Ellman's technique, which involves the interaction of thiol groups with 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) to produce a yellow-colored product quantifiable by spectrophotometry (Ellman, 1959). For the experiment, 50 μL of phosphate buffer (pH 7.4) and 60 μL of the sample were introduced into each well. Subsequently, 100 μL of DTNB reagent was added, and the mixture was incubated at 37°C for 10 min to facilitate the reaction. The absorbance of the reaction product was then quantified at 412 nm using an ELISA reader. The differences in OD expressed as a percentage between the normal control and other groups (sample) were calculated using the following formula (Fakhri et al., 2022b).

$$\text{Concentration difference } (\%) = \left(\left(C_{\text{control}} - C_{\text{sample}} \right) / C_{\text{sample}} \right) \times 100$$

2.5.3 Measurement of nitrite

The nitrite levels were assessed by the Griess reagent technique, a commonly used colorimetric test for identifying nitrite concentrations. Nitrate was first reduced to nitrite, which then reacted with the Griess reagent to produce a chromophoric azo molecule. The procedure commenced with deproteinization using zinc sulfate, followed by centrifugation at 10,000 rpm for 10 min. Next, 100 μL of vanadium chloride solution was added to the serum supernatant to convert nitrates into nitrites. The resulting mixture was then combined with Griess reagent (sulfanilamide and ethylene diamine dihydrochloride) and incubated for 30 min at 37°C. Finally, the color intensity was measured at a wavelength of 540 nm (Berkels et al., 2004).

2.6 Zymography

To assess gelatinase activity, serum samples containing 100 μg of total protein were loaded onto SDS-PAGE gels supplemented with 0.1% gelatin. The gel was electrophoresed at 150 V. Following this, the gel was washed with a buffer solution containing 2.5% Triton X-100 in 50 mM Tris-HCl for 1 h. The washed gel was then incubated at 37°C for 18 h in a buffer containing 10 mM calcium chloride ($CaCl_2$), 0.02% NaN_3, and 0.15 M sodium chloride (NaCl) in 50 mM HCl. After incubation, the gel was stained with Coomassie blue, which binds to proteins and allows the visualization of protein bands. To enhance the contrast between the stained protein bands and the clear areas, excess dye was removed from the gel using a solution of 5% acetic acid and 7% methanol. Finally, the bands were quantified using ImageJ software (Hashemi et al., 2024).

2.7 Western blot analysis

For Western blotting, hippocampal tissues were lysed in RIPA buffer and centrifuged at 14,000 rpm for 20 min at 4°C. According to the manufacturer's instructions, protein concentrations were determined using the Bradford Protein Quantification kit (DB0017, DNAbioTech, Iran). A total of 40 μg of lysate was loaded onto SDS-PAGE. The proteins were transferred to a PVDF membrane (Bio-Rad Laboratories, CA, United States) and blocked with 5% BSA (Sigma Aldrich, MO, United States) in 0.1% Tween 20 for 1 h. Membranes were then incubated overnight at 4°C with anti- NF-κB (1:1,000, Abcam), anti-Nrf2 (1:1,000, Biorbyt), and anti-β-actin antibodies (1:2500, Abcam). After washing with TBST, the membranes were incubated for 1 h with goat anti-rabbit IgG H&L (HRP) secondary antibody (1:10,000, Abcam). The protein bands were visualized using a chemiluminescence kit and X-ray films. Densitometric analysis was then conducted using ImageJ software (NIH, United States) (Fakhri et al., 2019b).

2.8 Histology

On the 14th day, whole brains were fixed in 10% formalin and subsequently dehydrated using ethanol at varying concentrations before being embedded in paraffin. Thin sections (5 μm) of the hippocampus were stained with hematoxylin and eosin (H&E). An Olympus CX23 light microscope, a Dino-Lite camera, and DinoCapture 2.0 software were used for imaging. The histopathological evaluation was performed by a blinded experimenter (El-Missiry et al., 2018).

2.9 Statistical analysis

Statistical analyses were performed using GraphPad Prism software (version 8.4.3). To assess the normality distribution of the data, the Shapiro test was conducted. One-way or two-way analysis of variance (ANOVA), followed by Tukey or Bonferroni *post hoc* tests, was employed. A *P*-value of less than 0.05 ($P < 0.05$) was considered statistically significant, and results are presented as mean ± standard error of the mean (SEM).

3 Results

3.1 Open-field test

The exploratory behavior of rats was assessed by the open-field test, focusing on the frequency of crossings (Figures 1A,B), grooming (Figures 1C,D), and rearing (Figures 1E,F) activities. Administration of scopolamine, a drug known to induce cognitive and motor impairments, led to significant reductions in all measured behaviors throughout the experiment in the Scop

10.3389/fphar.2025.1589751

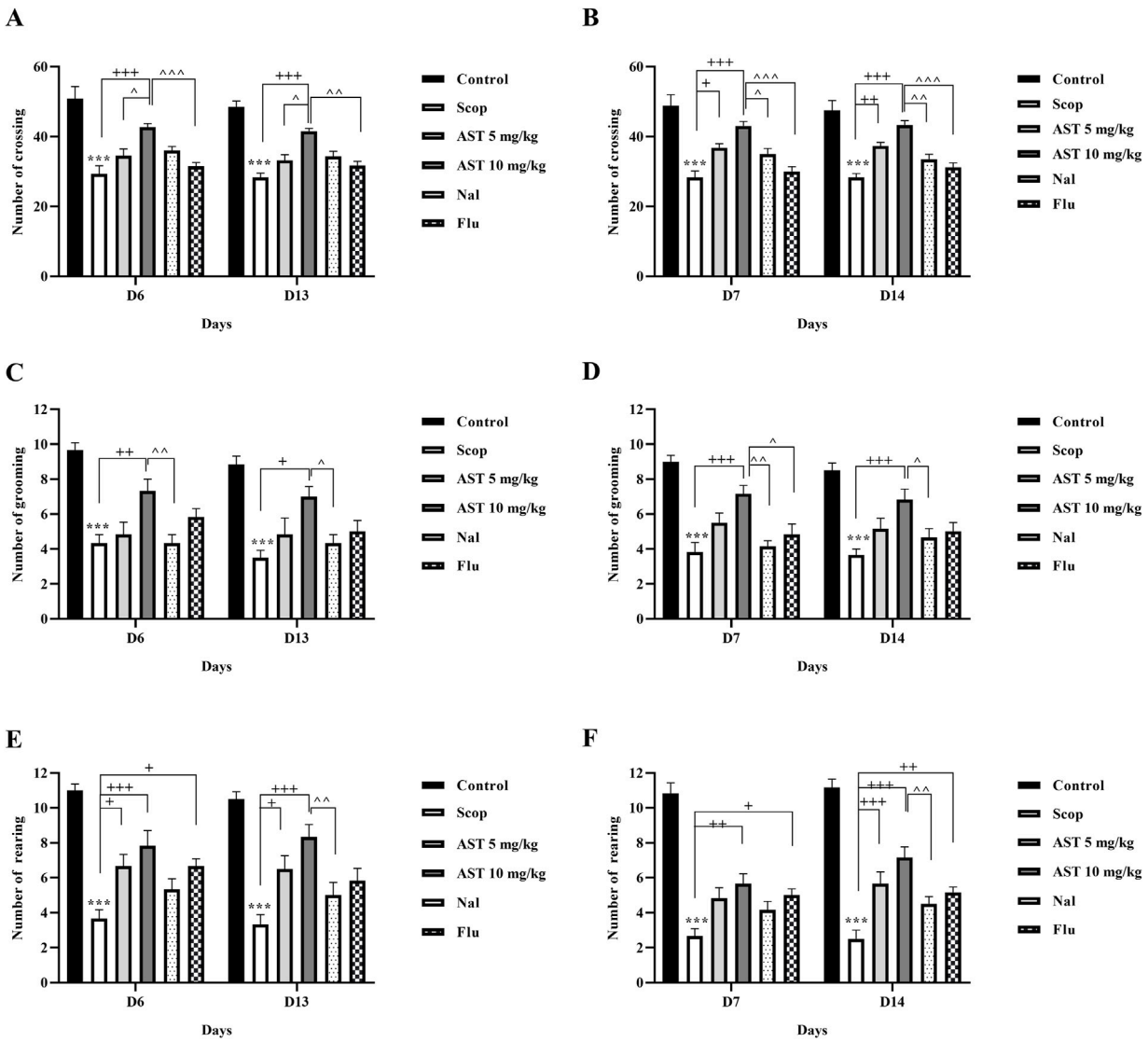


FIGURE 1
Effect of astaxanthin and co-administration with Flu and Nal on open field test in a rat model of AD. Day 6 and day 13: **(A)** number of crossings, **(C)** number of grooming, and **(E)** number of rearing. Day 7 and day 14: **(B)** number of crossings, **(D)** number of grooming, and **(F)** number of rearing. In Figure Flu: Flumazenil + AST 10 mg/kg; and Nal: Naloxone + AST 10 mg/kg; AST: Astaxanthin; Scop: Scopolamine. ***$P < 0.001$ compared with control group, $^{+}P < 0.05$, $^{++}P < 0.01$, $^{+++}P < 0.001$ compared with Scop group. $^{\wedge}P < 0.05$, $^{\wedge\wedge}P < 0.01$, $^{\wedge\wedge\wedge}P < 0.001$ compared with astaxanthin. Data are reported as mean ± SEM ($n = 6$).

group ($P < 0.001$). Conversely, administration of astaxanthin, particularly at 10 mg/kg, significantly improved impairments induced by scopolamine ($P < 0.05$). Pre-treatment with Flu (GABA-A receptor antagonist) and Na1 (opioid receptor antagonist), significantly reduced the therapeutic effects of astaxanthin on the test days ($P < 0.05$).

3.2 Passive avoidance test

The results from the shuttle test indicated that the negative control group (Scop group) showed a significant increase in ITL ($P < 0.001$, Figure 2A). Treatment with both doses of astaxanthin (5 and 10 mg/kg) resulted in a significant reduction in ITL on days 6 and 13 ($P < 0.001$). Furthermore, scopolamine treatment significantly decreased STL ($P < 0.001$). In contrast, astaxanthin treatment resulted in a significant increase in STL compared to the scopolamine group on days 7 and 14 ($P < 0.001$). Additionally, a significant reversal of the effects of astaxanthin (10 mg/kg) was observed in groups pre-treated with Flu and Nal ($P < 0.01$).

3.3 Elevated plus maze

The results indicated that scopolamine treatment resulted in a notable increase in ITL in the scopolamine group compared to the control group ($P < 0.01$, Figure 3A), suggesting an increased

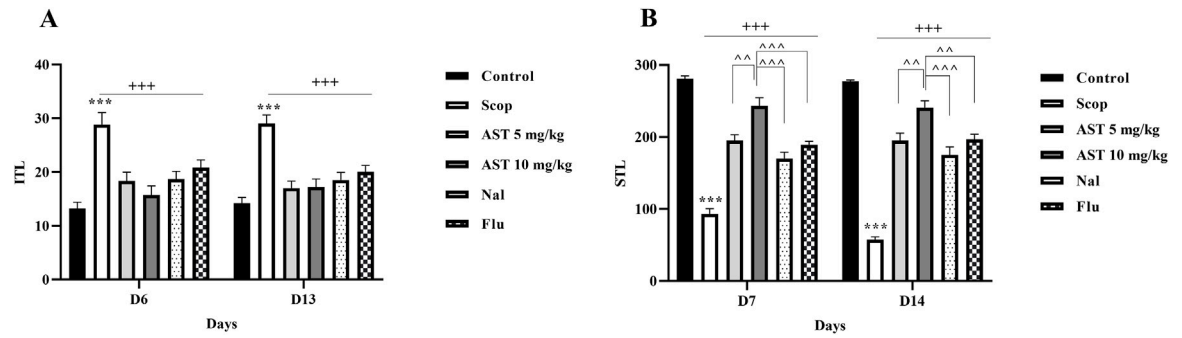


FIGURE 2
Effect of astaxanthin and co-administration with Flu and Nal on passive avoidance learning and memory in a rat model of AD. **(A)** Initial transfer latency (ITL-Day 6 and day 13) and **(B)** step-through latency (STL-Day 7 and day 14). In Figure Flu: Flumazenil + AST 10 mg/kg; and Nal: Naloxone + AST 10 mg/kg; AST: Astaxanthin; Scop: Scopolamine. ***$P < 0.001$ compared with control group, $^{+++}P < 0.001$, compared with Scop group, $^{\wedge}P < 0.01$, $^{\wedge\wedge\wedge}P < 0.001$ compared with astaxanthin. Data are reported as mean ± SEM ($n = 6$).

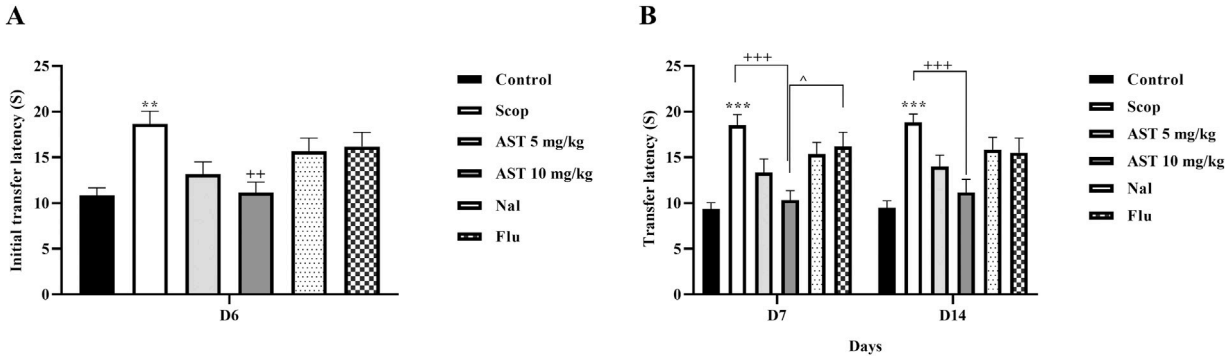


FIGURE 3
Effect of astaxanthin and co-administration with Flu and Nal on elevated plus maze and memory in a rat model of AD. **(A)** initial transfer latency (Day 6) and **(B)** transfer latency (Day 7 and day 14). In Figure Flu: Flumazenil + AST 10 mg/kg; and Nal: Naloxone + AST 10 mg/kg; AST: Astaxanthin; Scop: Scopolamine. **$P < 0.01$, ***$P < 0.001$ compared with control group, $^{++}P < 0.01$, $^{+++}P < 0.001$ compared with Scop group, $^{\wedge}P < 0.05$ compared with astaxanthin. Data are reported as mean ± SEM ($n = 6$).

anxiety or poor memory retention. In contrast, the administration of astaxanthin, especially at a dosage of 10 mg/kg, led to a significant reduction in ITL ($P < 0.01$), demonstrating its cognitive-enhancing properties. Notably, pre-treatment with the antagonists Flu and Nal reversed these positive effects. Furthermore, rats treated with scopolamine exhibited significant increases in TL on days 7 and 14 ($P < 0.001$, Figure 3B), indicating poor memory retention. In contrast, the astaxanthin-treated group showed significant decreases in TL, effectively counteracting the memory impairments caused by scopolamine ($P < 0.001$). Pre-treatment with the antagonists Flu and Nal diminished the positive effects of astaxanthin on TL, and the effects of Flu were statistically significant ($P < 0.05$).

3.4 Determination of antioxidant markers

Compared to the control group, the Scop group demonstrated a significant increase in serum nitrite levels ($P < 0.001$, Figure 4A), suggesting enhanced nitrite production. Furthermore, the Scop group displayed markedly lower levels of serum catalase ($P < 0.001$, Figure 4B) and glutathione ($P < 0.001$, Figure 4C). In contrast, treatment with astaxanthin, particularly at a concentration of 10 mg/kg, led to significant restoration of these biochemical markers. However, pre-treatment with the antagonists Flu and Nal reduced the beneficial effects of astaxanthin ($P < 0.05$).

3.5 Zymography

Compared to the control group, the Scop group demonstrated a significant increase in MMP-9 ($P < 0.001$, Figure 5A) and a reduction in MMP-2 levels ($P < 0.001$, Figure 5B). In contrast, treatment with astaxanthin, particularly at a concentration of 10 mg/kg, led to significant restoration of these levels ($P < 0.05$). However, pre-treatment with the antagonists Flu and Nal reduced the beneficial effects of astaxanthin ($P < 0.05$).

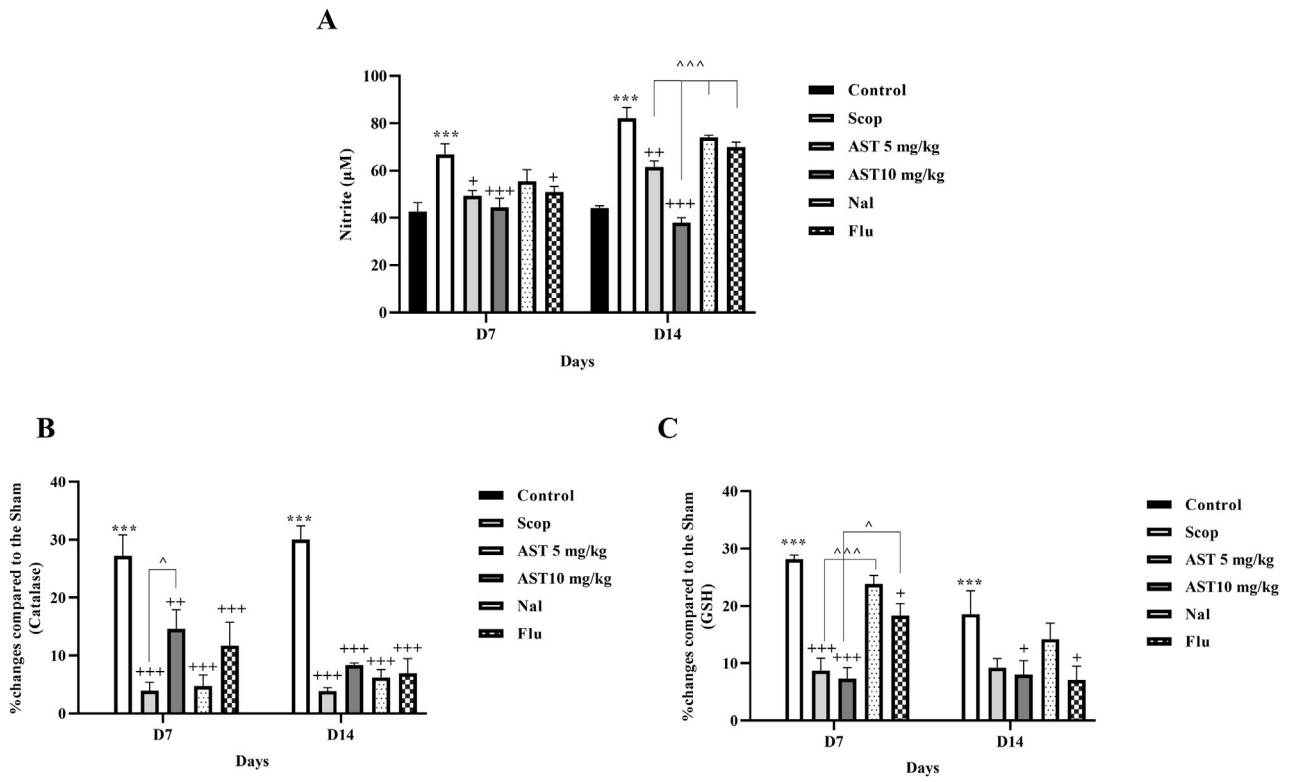


FIGURE 4
Effect of astaxanthin and co-administration with Flu and Nal on oxidative stress in a rat model of AD. **(A)** Nitrite, **(B)** catalase, and **(C)** GSH. In Figure Flu: Flumazenil + AST 10 mg/kg; and Nal: Naloxone + AST 10 mg/kg; AST: Astaxanthin; Scop: Scopolamine. ***$P < 0.001$ compared with control group, $^{+}P < 0.05$, $^{++}P < 0.01$, $^{+++}P < 0.001$ compared with Scop group, and $^{\wedge}P < 0.05$, $^{\wedge\wedge\wedge}P < 0.001$ compared with astaxanthin. Data are reported as mean ± SEM.

3.6 Histology

Administration of Scop led to significant neuronal loss in the hippocampal region (CA1, CA2, and DG). However, treatment with astaxanthin demonstrated a protective effect on these neurons, helping to preserve their function and integrity. On the other hand, the administration of Flu and Nal appeared to reverse the protective effects of astaxanthin (Figure 6).

3.7 Western blot

Following the induction of the scopolamine model of AD, we observed downregulation in the expression levels of Nrf2 ($P < 0.001$, Figure 7A) and upregulation of NF-κB ($P < 0.001$, Figure 7B) proteins in the hippocampal tissue of rats. However, the administration of astaxanthin at both doses effectively reversed these alterations ($P < 0.01$). Notably, pre-treatment with the antagonists Flu and Nal mitigated the positive effects of astaxanthin ($P < 0.05$).

4 Discussion

The current study examined the effects of astaxanthin in a scopolamine-induced rat model of AD. Behavioral assessments indicated that astaxanthin alleviated AD-related cognitive deficits, including the open field, passive avoidance, and elevated plus maze tests. Furthermore, we demonstrated its antioxidant properties by restoring levels of catalase and glutathione, as well as expressing Nrf-2, which was accompanied by a reduction in the oxidative stress marker nitrite. Our findings also underscored the anti-inflammatory effects of astaxanthin, as shown by a decrease in inflammatory MMP-9, while a decrease in anti-inflammatory MMP-2 and suppression of NF-κB. These biochemical changes were further validated by histological evaluations, which highlighted the neuroprotective effects of astaxanthin. However, it is important to note that the protective effects of astaxanthin were attenuated by Nal and Flu (Figure 8).

AD remains a formidable challenge in neurology, characterized by a complex interplay of pathogenic mechanisms, including oxidative stress, neuroinflammation, and synaptic dysfunction (Kobayashi et al., 2009; McMahon et al., 2010; de Castro et al., 2019; Teixeira et al., 2019; Fakhri et al., 2020). Scopolamine acts as a non-selective muscarinic acetylcholine receptor antagonist, reducing cholinergic activity. This mimics the cholinergic deficits seen in AD patients, where impaired neurotransmission contributes to memory loss and cognitive decline. The administration of scopolamine results in several pathological changes that are characteristic of AD, including the deposition of Aβ, hyperphosphorylation of tau proteins, and increased oxidative stress levels (Chen and Yeong, 2020; Yadang et al., 2020).

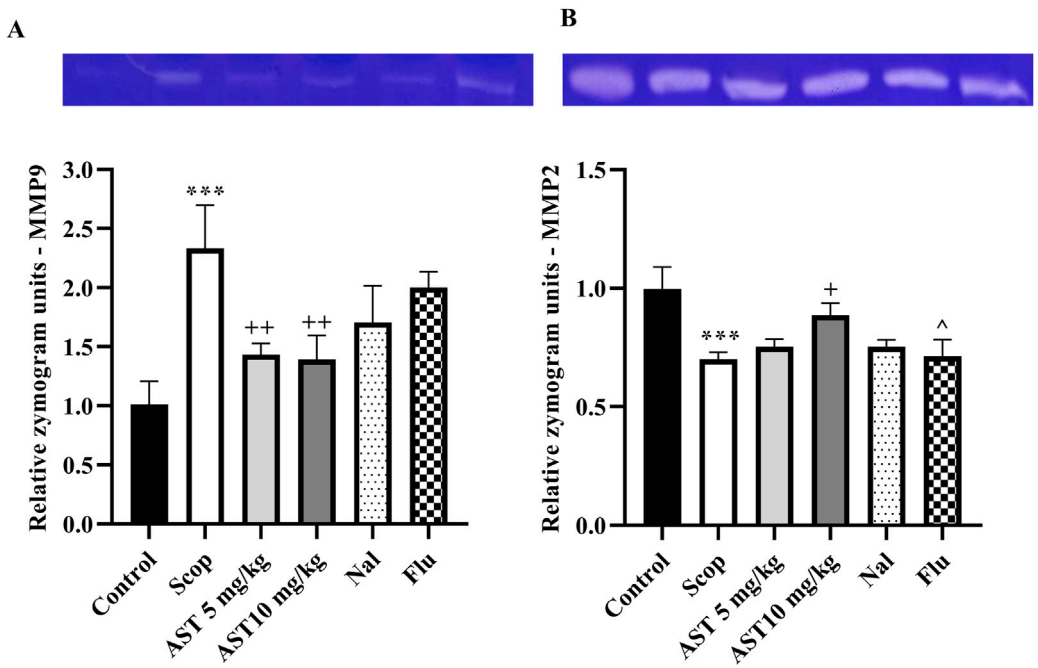


FIGURE 5
Effect of astaxanthin and co-administration with Flu and Nal on MMP in a rat model of AD. **(A)** MMP-9, and **(B)** MMP-2. In Figure Flu: Flumazenil + AST 10 mg/kg; and Nal: Naloxone + AST 10 mg/kg; AST: Astaxanthin; Scop: Scopolamine. ***$P < 0.001$ compared with control group, $^{+}P < 0.05$, $^{++}P < 0.01$, compared with Scop group, and $^{\wedge}P < 0.05$ compared with astaxanthin. Data are reported as mean ± SEM.

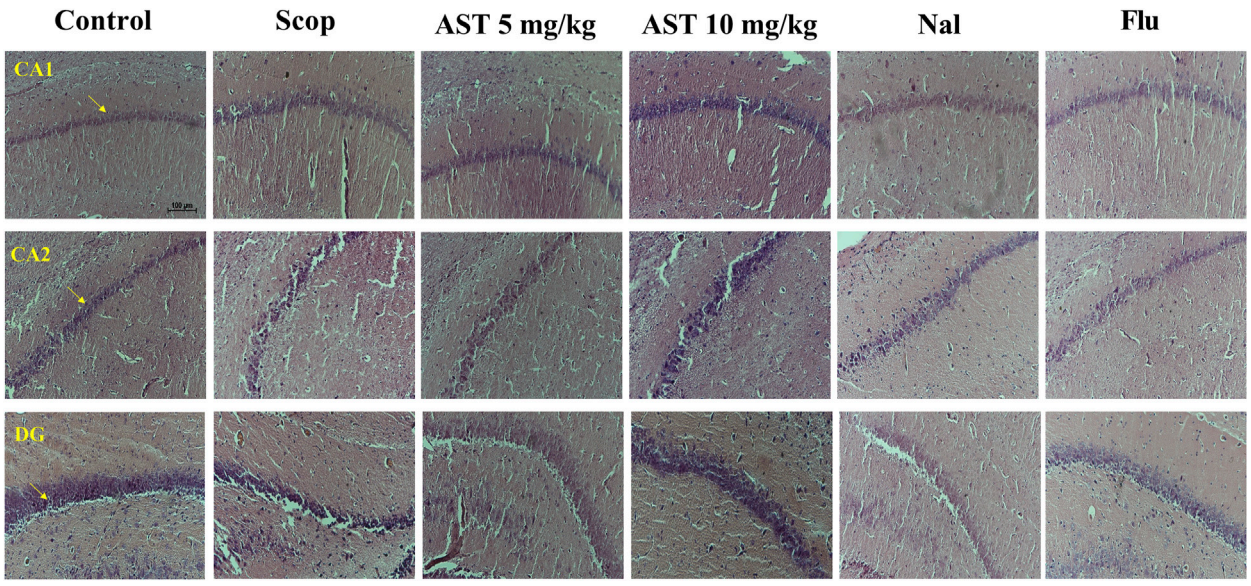


FIGURE 6
Effect of astaxanthin and co-administration with Flu and Nal on hippocampus tissue (CA1, CA2, and DG) changes in a rat model of AD, Arrows indicate healthy neurons (X20). In Figure Flu: Flumazenil + AST 10 mg/kg; and Nal: Naloxone + AST 10 mg/kg; AST: Astaxanthin; Scop: Scopolamine.

In AD, elevated oxidative stress is implicated in neuronal damage, synaptic dysfunction, and the accumulation of Aβ plaques and hyperphosphorylated tau protein, contributing to the disease's characteristic neurodegenerative processes (Zhao and Zhao, 2013; Huang et al., 2016). Nrf-2 is central to the cellular response to oxidative stress, a transcription factor that orchestrates the expression of various antioxidant and cytoprotective genes. Under normal physiological conditions, Nrf-2 is kept in the cytoplasm in an inactive form; however, under oxidative stress, it translocates to the nucleus, where it binds to antioxidant response

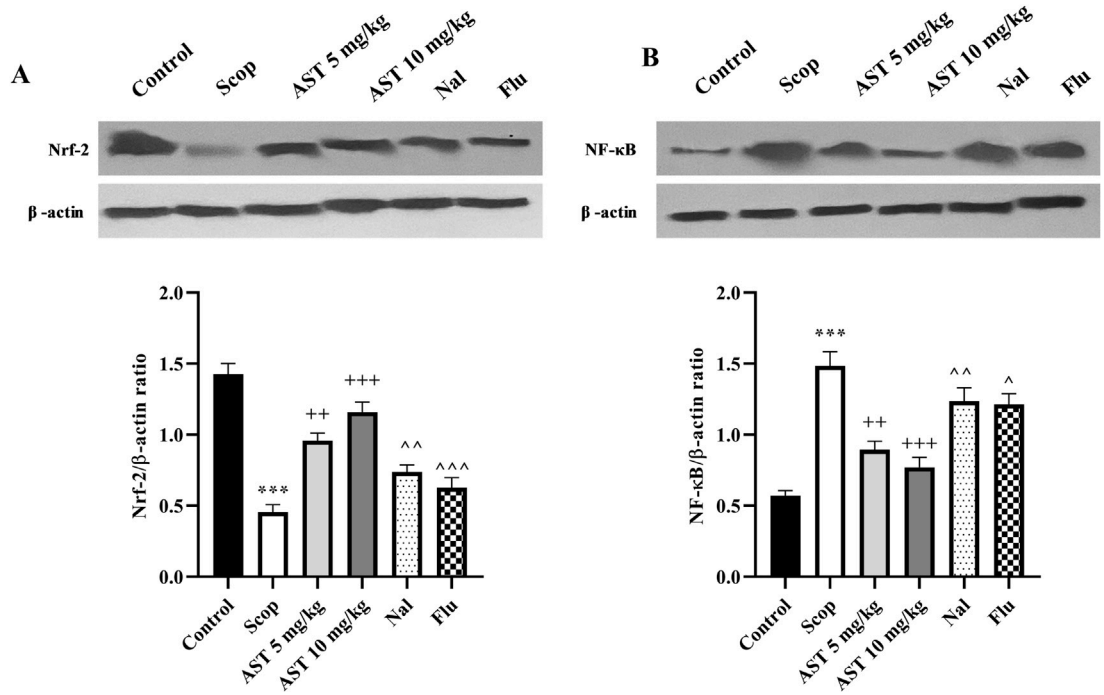


FIGURE 7
Effect of astaxanthin and co-administration with Flu and Nal on the expression levels of Nrf-2 and NF-κB in a rat model of AD. **(A)** Nrf-2, and **(B)** NF-κB. In Figure Flu: Flumazenil + AST 10 mg/kg; and Nal: Naloxone + AST 10 mg/kg; AST: Astaxanthin; Scop: Scopolamine. ***$P < 0.001$ compared with control group, $^{++}P < 0.01$, $^{+++}P < 0.001$, compared with Scop group, and $^{^}P < 0.05$, $^{^^}P < 0.01$, $^{^^^}P < 0.001$ compared with astaxanthin. Data are reported as mean ± SEM.

elements (AREs) in the promoters of target genes. This process changed the expression of critical antioxidant enzymes such as catalase and glutathione (Ngo and Duennwald, 2022; Chu et al., 2024). In the brains of AD patients, the activity of antioxidant enzymes such as glutathione peroxidase is often reduced in affected brain regions, highlighting the compromised ability to counteract oxidative stress (Jurcău et al., 2022). Oxidative stress can initiate inflammatory responses by activating NF-κB. Under oxidative stress, ROS can induce the phosphorylation and subsequent degradation of IκB proteins, which normally inhibit NF-κB by sequestering it in the cytoplasm. Once IκB is degraded, NF-κB translocates to the nucleus, where it activates the transcription of pro-inflammatory genes (Lingappan, 2018). On the other hand, NF-κB can enhance the transcription of genes encoding MMPs, particularly MMP-9 (Hsieh et al., 2010). MMP-9 levels are often elevated in the brains of AD patients. It has been shown to correlate with cognitive decline and may contribute to neuroinflammation and synaptic dysfunction (Wang et al., 2014). While MMP-2 activity is decreased in AD patients and those with mild cognitive impairment, suggesting a potential protective role against Aβ accumulation (Lim et al., 2011). Those results were in line with our current study and our previous reports on the anti-inflammatory roles of MMP-2 and the inflammatory potentials of MMP-9 in AD (Zarneshan et al., 2025).

Given the critical role of oxidative stress in AD, various treatment strategies have been explored, including the use of antioxidants. Antioxidants are compounds that can mitigate oxidative damage, potentially slowing the progression of AD and improving cognitive function (Pritam et al., 2022). Phytochemicals and herbal medicines are potent antioxidants, metal chelators, and free radical scavengers, by suppressing lipid peroxidation (Gogebakan et al., 2012). For instance, there are great antioxidant properties behind the neuroprotective role of polyphenols (Daglia et al., 2014). Additionally, these compounds regulate inflammatory biomarkers, oxidative stress, and endothelial function towards neuroprotection. Of other plant secondary metabolites, iridoids showed anti-AD potentials with antioxidant effects (Hamedi et al., 2020; Kavyani et al., 2024). Natural products have also shown antioxidant properties through total antioxidant status, total oxidant status and levels, and oxidative stress index (Sevindik et al., 2020). These compounds also showed antioxidant potential by combating nitric oxide and downstream mediators (Selamoglu-Talas et al., 2015). Nutritional deficiencies are also in a near linkage with psychiatric disorders such as schizophrenia, anxiety, and depression (Vasile et al., 2024). Astaxanthin is considered one of the most powerful super antioxidants in nature, significantly more effective than vitamin C, vitamin E, and other carotenoids. Its unique molecular structure allows it to quench free radicals, reduce oxidative stress, and protect cells from damage (Fakhri et al., 2018). We previously showed the neuroprotective effects of astaxanthin (at the same doses of 5, and 10 mg/kg, i.p.) in a rat model of chronic constriction injury (Hashemi et al., 2024). Similarly, our research team confirmed the antinociceptive role of astaxanthin through nitric oxide pathway at i.p. doses of 5, and 10 mg/kg (Mohammadi et al., 2021). We also demonstrated the antioxidant properties of

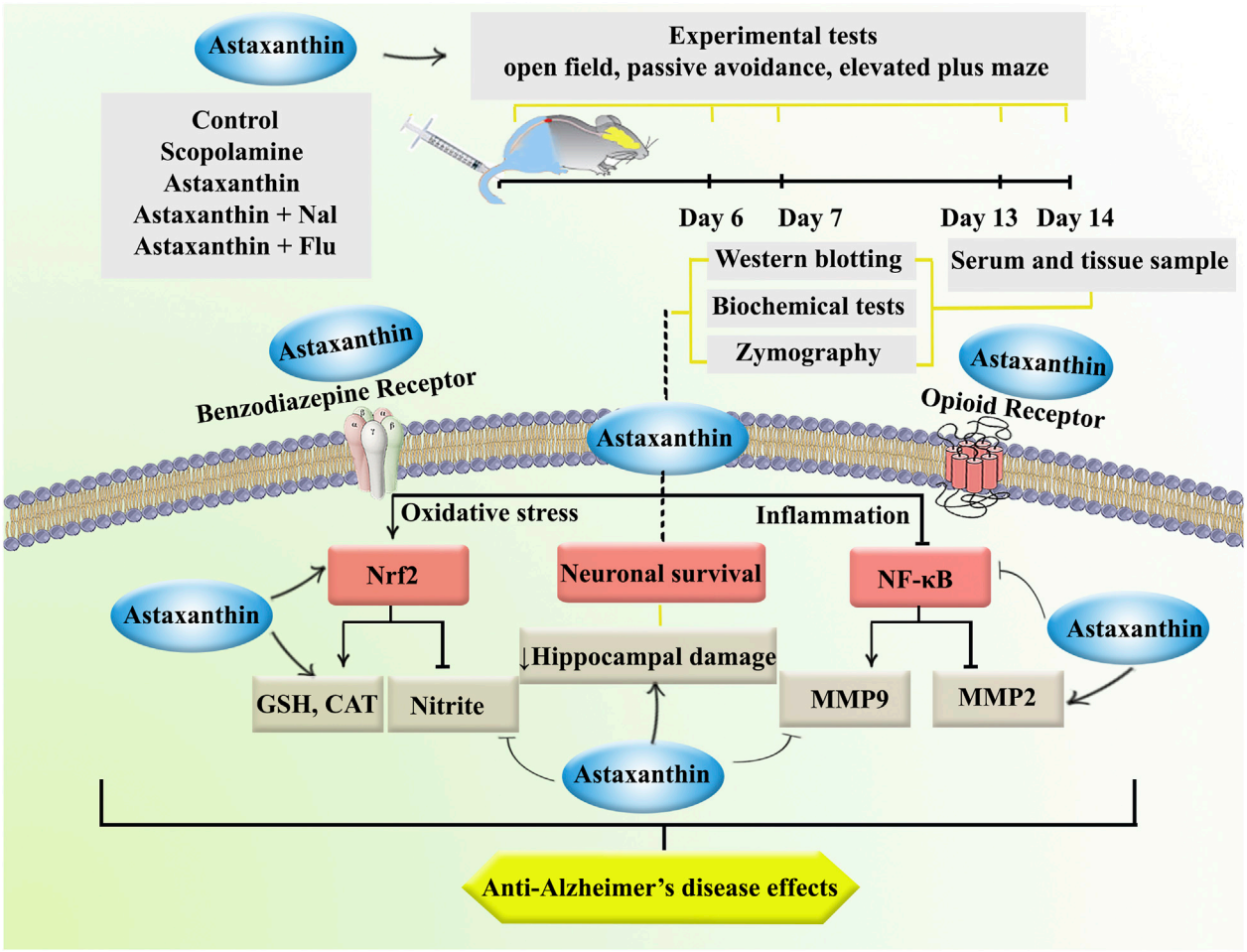


FIGURE 8
The study procedure and anti-AD effects of astaxanthin through opioid/benzodiazepine receptors by regulating Nrf2, NF-κB, and interconnected pathways.

astaxanthin solid lipid nanoparticles (5, and 10 mg/kg, i.p.) against acute kidney injury in rats (Yarmohammadi et al., 2025). The administration of astaxanthin leads to a notable enhancement in memory and cognitive performance. A rat model of AD using hydrated aluminum chloride indicated that astaxanthin treatment can lead to a dose-dependent (5, 10, and 15 mg/kg) reduction in Aβ1-42 levels in brain tissues, thereby potentially decreasing amyloid plaque formation (Hafez et al., 2021). Magadmi et al. reported that oral administration of astaxanthin was associated with a beneficial effect on short-term memory and reduced acetylcholinesterase activity in the brain (Magadmi et al., 2024). A study utilized a vascular dementia mouse model established through unilateral common carotid artery occlusion. Mice were administered astaxanthin for 30 days, and cognitive function was assessed using the Morris water maze and object recognition tests. Results indicated that astaxanthin treatment significantly improved cognitive performance, as evidenced by increased superoxide dismutase (SOD) activity and reduced malondialdehyde (MDA) and IL-1β levels, markers of oxidative stress and inflammatory response (Zhu et al., 2020). Also, a study involving healthy adults reported that astaxanthin supplementation resulted in significant improvements in composite and verbal memory after 12 weeks compared to a placebo group. Participants noted fewer memory-related issues, such as trouble recalling names (Sekikawa et al., 2020). Astaxanthin's unique chemical structure allows it to effectively cross the BBB, reaching CNS regions such as the hippocampus and cortex. This property is crucial for targeting neurodegenerative diseases like AD (Si and Zhu, 2022; Medoro et al., 2023). Studies using nano-emulsion formulations have quantified astaxanthin in different brain regions of animal models. For example, higher concentrations of astaxanthin were observed in the hippocampus after oral administration (160 mg/kg/day), suggesting its potential for enhancing memory-related functions (Chik et al., 2022).

Astaxanthin's bioavailability can be influenced by its formulation, as it is a lipophilic compound. Studies have shown that astaxanthin is better absorbed when consumed with fats. Micro-encapsulation and emulsification techniques can enhance its bioavailability. A clinical trial indicated that astaxanthin can be absorbed effectively in humans, with peak plasma concentrations occurring within 1–4 h after ingestion (Mercke Odeberg et al., 2003). The elimination half-life of astaxanthin varies but is generally around 16 h in humans. This suggests that astaxanthin may

require multiple dosing for sustained effects, particularly for chronic conditions like AD (Singh et al., 2020).

Our investigation revealed a significant reduction in the levels of catalase, glutathione, and MMP-2, as well as in the expression of the protein Nrf-2, in the scopolamine-treated group. Conversely, there was a notable increase in nitrite, MMP-9 levels, and the expression of the protein NF-κB.

This finding aligns with existing research indicating that scopolamine treatment can lead to oxidative stress and neuroinflammation. For instance, a study found that scopolamine downregulated antioxidant enzymes such as superoxide dismutase and catalase, while also decreasing Nrf2 expression, which is crucial for regulating antioxidant responses (Venkatesan et al., 2016). Scopolamine disrupts cholinergic signaling by antagonizing acetylcholine receptors, which can lead to reduced activation of the Nrf2 signaling pathway. Studies also suggested that scopolamine-induced cognitive impairments may be linked to its suppression of Nrf2 activity and related treatments activating Nrf2 signaling pathways (Venkatesan et al., 2016; Li et al., 2024). Scopolamine has also been linked to neuroinflammatory processes, which can further affect Nrf2 activity. Inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and IL-6 modulate Nrf2 signaling, and scopolamine-induced inflammation may exacerbate reductions in Nrf2 expression. Some other therapeutic candidates demonstrated neuroprotective effects by reducing inflammatory markers (e.g., NF-κB and TNF-α) while increasing Nrf2 activity in a scopolamine-induced AD model (Faldu and Shah, 2024). Also, several studies have shown that scopolamine administration markedly increases oxidative stress by reducing catalase and glutathione levels, while elevating nitric oxide levels, leading to brain damage (Chen et al., 2014; Chen and Yeong, 2020; Yadang et al., 2020). Moosavi et al. found that treatment with scopolamine led to a change in the levels of MMP-2 and MMP-9 in the hippocampus (Moosavi et al., 2018). Furthermore, an increase in NF-κB expression was observed in the scopolamine-treated group (El-Marasy et al., 2018; Joseph et al., 2020).

The evidence supports that enhancing antioxidant defenses through dietary means or supplementation could play a significant role in preventing AD and managing cognitive decline (Arslan et al., 2020; Sidiropoulou et al., 2023). In further studies, we found that treatment with astaxanthin (10 mg/kg) notably restored levels of catalase, glutathione, and MMP-2, while also enhancing the expression of the protein Nrf-2. Simultaneously, it reduced nitrite levels, MMP-9 levels, and the expression of the protein NF-κB, further confirming the strong antioxidant properties of astaxanthin. Recent evidence highlighted astaxanthin's protective role against diseases linked to oxidative stress and inflammation, focusing on its regulation of key redox-sensitive transcription factors, Nrf2 and NF-κB (Davinelli et al., 2022; Kanwugu and Glukhareva, 2023).

Opioid receptors are G-protein-coupled receptors that influence neurotransmitter release and neuronal excitability. Their signaling pathways have been implicated in the modulation of several neurodegenerative processes, including those associated with AD. Studies indicate that dysregulations in opioid receptor signaling can lead to increased expression of β-site amyloid precursor protein cleaving enzyme 1 (BACE1) and γ-secretase, both of which are crucial for the amyloidogenic pathway that produces toxic Aβ peptide (Tanguturi and Streicher, 2023). We have previously shown that Nal may diminish the potential effects of astaxanthin in pain relief (Fayazzadeh et al., 2024). A multicenter trial involving 54 subjects with clinically diagnosed AD tested the effects of intravenous Nal at doses of 1 mg, 10 mg, and even 30 mg. The results indicated no significant improvement in neuropsychological performance across these doses (Fayazzadeh et al., 2024). Another study administered Nal hydrochloride in varying doses (5 μg/kg, 0.1 mg/kg, and 2.0 mg/kg) to 12 patients with dementia of the AD type. The findings revealed no improvements in motor skills, attention, memory, or learning. Notably, some patients exhibited increased inappropriate verbal behavior and irritability after receiving the lower dose (Tariot, 1986).

GABA is crucial for regulating neuronal excitability and maintaining the balance between excitation and inhibition in the brain. In the context of AD, studies have shown that GABAergic dysfunction can lead to hyperactivity within neural networks, particularly in the hippocampus, a region critical for memory formation. This hyperactivity is often observed even before clinical symptoms manifest, suggesting that GABAergic dysregulation could serve as an early biomarker for cognitive decline associated with AD (Jiménez-Balado and Eich, 2021). Systematic reviews have consistently reported lower levels of GABA in both the brain and cerebrospinal fluid (CSF) of AD patients compared to healthy controls. For instance, a meta-analysis found standardized mean differences indicating significant reductions in GABA levels and related components such as GAD65/67 and GABA A receptors (Carello-Collar et al., 2023). Flu has been evaluated for its cognitive effects on patients with AD. Some studies suggest that while Flu might improve memory retrieval in certain contexts, it can also lead to cognitive slowing or adverse effects, indicating a complex relationship between Flu administration and cognitive performance in AD patients (Ott et al., 1996; Raut et al., 2016). Studies investigating the combination of Flu and astaxanthin suggested that pre-treatment with Flu partially blocks the anxiety-reducing effects of astaxanthin (Jayasheela et al., 2021). To investigate the underlying mechanisms contributing to the neuroprotective effects of astaxanthin, we pretreated the rats with Flu and Nal, which inhibit GABAergic and opioid pathways, respectively, before administering an effective dose of astaxanthin (10 mg/kg). The findings revealed that the neuroprotective effects of astaxanthin were significantly reduced by both Nal and Flu.

Previous research has shown that the administration of scopolamine can reduce the number of neurons in the hippocampus and disrupt normal neurogenesis, which is critical for cognition, memory, and learning (Yan et al., 2014; Lee et al., 2017). Our histological findings showed a visual reduction in hippocampal neurons, and treatment with astaxanthin partially preserved them. In experimental models, astaxanthin treatment has been linked to improved cognitive performance and a reduction in neuronal loss in mouse models of AD, attributed to the mitigation of oxidative stress and inflammation in the hippocampus (Magadmi et al., 2024).

5 Conclusion

The present study highlights the neuroprotective effects of astaxanthin in a scopolamine-induced rat model of AD. The results indicate that astaxanthin treatment significantly ameliorates cognitive deficits. The observed enhancements in cognitive function were accompanied by notable biochemical changes, including increased levels of antioxidant markers such as catalase and glutathione, and a reduction in nitrite levels, indicating a reduction in oxidative stress. Furthermore, astaxanthin appears to exert neuroprotective effects by modulating the expression of MMP-2, MMP-9, and the transcription factors Nrf2 and NF-κB, and preservation of neurons. Moreover, the interaction of astaxanthin with key neurotransmitter systems, specifically through the modulation of GABA-A and opioid receptors, emphasizes its multi-targeted approach in addressing the complex pathophysiology of AD. The partial reversal of astaxanthin's effects by the antagonists Flu and Nal suggests that these pathways may significantly contribute to the neuroprotective mechanisms of astaxanthin.

While the data are promising, further research is needed to fully elucidate the precise mechanisms by which astaxanthin exerts its effects in the context of AD. Future studies should aim to investigate the long-term effects of astaxanthin administration and other mechanisms, as well as sex differences involved in the pathogenesis of AD. Additionally, providing novel delivery systems is appreciated to drawback possible pharmacokinetic limitations (Fakhri et al., 2022a).

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was approved by Dr. Mahmoodreza Moradi, Committee Director, Kermanshah University of Medical Sciences Dr. Reza Khodarahmi, Committee Secretary, Kermanshah University of Medical Sciences. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

ZR: Investigation, Writing – original draft. SF: Conceptualization, Formal Analysis, Methodology, Software, Supervision, Writing – original draft, Writing – review and editing. FA: Formal Analysis, Software, Writing – original draft. MR: Writing – original draft. AK: Investigation, Writing – original draft. MA: Writing – original draft. JE: Writing – original draft, Writing – review and editing.

Funding

The author(s) declare that financial support was received for the research and/or publication of this article. The authors appreciate Kermanshah University of Medical Sciences for the special Grant No. 4000755.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

Generative AI statement

The author(s) declare that no Generative AI was used in the creation of this manuscript.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

Aebi, H. (1984). Catalase *in vitro*. *Methods Enzymol.* 105, 121–126. doi:10.1016/S0076-6879(84)05016-3

Arslan, J., Jamshed, H., and Qureshi, H. (2020). Early detection and prevention of alzheimer's disease: role of oxidative markers and natural antioxidants. *Front. Aging Neurosci.* 12, 231. doi:10.3389/fnagi.2020.00231

Bai, R., Guo, J., Ye, X.-Y., Xie, Y., and Xie, T. (2022). Oxidative stress: the core pathogenesis and mechanism of Alzheimer's disease. *Ageing Res. Rev.* 77, 101619. doi:10.1016/j.arr.2022.101619

Behl, T., Kaur, G., Sehgal, A., Bhardwaj, S., Singh, S., Buhas, C., et al. (2021). Multifaceted role of matrix metalloproteinases in neurodegenerative diseases: pathophysiological and therapeutic perspectives. *Int. J. Mol. Sci.* 22, 1413. doi:10.3390/ijms22031413

Berkels, R., Purol-Schnabel, S., and Roesen, R. (2004). Measurement of nitric oxide by reconversion of nitrate/nitrite to NO. *Methods Mol. Biol.* 279, 1–8. doi:10.1385/1-59259-807-2:001

Carello-Collar, G., Bellaver, B., Ferreira, P. C. L., Ferrari-Souza, J. P., Ramos, V. G., Therriault, J., et al. (2023). The GABAergic system in Alzheimer's disease: a systematic review with meta-analysis. *Mol. Psychiatry* 28, 5025–5036. doi:10.1038/s41380-023-02140-w

Chen, C., Li, X.-H., Zhang, S., Tu, Y., Wang, Y.-M., and Sun, H.-T. (2014). 7,8-dihydroxyflavone ameliorates scopolamine-induced Alzheimer-like pathologic dysfunction. *Rejuvenation Res.* 17, 249–254. doi:10.1089/rej.2013.1519

Chen, W. N., and Yeong, K. Y. (2020). Scopolamine, a toxin-induced experimental model, used for research in Alzheimer's disease. *CNS Neurol. Disord. Drug Targets* 19, 85–93. doi:10.2174/1871527319666200214104331

Chik, M. W., Mohd Affandi, M. M. R. M., and Singh, G. K. S. (2022). Detection of astaxanthin at different regions of the brain in rats treated with astaxanthin nanoemulsion. *J. Pharm. Bioallied Sci.* 14, 25–30. doi:10.4103/jpbs.jpbs_464_21

Chu, C.-T., Uruno, A., Katsuoka, F., and Yamamoto, M. (2024). Role of NRF2 in pathogenesis of Alzheimer's disease. *Antioxidants* 13, 1529. doi:10.3390/antiox13121529

Daglia, M., Di Lorenzo, A., Nabavi, S. F., Talas, Z. S., and Nabavi, S. M. (2014). Polyphenols: well beyond the antioxidant capacity: gallic acid and related compounds as neuroprotective agents: you are what you eat. *Curr. Pharm. Biotechnol.* 15, 362–372. doi:10.2174/1389201015041408251207 37

Danduga, R. C. S. R., and Kola, P. K. (2024). Elevated plus maze for assessment of anxiety and memory in rodents. *Methods Mol. Biol.* 2761, 93–96. doi:10.1007/978-1-0716-3662-6_8

Davies, D. A., Adlimoghaddam, A., and Albensi, B. C. (2021). Role of Nrf2 in synaptic plasticity and memory in alzheimer's disease. *Cells* 10, 1884. doi:10.3390/cells10081884

Davinelli, S., Saso, L., D'Angeli, F., Calabrese, V., Intrieri, M., and Scapagnini, G. (2022). Astaxanthin as a modulator of Nrf2, NF-κB, and their crosstalk: molecular mechanisms and possible clinical applications. *Molecules* 27, 502. doi:10.3390/molecules27020502

de Castro, A. A., Soares, F. V., Pereira, A. F., Polisel, D. A., Caetano, M. S., Leal, D. H. S., et al. (2019). Non-conventional compounds with potential therapeutic effects against Alzheimer's disease. *Expert Rev. Neurother.* 19, 375–395. doi:10.1080/14737175.2019.1608823

Duits, F. H., Hernandez-Guillamon, M., Montaner, J., Goos, J. D. C., Montañola, A., Wattjes, M. P., et al. (2015). Matrix metalloproteinases in Alzheimer's disease and concurrent cerebral microbleeds. *J. Alzheimers. Dis.* 48, 711–720. doi:10.3233/JAD-143186

Eichenbaum, H. (2004). Hippocampus: cognitive processes and neural representations that underlie declarative memory. *Neuron* 44, 109–120. doi:10.1016/j.neuron.2004.08.028

Ellman, G. L. (1959). Tissue sulfhydryl groups. *Arch. Biochem. Biophys.* 82, 70–77. doi:10.1016/0003-9861(59)90090-6

El-Marasy, S. A., Abd-Elsalam, R. M., and Ahmed-Farid, O. A. (2018). Ameliorative effect of silymarin on scopolamine-induced dementia in rats. *Open Access Maced. J. Med. Sci.* 6, 1215–1224. doi:10.3889/oamjms.2018.257

El-Missiry, M. A., Othman, A. I., El-Sawy, M. R., and Lebede, M. F. (2018). Neuroprotective effect of epigallocatechin-3-gallate (EGCG) on radiation-induced damage and apoptosis in the rat hippocampus. *Int. J. Radiat. Biol.* 94, 798–808. doi:10.1080/09553002.2018.1492755

El Sayed, N. S., and Ghoneum, M. H. (2020). Antia, a natural antioxidant product, attenuates cognitive dysfunction in streptozotocin-induced mouse model of sporadic alzheimer's disease by targeting the amyloidogenic, inflammatory, autophagy, and oxidative stress pathways. *Oxid. Med. Cell. Longev.* 2020, 4386562. doi:10.1155/2020/4386562

Fakhri, S., Abbaszadeh, F., Dargahi, L., and Jorjani, M. (2018). Astaxanthin: a mechanistic review on its biological activities and health benefits. *Pharmacol. Res.* 136, 1–20. doi:10.1016/j.phrs.2018.08.012

Fakhri, S., Abdian, S., Zarneshan, S. N., Moradi, S. Z., Farzaei, M. H., and Abdollahi, M. (2022a). Nanoparticles in combating neuronal dysregulated signaling pathways: recent approaches to the nanoformulations of phytochemicals and synthetic drugs against neurodegenerative diseases. *Int. J. Nanomedicine* 17, 299–331. doi:10.2147/IJN.S347187

Fakhri, S., Aneva, I. Y., Farzaei, M. H., and Sobarzo-Sánchez, E. (2019a). The neuroprotective effects of astaxanthin: therapeutic targets and clinical perspective. *Molecules* 24, 2640. doi:10.3390/molecules24142640

Fakhri, S., Dargahi, L., Abbaszadeh, F., and Jorjani, M. (2019b). Effects of astaxanthin on sensory-motor function in a compression model of spinal cord injury: involvement of ERK and AKT signalling pathway. *Eur. J. Pain* 23, 750–764. doi:10.1002/ejp.1342

Fakhri, S., Pesce, M., Patruno, A., Moradi, S. Z., Iranpanah, A., Farzaei, M. H., et al. (2020). Attenuation of nrf2/keap1/ARE in alzheimer's disease by plant secondary metabolites: a mechanistic review. *Molecules* 25, 4926. doi:10.3390/molecules25214926

Fakhri, S., Piri, S., Farzaei, M. H., and Sobarzo-Sánchez, E. (2021a). "Recent updates on the neuroprotective role of carotenoids: astaxanthin and beyond," in *Global perspectives on astaxanthin* (Elsevier), 719–740. doi:10.1016/B978-0-12-823304-7.00035-0

Fakhri, S., Sabouri, S., Kiani, A., Farzaei, M. H., Rashidi, K., Mohammadi-Farani, A., et al. (2022b). Intrathecal administration of naringenin improves motor dysfunction and neuropathic pain following compression spinal cord injury in rats: relevance to its antioxidant and anti-inflammatory activities. *Korean J. Pain* 35, 291–302. doi:10.3344/kjp.2022.35.3.291

Fakhri, S., Yarmohammadi, A., Yarmohammadi, M., Farzaei, M. H., and Echeverria, J. (2021b). Marine natural products: promising candidates in the modulation of gut-brain Axis towards neuroprotection. *Mar. Drugs* 19, 165. doi:10.3390/md19030165

Faldu, K. G., and Shah, J. S. (2024). Ambroxol improves amyloidogenic, NF-κB, and Nrf2 pathways in a scopolamine-induced cognitive impairment rat model of alzheimer's disease. *Drug Dev. Res.* 85, e70017. doi:10.1002/ddr.70017

Fayazzadeh, S., Fakhri, S., Abbaszadeh, F., and Farzaei, M. H. (2024). Role of l-arginine/nitric oxide/cyclic GMP/K ATP channel signaling pathway and opioid receptors in the antinociceptive effect of rutin in mice. *Behav. Pharmacol.* 35, 399–407. doi:10.1097/FBP.0000000000000792

Galasso, C., Orefice, I., Pellone, P., Cirino, P., Miele, R., Ianora, A., et al. (2018). On the neuroprotective role of astaxanthin: new perspectives? *Mar. Drugs* 16, 247. doi:10.3390/md16080247

Ghafarimoghadam, M., Mashayekh, R., Gholami, M., Fereydani, P., Shelley-Tremblay, J., Kandezi, N., et al. (2022). A review of behavioral methods for the evaluation of cognitive performance in animal models: current techniques and links to human cognition. *Physiol. Behav.* 244, 113652. doi:10.1016/j.physbeh.2021.113652

Gogebakan, A., Talas, Z. S., Ozdemir, I., and Sahna, E. (2012). Role of propolis on tyrosine hydroxylase activity and blood pressure in nitric oxide synthase-inhibited hypertensive rats. *Clin. Exp. Hypertens.* 34, 424–428. doi:10.3109/10641963.2012.665542

Gould, T., Dao, D., and Kovacsics, C. (2009). The open field test mood and anxiety related phenotypes in mice. *Neuromethods* 42, 1–20.

Hafez, H. A., Kamel, M. A., Osman, M. Y., Osman, H. M., Elblehi, S. S., and Mahmoud, S. A. (2021). Ameliorative effects of astaxanthin on brain tissues of alzheimer's disease-like model: cross talk between neuronal-specific microRNA-124 and related pathways. *Mol. Cell. Biochem.* 476, 2233–2249. doi:10.1007/s11010-021-04079-4

Hamedi, A., Zengin, G., Aktumsek, A., Selamoglu, Z., and Pasdaran, A. (2020). *In vitro* and *in silico* approach to determine neuroprotective properties of iridoid glycosides from aerial parts of Scrophularia amplexicaulis by investigating their cholinesterase inhibition and anti-oxidant activities. *Biointerface Res. Appl. Chem.* 10, 5429–5454.

Hashemi, B., Fakhri, S., Kiani, A., Abbaszadeh, F., Miraghaee, S., Mohammadi, M., et al. (2024). Anti-neuropathic effects of astaxanthin in a rat model of chronic constriction injury: passing through opioid/benzodiazepine receptors and relevance to its antioxidant and anti-inflammatory effects. *Front. Pharmacol.* 15, 1467788. doi:10.3389/fphar.2024.1467788

Hsieh, H.-L., Wang, H.-H., Wu, W.-B., Chu, P.-J., and Yang, C.-M. (2010). Transforming growth factor-β1 induces matrix metalloproteinase-9 and cell migration in astrocytes: roles of ROS-dependent ERK- and JNK-NF-κB pathways. *J. Neuroinflammation* 7, 88. doi:10.1186/1742-2094-7-88

Huang, W.-J., Zhang, X., and Chen, W.-W. (2016). Role of oxidative stress in Alzheimer's disease. *Biomed. Rep.* 4, 519–522. doi:10.3892/br.2016.630

Ishak, S., Allouche, M., Alotaibi, G. S., Alwthery, N. S., Al-Subaie, R. A., Al-Hoshani, N., et al. (2024). Experimental and computational assessment of antiparkinson medication effects on meiofauna: case study of benserazide and trihexyphenidyl. *Mar. Pollut. Bull.* 205, 116668. doi:10.1016/j.marpolbul.2024.116668

Jayasheela, J., Kumar, A., Topno, I., and Santhanalakshm, S. (2021). Evaluation of antianxiety-like activity of astaxanthin and its possible mechanism of action using animal models. *Res. J. Pharm. Biol. Chem. Sci. Evalu* 12, 37–47. doi:10.33887/rjpbcs/2021.12.1.5

Jiménez-Balado, J., and Eich, T. S. (2021). GABAergic dysfunction, neural network hyperactivity and memory impairments in human aging and Alzheimer's disease. *Semin. Cell Dev. Biol.* 116, 146–159. doi:10.1016/j.semcdb.2021.01.005

Joseph, E., Villalobos-Acosta, D. M. Á., Torres-Ramos, M. A., Farfán-García, E. D., Gómez-López, M., Miliar-García, Á., et al. (2020). Neuroprotective effects of apocynin and galantamine during the chronic administration of scopolamine in an Alzheimer's disease model. *J. Mol. Neurosci.* 70, 180–193. doi:10.1007/s12031-019-01426-5

Jurcău, M. C., Andronie-Cioara, F. L., Jurcău, A., Marcu, F., Țiț, D. M., Pașcalău, N., et al. (2022). The link between oxidative stress, mitochondrial dysfunction and neuroinflammation in the pathophysiology of Alzheimer's disease: therapeutic implications and future perspectives. *Antioxidants (Basel, Switzerland)* 11, 2167. doi:10.3390/antiox11112167

Kanwugu, O. N., and Glukhareva, T. V. (2023). Activation of Nrf2 pathway as a protective mechanism against oxidative stress-induced diseases: potential of astaxanthin. *Arch. Biochem. Biophys.* 741, 109601. doi:10.1016/j.abb.2023.109601

Kavyani, Z., Najafi, K., Naghsh, N., Karvane, H. B., and Musazadeh, V. (2024). The effects of curcumin supplementation on biomarkers of inflammation, oxidative stress, and endothelial function: a meta-analysis of meta-analyses. *Prostagl. Other Lipid Mediat* 174, 106867. doi:10.1016/j.prostaglandins.2024.106867

Khan, H., Tundis, R., Ullah, H., Aschner, M., Belwal, T., Mirzaei, H., et al. (2020). Flavonoids targeting NRF2 in neurodegenerative disorders. *Food Chem. Toxicol.* 146, 111817. doi:10.1016/j.fct.2020.111817

Klenowski, P., Morgan, M., and Bartlett, S. E. (2015). The role of δ-opioid receptors in learning and memory underlying the development of addiction. *Br. J. Pharmacol.* 172, 297–310. doi:10.1111/bph.12618

Kobayashi, M., Li, L., Iwamoto, N., Nakajima-Takagi, Y., Kaneko, H., Nakayama, Y., et al. (2009). The antioxidant defense system Keap1-Nrf2 comprises a multiple sensing mechanism for responding to a wide range of chemical compounds. *Mol. Cell. Biol.* 29, 493–502. doi:10.1128/MCB.01080-08

Lee, J., Park, J., Ahn, J., Park, J., Kim, I., Cho, J., et al. (2017). Effects of chronic scopolamine treatment on cognitive impairment and neurofilament expression in the mouse hippocampus. *Mol. Med. Rep.* 17, 1625–1632. doi:10.3892/mmr.2017.8082

Li, X., Zheng, K., Chen, H., and Li, W. (2024). Ginsenos*ide re* regulates oxidative stress through the PI3K/Akt/Nrf2 signaling pathway in mice with scopolamine-induced memory impairments. *Curr. Issues Mol. Biol.* 46, 11359–11374. doi:10.3390/cimb46100677

Lim, N. K.-H., Villemagne, V. L., Soon, C. P. W., Laughton, K. M., Rowe, C. C., McLean, C. A., et al. (2011). Investigation of matrix metalloproteinases, MMP-2 and MMP-9, in plasma reveals a decrease of MMP-2 in Alzheimer's disease. *J. Alzheimer's Dis.* 26, 779–786. doi:10.3233/JAD-2011-101974

Lingappan, K. (2018). NF-κB in oxidative stress. *Curr. Opin. Toxicol.* 7, 81–86. doi:10.1016/j.cotox.2017.11.002

Magadmi, R., Nassibi, S., Kamel, F., Al-Rafiah, A. R., Bakhshwin, D., Jamal, M., et al. (2024). The protective effect of Astaxanthin on scopolamine - induced Alzheimer's model in mice. *Neurosciences* 29, 103–112. doi:10.17712/nsj.2024.2.20230060

McMahon, M., Lamont, D. J., Beattie, K. A., and Hayes, J. D. (2010). Keap1 perceives stress via three sensors for the endogenous signaling molecules nitric oxide, zinc, and alkenals. *Proc. Natl. Acad. Sci. U. S. A.* 107, 18838–18843. doi:10.1073/pnas.1007387107

Medoro, A., Davinelli, S., Milella, L., Willcox, B. J., Allsopp, R. C., Scapagnini, G., et al. (2023). Dietary astaxanthin: a promising antioxidant and anti-inflammatory agent for brain aging and adult neurogenesis. *Mar. Drugs* 21, 643. doi:10.3390/md21120643

Mercke Odeberg, J., Lignell, A., Pettersson, A., and Höglund, P. (2003). Oral bioavailability of the antioxidant astaxanthin in humans is enhanced by incorporation of lipid based formulations. *Eur. J. Pharm. Sci.* 19, 299–304. doi:10.1016/s0928-0987(03)00135-0

Mohammadi, S., Fakhri, S., Mohammadi-Farani, A., Farzaei, M. H., and Abbaszadeh, F. (2021). Astaxanthin engages the l-arginine/NO/cGMP/KATP channel signaling pathway toward antinociceptive effects. *Behav. Pharmacol.* 32, 607–614. doi:10.1097/FBP.0000000000000655

Moneim, A. E. A. (2015). Oxidant/antioxidant imbalance and the risk of Alzheimer's disease. *Curr. Alzheimer Res.* 12, 335–349. doi:10.2174/1567205012666150325182702

Moosavi, M., SoukhakLari, R., Moezi, L., and Pirsalami, F. (2018). Scopolamine-induced passive avoidance memory retrieval deficit is accompanied with hippocampal MMP2, MMP-9 and MAPKs alteration. *Eur. J. Pharmacol.* 819, 248–253. doi:10.1016/j.ejphar.2017.12.007

Ngo, V., and Duennwald, M. L. (2022). Nrf2 and oxidative stress: a general overview of mechanisms and implications in human disease. *Antioxidants (Basel, Switzerland)* 11, 2345. doi:10.3390/antiox11122345

Ott, B. R., Thompson, J. A., and Whelihan, W. M. (1996). Cognitive effects of flumazenil in patients with Alzheimer's disease. *J. Clin. Psychopharmacol.* 16, 400–402. doi:10.1097/00004714-199610000-00010

Pellissier, L. P., Pujol, C. N., Becker, J. A. J., and Le Merrer, J. (2018). Delta opioid receptors: learning and motivation. *Handb. Exp. Pharmacol.* 247, 227–260. doi:10.1007/164_2016_89

Pritam, P., Deka, R., Bhardwaj, A., Srivastava, R., Kumar, D., Jha, A. K., et al. (2022). Antioxidants in Alzheimer's disease: current therapeutic significance and future prospects. *Biol. (Basel).* 11, 212. doi:10.3390/biology11020212

Prut, L., and Belzung, C. (2003). The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: a review. *Eur. J. Pharmacol.* 463, 3–33. doi:10.1016/s0014-2999(03)01272-x

Rahman, S. O., Panda, B. P., Parvez, S., Kaundal, M., Hussain, S., Akhtar, M., et al. (2019). Neuroprotective role of astaxanthin in hippocampal insulin resistance induced by Aβ peptides in animal model of Alzheimer's disease. *Biomed. Pharmacother.* 110, 47–58. doi:10.1016/j.biopha.2018.11.043

Raut, S. B., Bolegave, S. S., and Marathe, P. A. (2016). Effect of flumazenil on memory retrieval determined by trial-to-criteria inhibitory avoidance method in mice. *J. Pharmacol. Pharmacother.* 7, 24–26. doi:10.4103/0976-500X.179354

Sakimoto, Y., Oo, P. M.-T., Goshima, M., Kanehisa, I., Tsukada, Y., and Mitsushima, D. (2021). Significance of GABAA receptor for cognitive function and hippocampal pathology. *Int. J. Mol. Sci.* 22, 12456. doi:10.3390/ijms222212456

Scheltens, P., De Strooper, B., Kivipelto, M., Holstege, H., Chételat, G., Teunissen, C. E., et al. (2021). Alzheimer's disease. *Lancet (London, England)* 397, 1577–1590. doi:10.1016/S0140-6736(20)32205-4

Sekikawa, T., Kizawa, Y., Li, Y., and Takara, T. (2020). Cognitive function improvement with astaxanthin and tocotrienol intake: a randomized, double-blind, placebo-controlled study. *J. Clin. Biochem. Nutr.* 67, 307–316. doi:10.3164/jcbn.19-116

Selamoglu-Talas, Z., Ozdemir, I., Ciftci, O., and Cakir, O. (2015). Propolis attenuates oxidative injury in brain and lung of nitric oxide synthase inhibited rats. *J. Pharm. Care* 1 (2), 45–50. Available online at: https://jpc.tums.ac.ir/index.php/jpc/article/view/11. Spring 2013.

Sevindik, M., Akgul, H., Selamoglu, Z., and Braidy, N. (2020). Antioxidant, antimicrobial and neuroprotective effects of Octaviania asterosperma *in vitro*. *Mycology* 12, 128–138. doi:10.1080/21501203.2020.1816584

Si, P., and Zhu, C. (2022). Biological and neurological activities of astaxanthin (Review). *Mol. Med. Rep.* 26, 300. doi:10.3892/mmr.2022.12816

Sidiropoulou, G. A., Metaxas, A., and Kourti, M. (2023). Natural antioxidants that act against Alzheimer's disease through modulation of the NRF2 pathway: a focus on their molecular mechanisms of action. *Front. Endocrinol. (Lausanne).* 14, 1217730. doi:10.3389/fendo.2023.1217730

Singh, K. N., Patil, S., and Barkate, H. (2020). Protective effects of astaxanthin on skin: recent scientific evidence, possible mechanisms, and potential indications. *J. Cosmet. Dermatol.* 19, 22–27. doi:10.1111/jocd.13019

Smirnov, D., and Galasko, D. (2022). Dynamics of neuroinflammation in Alzheimer's disease. *Lancet. Neurol.* 21, 297–298. doi:10.1016/S1474-4422(22)00087-4

Sun, E., Motolani, A., Campos, L., and Lu, T. (2022). The pivotal role of NF-kB in the pathogenesis and therapeutics of Alzheimer's disease. *Int. J. Mol. Sci.* 23, 8972. doi:10.3390/ijms23168972

Taksima, T., Chonpathompikunlert, P., Sroyraya, M., Hutamekalin, P., Limpawattana, M., and Klaypradit, W. (2019). Effects of astaxanthin from shrimp shell on oxidative stress and behavior in animal model of Alzheimer's disease. *Mar. Drugs* 17, 628. doi:10.3390/md17110628

Tanguturi, P., and Streicher, J. M. (2023). The role of opioid receptors in modulating Alzheimer's Disease. *Front. Pharmacol.* 14, 1056402. doi:10.3389/fphar.2023.1056402

Tariot, P. N., Sunderland, T., Weingartner, H., Murphy, D. L., Cohen, M. R., and Cohen, R. M. (1986). Naloxone and Alzheimer's disease. Cognitive and behavioral effects of a range of doses. *Arch. Gen. Psychiatry* 43, 727–732. doi:10.1001/archpsyc.1986.01800080013002

Teixeira, J. P., de Castro, A. A., Soares, F. V., da Cunha, E. F. F., and Ramalho, T. C. (2019). Future therapeutic perspectives into the Alzheimer's disease targeting the oxidative stress hypothesis. *Molecules* 24, 4410. doi:10.3390/molecules24234410

Teng, L., Zhao, J., Wang, F., Ma, L., and Pei, G. (2010). A GPCR/secretase complex regulates beta- and gamma-secretase specificity for Abeta production and contributes to AD pathogenesis. *Cell Res.* 20, 138–153. doi:10.1038/cr.2010.3

Thakur, S., Dhapola, R., Sarma, P., Medhi, B., and Reddy, D. H. (2023). Neuroinflammation in Alzheimer's disease: current progress in molecular signaling and therapeutics. *Inflammation* 46, 1–17. doi:10.1007/s10753-022-01721-1

Vasile, A., Ciobîcă, A., Kamal, F. Z., Mavroudis, I., Plăvan, G., Selamoglu, Z., et al. (2024). Latest developments on the connections between diet and most of the neuropsychiatric disorders. *Bull. Integr. Psychiatry* 101, 105–119. doi:10.36219/BPI.2024.2.11

Venkatesan, R., Subedi, L., Yeo, E.-J., and Kim, S. Y. (2016). Lactucopicrin ameliorates oxidative stress mediated by scopolamine-induced neurotoxicity through activation of the NRF2 pathway. *Neurochem. Int.* 99, 133–146. doi:10.1016/j.neuint.2016.06.010

Wang, X.-X., Tan, M.-S., Yu, J.-T., and Tan, L. (2014). Matrix metalloproteinases and their multiple roles in Alzheimer's disease. *Biomed. Res. Int.* 2014, 908636–908638. doi:10.1155/2014/908636

Yadang, F. S. A., Nguezeye, Y., Kom, C. W., Betote, P. H. D., Mamat, A., Tchokouaha, L. R. Y., et al. (2020). Scopolamine-induced memory impairment in mice: neuroprotective effects of *Carissa edulis* (forssk.) valh (apocynaceae) aqueous extract. *Int. J. Alzheimers. Dis.* 2020, 6372059. doi:10.1155/2020/6372059

Yan, B., Park, J., Chen, B., Cho, J.-H., Kim, I., Ahn, J., et al. (2014). Long-term administration of scopolamine interferes with nerve cell proliferation, differentiation and migration in adult mouse hippocampal dentate gyrus, but it does not induce cell death. *Neural Regen. Res.* 9, 1731–1739. doi:10.4103/1673-5374.143415

Yarmohammadi, A., Arkan, E., Najafi, H., Abbaszadeh, F., Rashidi, K., Piri, S., et al. (2025). Protective effects of astaxanthin solid lipid nanoparticle as a promising candidate against acute kidney injury in rats. *Naunyn. Schmiedeb. Arch. Pharmacol.* 398, 4491–4502. doi:10.1007/s00210-024-03543-4

Zarneshan, S. N., Arkan, E., Kiani, A., Hosseini, S. Z., Abbaszadeh, F., and Fakhri, S. (2025). Protective effects of polydatin amphiphilic chitosan nanocarriers against an aluminum chloride-induced model of Alzheimer's disease in rats: relevance to its anti-inflammatory and antioxidant effects. *Naunyn. Schmiedeb. Arch. Pharmacol.* doi:10.1007/s00210-024-03696-2

Zhao, Y., and Zhao, B. (2013). Oxidative stress and the pathogenesis of Alzheimer's disease. *Oxid. Med. Cell. Longev.* 2013, 316523. doi:10.1155/2013/316523

Zhu, N., Liang, X., Zhang, M., Yin, X., Yang, H., Zhi, Y., et al. (2020). Astaxanthin protects cognitive function of vascular dementia. *Behav. Brain Funct.* 16, 10. doi:10.1186/s12993-020-00172-8

frontiers | Frontiers in Pharmacology

TYPE Review
PUBLISHED 29 May 2025
DOI 10.3389/fphar.2025.1586345

Check for updates

OPEN ACCESS

EDITED BY
Huazheng Liang,
Monash University - Southeast University Joint Research Institute (Suzhou), China

REVIEWED BY
Ayesha Wadood,
Nuclear Institute for Agriculture and Biology, Pakistan
Alexandra Daniela Rotaru-Zavaleanu,
University of Medicine and Pharmacy of Craiova, Romania
Albeiro Marrugo Padilla,
University of Cartagena, Colombia

*CORRESPONDENCE
Jianwen Guo,
jianwen_guo@qq.com

†These authors have contributed equally to this work

RECEIVED 02 March 2025
ACCEPTED 15 May 2025
PUBLISHED 29 May 2025

CITATION
Zheng Y, Tan X, Wang X, Mao R and Guo J (2025) Targeting ferroptosis with natural products in stroke: therapeutic mechanisms and translational opportunities.
Front. Pharmacol. 16:1586345.
doi: 10.3389/fphar.2025.1586345

COPYRIGHT
© 2025 Zheng, Tan, Wang, Mao and Guo. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

Targeting ferroptosis with natural products in stroke: therapeutic mechanisms and translational opportunities

Yingyi Zheng[1†], Xi Tan[1,2†], Xiaojie Wang[2], Rui Mao[1] and Jianwen Guo[2*]

[1]The Second Clinical College of Guangzhou University of Chinese Medicine, Guangzhou, China, [2]The Second Affiliated Hospital of Guangzhou University of Chinese Medicine, Guangzhou, China

Ferroptosis is a new type of controlled cell death. It is distinguished by its reliance on iron and the production of lipid peroxidation. The role of ferroptosis in stroke has attracted a lot of attention recently. The purpose of this review is to clarify the connection between ferroptosis and stroke and to investigate the potential contribution of natural products to the clinical management of stroke and the discovery of novel medications. In this review, we summarize in detail the mechanism of ferroptosis after stroke, especially the relevant targets of ferroptosis after stroke. Furthermore, we summarize the natural products and herbal medicine currently employed in ferroptosis along with their mechanisms of action, highlighting the potential and challenges of clinical translation. We included 55 articles and classified them. After systematic screening, We think that ginkgolide B, kellerin, loureirin C, quercetin, icariside II, salvianolic acid A, berberine, Dl-3-n-butylphthalide is an effective candidate drug for the treatment of stroke.

KEYWORDS

natural products, stroke, ferroptosis, Phytomedicine, intracerebral hemorrhage, ischemic stroke

1 Introduction

Stroke, which contributes to a growing portion of the global medical burden, can be broadly classified into ischemic stroke and hemorrhagic stroke, with the latter incorporating intracerebral hemorrhage (ICH) and subarachnoid hemorrhage (SAH) (GBD, 2021 Diseases and Injuries Collaborators, 2024). Ischemic stroke is characterized by brain, spinal cord, or retinal infarction, representing approximately 85% of all strokes globally (Tsao et al., 2023). In accordance with the secondary stroke prevention guidelines established by the American Heart Association/American College of Cardiology, antiplatelet medications, including aspirin, clopidogrel, and dipyridamole, have become the primary therapeutic alternative (Medina-Inojosa et al., 2023). Nevertheless, a substantial problem has emerged: certain patients exhibit resistance to aspirin, which could potentially undermine the efficacy of these medications (Venketasubramanian et al., 2022). Given this predicament, it is imperative to create novel therapeutic agents that are effective for individuals who are resistant to conventional remedies.

Ferroptosis, a type of programmed cell death associated with stroke, was initially identified in 2012 and displays distinctive features, such as changes in the structure and

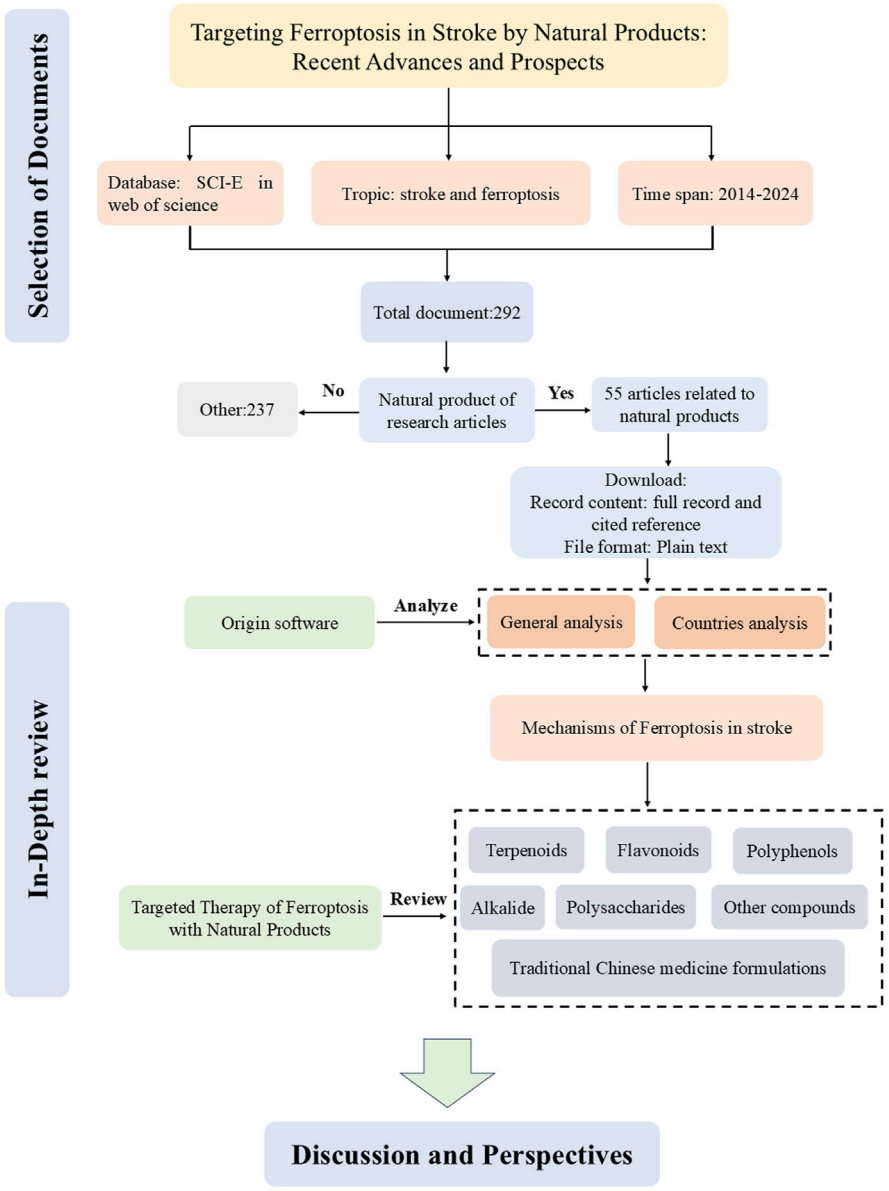


FIGURE 1
The flow chart of review.

density of mitochondria (Dixon et al., 2012; Xu et al., 2023a). Thus, a complete understanding of the function of ferroptosis in stroke could serve to offer new intervention targets. Natural products are the metabolites or plant metabolites of insects, microbes, marine, plant, and animal extracts, as well as many more endogenous chemical plant metabolites and metabolites found in human and animal bodies (Xing et al., 2023). They are proven to be a valuable source of new drugs (Liang et al., 2021). Many natural products have shown good therapeutic effects on stroke (Liu et al., 2023a). In this review, we summarize the mechanisms of ferroptosis in stroke and in detail the natural products of anti-ferroptosis therapy. Figure 1 shows a flowchart summarizing natural products that target ferroptosis after strokes.

2 Research trends on ferroptosis in stroke

Ferroptosis is a novel form of regulatory cell death associated with the formation of iron-dependent reactive oxygen species (ROS) and lipid peroxides (Dixon et al., 2012). Disordered iron metabolism in cells leads to the generation of excessive iron ions, which produce a significant quantity of ROS via the Fenton reaction (Dixon et al., 2012). ROS target polyunsaturated fatty acids (PUFAs) within the lipid membrane, generating lipid peroxides, compromising the integrity of the cell membrane, disrupting mitochondrial activity, and ultimately resulting in ferroptosis (Dixon et al., 2012). Figure 2 illustrates the distinction between ferroptosis cells and normal cells.

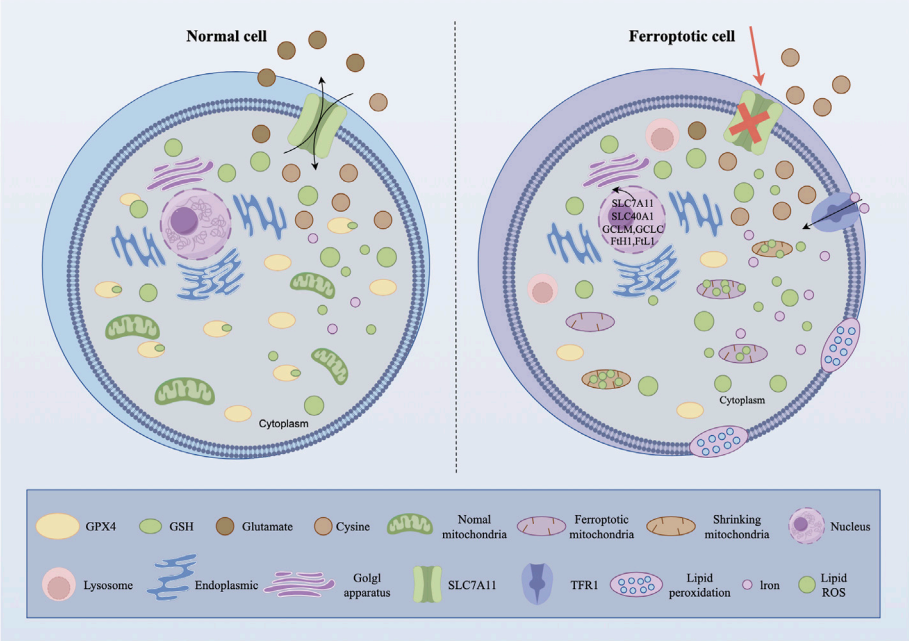


FIGURE 2
Ferroptosis causes damage to cells (figure made by figdraw).

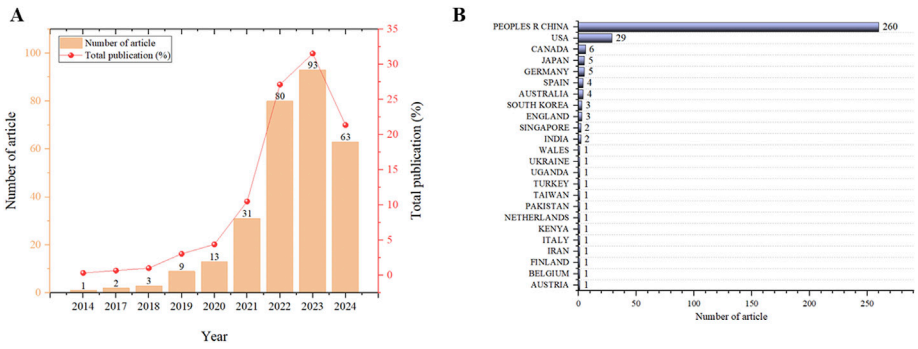


FIGURE 3
Scientometric study of ferroptosis on stroke. **(A)** Number of publications by year. **(B)** Number of publications by country or region.

The terms, "ferroptosis" and "stroke" as topic, were searched in Web of Science (SCI-E) core collection database. The article was refined and used for scientometric analysis. The time span is from 1 January 2014, to 31 December 2024. Original articles related to natural products were included in our study after being read in full by the researchers. The first paper, published in 2014, was titled "(−)-Epicatechin protects hemorrhagic brain via synergistic Nrf2 pathways" (Figure 3A). Most papers were published in the year 2023, while the most cited year was 2021. So far, research in the field of the therapeutic on the ferroptosis of stroke on natural products is increasingly ongoing. A total of 24 countries contributed to the publications included in this study. Among them, China published the most papers, followed by American, Canada, Japan, Germany, Spain, Australia, South Korea, England, and Singapore (Figure 3B). Next, we conducted a thorough review of these 293 articles, and 55 of them were included in our study (Figure 1).

3 Mechanisms of ferroptosis in ICH

The accumulation of iron ions produced by red blood cells (RBCs) lysis after ICH is the main cause of ferroptosis (Figure 4). The lysis of RBCs in the hematoma is the most contributor of free hemoglobin, followed by the release of heme, which is subsequently degraded into iron, biliverdin, and carbon monoxide (Zhang et al., 2021). Iron ions accumulate after 24 h of ICH, and excessive accumulation is an important characteristic of ferroptosis (Sun et al., 2022). Under physiological conditions, the plasma membrane's transferrin receptor TFR1 internalizes to facilitate

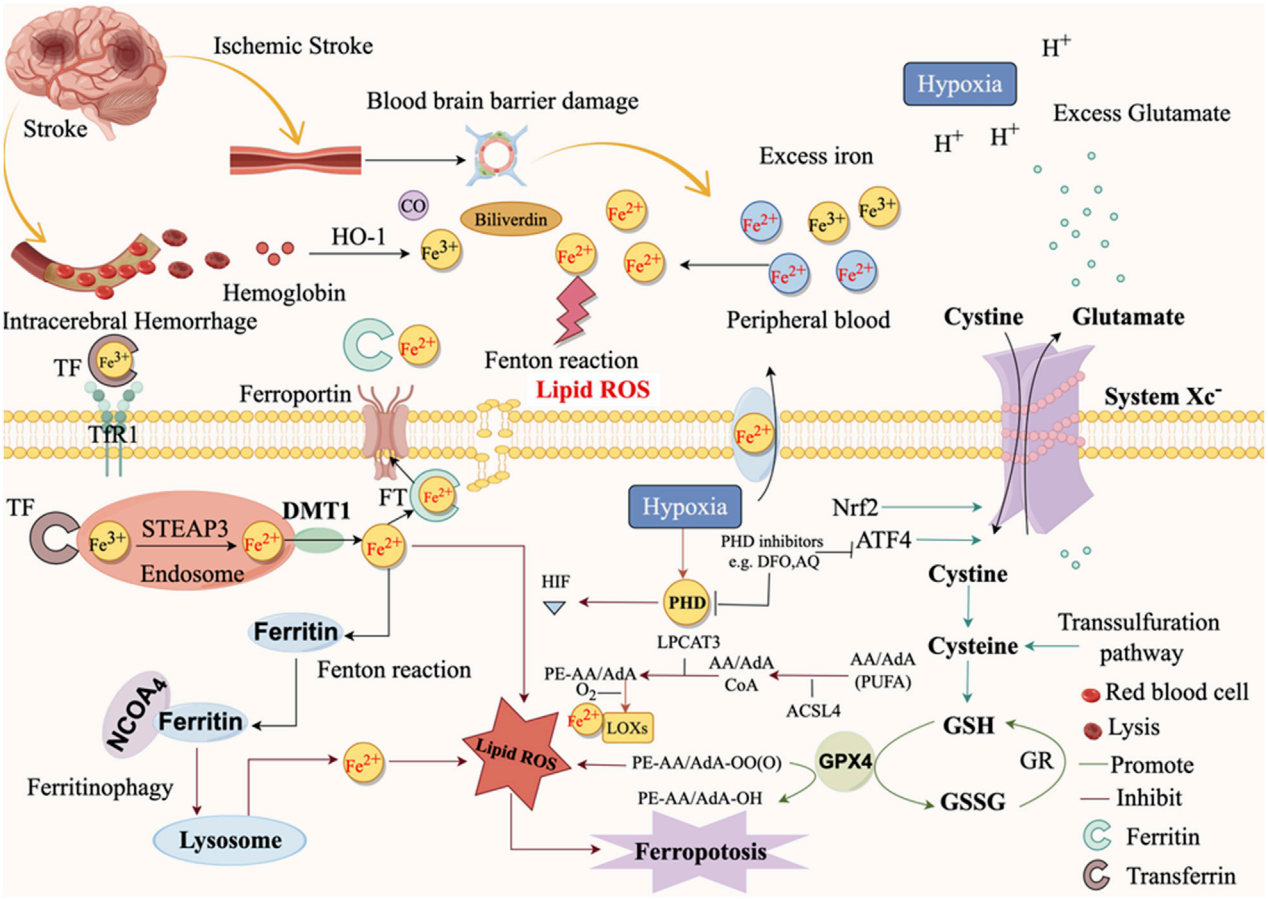


FIGURE 4
Mechanisms of ferroptosis after stroke (figure made by figdraw).

transport (Zhao et al., 2021a). The iron output protein FPN1, which couples with multicopper iron oxidase (like ceruloplasmin) on the plasma membrane, mediates the intracellular iron output (Zhao et al., 2021a). Alternatively, multivesicular and extracellular vesicles containing ferritin can also excrete iron out of the cell (Zhao et al., 2021a). Under ICH conditions, iron is transported to the extracellular space through the iron export protein FPN in microglia, causing a large amount of toxic iron to enter the brain tissue (Hvidberg et al., 2005).

To be specific, two-molecule Fe^{3+} participates in the transport of iron by binding to one molecule of transferrin, which transports Fe^{3+} to the intracellular by binding to the membrane protein transferrin receptor 1 (TFR1) on the surface of neurons to form a Tf-Fe^{3+}-TFR1 complex. Fe^{3+} is then reduced to Fe^{2+} by the Six-Transmembrane Epithelial Antigen of Prostate 3 (STEAP3). Endosomes release Fe^{2+} into the cytoplasmic labile iron pool (LIP), which requires DMT1/Solute Carrier Family 11 Member 2 (SLC11A2) regulation (Chen et al., 2020; Yao et al., 2021; Zhou et al., 2020) (Figure 3). The synthesis of the essential enzyme lipoxygenases (LOXs) in lipid peroxidation (LPO) can be facilitated by Fe^{2+} in the LIP, which leads to ferroptosis. Furthermore, Fe^{2+} will engage in the Fenton reaction with hydrogen peroxide to generate hydroxyl radicals, which will in turn damage cellular plant metabolites and induce ferroptosis (Wan et al., 2019). ROS can be generated by iron-catalyzed enzymes, which in turn can induce lipid auto-oxidation, thereby promoting ferroptosis (Chen et al., 2020). Nuclear receptor coactivator 4 (NCOA4) also mediates ferritinophagy by binding to ferritin and subsequently transporting iron-bound ferritin to the autophagosome for lysosomal degradation and iron release. NCOA4 knockdown can prevent LPO and ferroptosis by reducing the amount of iron in the intracellular LIP (Fang et al., 2021). Through NCOA4, autophagy-related 5 (ATG5) and autophagy-related 7 (ATG7) promote ferroptosis by degradation of ferritin (Hou et al., 2016). The expression of TFR1 can be stimulated by nitrogen fixation 1 (NFS1), an iron-sulfur cluster biosynthetic enzyme, to improve ferroptosis (Alvarez et al., 2017). Further, heat shock factor-binding Protein 1 (HSPB1) is highly inducible after treatment with Erastin. Once activated, HSBP1 reduces iron levels by inhibiting TFR1 expression (Sun et al., 2015).

LPO is a critical mechanism that directly initiates ferroptosis. It is the process by which oxygen binds to lipids to generate lipid peroxides by forming peroxyl radicals. The Fenton reaction, which is initiated by iron stimulation, generates lipid ROS, which subsequently induces LPO and ultimately results in ferroptosis (Bai et al., 2019). Furthermore, the oxidation and esterification of PUFAs generate lipid peroxides, which in turn induce LPO (Yang

TABLE 1 Genes associated with ferroptosis after ICH.

S.N.	Gene name	Cell/Animal model	Pathway/Axis	Ref
1	Nrf2	SD, autologous blood	Nrf2/ARE-GPX4	Duan et al. (2022)
2	HDAC1/2	C57, autologous blood BV2 HT22, hemin	Nrf2/HO1	Jiang et al. (2024)
3	SOX10	HT22, hemin	miR-29a-3p/ACSL4	Chen et al. (2023)
4	ACSL4	C57, collagenase VII-S HT22, hemin	HOTAIR/UPF1/ACSL4	Jin et al. (2021)
5	lncRNA H19	BMVECs, hemin	miR-106b-5p/ACSL4	Chen et al. (2021a)
6	GPX4	SD, collagenase VII BV2, hemoglobin	AMPK/mTORC1	Xie et al. (2023)
7	H3K9me3	C57, collagenase VII N2A/SK-N-SH, hemin	Suv39h1-mediated H3K9me3, repress Tfr1	Lan et al. (2023)
8	Nedd4	C57, collagenase VII HT22, hemin	Nedd4/DMT1	Lv et al. (2024)
9	FOXO3	C57, collagenase VII HT22, hemin	FOXO3/NOX4	Qin et al. (2022)
10	CISD2	C57, collagenase VII	AKT/mTOR	Li et al. (2023a)
11	H_2S	BV2, hemin	CBS/H2S	Yu et al. (2023)
12	circAFF1	C57, collagenase VII Neurons, Hemoglobin	miR-140-5p/GSK-3β Wnt/β-catenin	Yin et al. (2022)
13	SLC7A11	HT-22, FAC	miR-122-5p/TP53/SLC7A11	Zhao et al. (2021b)
14	METTL3	C57, collagenase VII BMVECs, hemin/OGD	N6-methyladenosine/GPX4	Zhang et al. (2022a)
15	FSP1	C57, autologous blood	GPX4 pathway	Wang et al. (2022a)
16	Fpn	Fpn-floxed mice autologous blood	miR-124/Fpn	Bao et al. (2020)
17	IRP	C57, collagenase VII Neurons, hemin	miR-19b-3p	Yi and Tang (2021)

et al., 2016). LPO can attack cells by destroying proteins, DNA, and lipid membranes and activating ferroptosis (Salvador, 2010). Inhibiting LPO has been a critical and effective strategy for safeguarding ICH by reducing ferroptosis. We have summarized the related genes of ferroptosis after ICH, as shown in Table 1.

4 Mechanisms of ferroptosis in ischemic stroke

Following ischemic stroke, the BBB becomes compromised because of the breakdown of tight junction protein (Figure 4). This leads to the release of Fe^{3+} from the blood into the brain tissue, facilitated by TF and TFR1 (Zhao et al., 2023a). Afterwards, there is an excessive amount of Fe^{2+} which is then decreased and moved to the cytoplasm. ROS, which are generated quickly via the Fenton reaction, facilitate the degradation of nucleic acids, proteins, and membranes, leading to the occurrence of ferroptosis (Figure 4). When cerebral ischemia reperfusion occurs, the release of excitatory amino acids, represented by Glu, increases and accumulates in the synaptic cristae. The uptake of glutamate within cells reduces while the release of glutamate outside of cells increases, resulting in the inhibition of the system Xc^- (Xu et al., 2023a). Some studies have found through metabolomics that gamma-glutamyl dipeptide or tripeptide was found in the cell under Cys deprivation. These metabolites reduced the level of Glu and alleviated the sensitivity to ferroptosis (Kagan et al., 2017). But on the other hand, for cysteine-deprived cells, the catabolism of Gln promoted the synthesis of PUFA, and its decomposition products such as α-ketoglutaric acid could also increase the accumulation of lipid peroxides. These effects of glutamine restored the sensitivity of cells to cysteine-deficient ferroptosis (Gao et al., 2019). It is important to mention that while Gln can be broken down into Glu by the enzymes GLS1 and GLS2, only the catalysis of GLS2 is linked to ferroptosis (Gao et al., 2015).

Prior to the proposal of the notion of ferroptosis, there have been multiple instances of abnormal buildup of iron in ischemic brain tissue (Dietrich and Bradley, 1988; Kondo et al., 1995). Research indicates that neurological damage following an ischemic stroke is associated with dysregulation of brain iron metabolism and transport (Selim and Ratan, 2004). The impaired BBB facilitates the entry of circulating iron into brain tissue following an ischemic stroke, while neurons also experience an increase in iron uptake (Bu et al., 2021). The early extravasation of circulating transferrin into ischemic brain parenchyma is induced by cerebral ischemia-reperfusion (I/R), which also activates NF-κB and promotes the

TABLE 2 Targets associated with ferroptosis after ischemic stroke.

S.N.	Targets name	Model	Effect on ischemic stroke	Ref
1	Tau	C57, SD MCAO	Tau suppression prevents ferroptosis by iron-targeting interventions	Tuo et al. (2017)
2	LncRNA PVT1	PC12, C57 MCAO	PVT1 regulated ferroptosis through miR-214-mediated TFR1 and TP53 expression	Lu et al. (2020)
3	SSAT1	C57; tMCAO	The activation of SSAT1/ALOX15 axis may aggravate ischemic stroke injury via triggering neuronal ferroptosis	Zhao et al. (2022)
4	Thrombin	Human blood N27 cell, OGD/R C57, SD, MCAO	Thrombin-ACSL4 axis ameliorate ferroptotic neuronal injury	Tuo et al. (2022)
5	UBIAD1	Primary cell OGD/R SD, MCAO	UBIAD1 improved brain tissue impairment and neuronal death	Huang et al. (2022)
6	PGE2	Human samples	Inhibit iron accumulation and LPO	Xu et al. (2022)
7	FtMt	C57, MCAO	Ameliorate inflammation and hepcidin-mediated decreases in ferroportin1	Wang et al. (2021)
8	USP14	Primary cell, OGD/R C57, MCAO	Inhibition of USP14 reduces NCOA4 expression and protects neurons from ferroptosis	Li et al. (2021)
9	SIRT6	HT-22, OGD/R C57, MCAO	FTH1 and NCOA4 via SIRT6 influence ferritin autophagy	Yu et al. (2024)
10	mTOR	SD, MCAO	mTOR/SREBP1 Pathway inhibit ferroptosis	Lang et al. (2024)
11	ACSL4	PC12, OGD/R SD, MCAO	Inhibits ferroptosis by facilitating ACSL4 ubiquitination degradation	Ou et al. (2024)

expression of the DMT1 1B subtype, thereby enhancing iron uptake (DeGregorio-Rocasolano et al., 2018). In addition to facilitating iron uptake, cerebral ischemia also increases intracellular iron storage. The concentration of ferritin in the plasma and cerebral spinal fluid of individuals with ischemic stroke increases within 24 h. This rise is linked to early neurological impairment and might potentially result in hemorrhagic transformation and severe brain oedema following tPA thrombolysis (Liu, 2021). Interestingly, researchers found that ferritin overexpression attenuated Tau phosphorylation and ROS production, reversed the decreases of glutathione (GSH) and SLC7A11 in rats' hippocampal neurons after middle cerebral artery occlusion (MCAO), and had a protective effect on motor injury and memory deficits (Chen et al., 2021b). We have summarized the related genes of ferroptosis after ischemic stroke, as shown in Table 2.

5 Targeted therapy of ferroptosis with natural products

Whether it is ischemic stroke or ICH, there is still no perfect treatment plan, and there are still many shortcomings. Based on this, many researchers are looking for traditional medicine (Hilkens et al., 2024). Ferroptosis is a distinct mechanism that modulates cellular death and is integral to the pathophysiological processes of neurodegenerative disorders and stroke (Wang et al., 2023a). An increasing amount of evidence indicates that ferroptosis is a significant contributor to neurodegenerative disorders and stroke, making pharmacological inhibition of ferroptosis a viable therapeutic target for these conditions (Wang et al., 2023a). The following table provides a list of studies of natural products that are effective anti-ferroptosis in stroke models (Table 3).

5.1 Terpenoids

The structure of terpene compounds is built from isoprene units (C5 units), which are related to methylglutaric acid. These oxygen-containing derivatives can be alcohol, aldehydes, ketones, carboxylic acids, esters, among others. Terpenes are widely found in nature and are the main plant metabolites of essence, resin, pigment, among others, which constitute some plants. Terpenoids also include hormones and vitamins from animals. Recently, many scholars have found that terpenoids have anti-ferroptosis effects.

Ginkgolide B (PubChem CID: 65243) is a terpenoid compound that is generated from plants, namely, from *Ginkgo biloba L.* It exhibits a range of pharmacological effects, including inhibiting platelet aggregation, reducing inflammation, acting as an antioxidant, and scavenging free radicals (Chen et al., 2022b). Ginkgolide B inhibits the overproduction of ROS, MDA, ACSL4, NCOA4, and Fe^{2+}, while simultaneously increasing the activities of antioxidative enzymes GPX4 and SOD, and FITH1 (Yang et al., 2024a). It achieves its anti-ferroptosis effect by disrupting the NCOA4-FTH1 interaction (Yang et al., 2024a). In addition, molecular docking, and microscale thermophoresis assay were conducted to explore the combination of Ginkgolide B and NCOA4. Therefore, ginkgolide B may be a candidate for the treatment of ferroptosis after stroke.

Astragaloside IV (PubChem CID:13943297) is a lanolin alcohol-derived tetracyclic triterpene saponin extracted from

TABLE 3 Natural products associated with ferroptosis after stroke.

Name	Model	Dose	Influence index/pathway	Ref
Terpenoids				
Ginkgolide B	PC12 OGD/R SD MCAO	10, 20, 40 μM 1,2,4 mg/kg i.p	ACSL4↓, NCOA4↓, FTH1↑, GPX4↑ MDA↓, ACSL4↓, Fe^{2+}↓, SOD↑ NCOA4-3 FTH1 interaction	Yang et al. (2024a)
Astragaloside IV	SD MCAO SD SAH SH-SY5Y OGD/R SD MCAO HT-22 Neuro-2a OGD/R C57 MCAO	20 mg/kg i.p 20 mg/kg i.p 40 μg/mL 28 mg/kg i.p 6.25,12.5 and 25 μmol/L 20 mg/kg i.p	ROS↓, MDA↓, GPX4↑, SLC7A11↑ Fe^{2+}↓, GSH↑ Nrf2/HO-1 signaling pathway MDA↓, ROS↓, Fe^{2+}↓, GPX4↑ SLC7A11↑, GSH↑ Nrf2/HO-1 signaling pathway LDH↓, Fe^{2+}↓, MDA↓, ROS↓ GPX4↑ P62/Keap1/Nrf2 pathway LDH↓, MDA↓, GSH↑, Fe^{2+}↓ GPX4↑, SLC7A11↑, ACSL4↓ Fto↑, Atf3↑ ACSL4 axis	Zhang et al. (2023a) Liu et al. (2022a) Wang et al. (2023b) Jin et al. (2023)
β-Caryophyllene	Primary astrocytes OGD/R SD, MCAO	10, 20, 40 μM 204,306 and 408 mg/kg i.g	ACSL4↓, COX2↓, 4-HNE↓, MDA↓ Nrf2/HO-1 signaling pathway	Hu et al. (2022)
Ginsenoside Rd	bEnd.3 OGD/R SD MCAO/R	5,10,20 μM 1.5,3,6 mg/mL i.p	ZO-1↑, occludin↑, claudin-5↑ ACSL4↓, COX2↓, GPX4↑, xCT↑ GSH↑, MDA↓, Fe^{2+}↓, NRG1↑ PI3K/Akt/mTOR signaling pathway	Hu et al. (2024)
Withaferin A	SH-SY5Y Hemin C57 ICH	10 μM 0.1,1,5 μg/kg i.c.v	SOD↑, GSH-Px ↑, MDA↓, LDH↓, HO-1↑, Nrf2↑ Nrf2/HO-1 signaling pathway	Zhou et al. (2023a)
Rehmannioside A	SH-SY5Y OGD/R SD MCAO	80 μM 80 mg/kg i.p	GPX4↑, SLC7A11↑, SOD↑, MDA↓, GSH↑, ROS↓, MPO↓, GSSG↓ PI3K/AKT/Nrf2 signaling pathway	Fu et al. (2022)
Artesunate	BV2 Haemoglobin SD ICH	5 μM 20,50,70 mg/kg i.p	GPX4↑, ACSL4↓, Fe^{2+}↓, ROS↓ AMPK/mTORC1/GPX4 pathway	Xie et al. (2023)
Kellerin	SH-SY5Y OGD/R C57 MCAO	0.1,1,10 μM 10,20 mg/kg i.g	GPX4↑, COX2↓, GSH↑, MDA↓ Fe^{2+}↓, Akt/Nrf2 pathway	Mi et al. (2024)
Loureirin C	SH-SY5Y OGD/R C57 MCAO	1,5,10 μM 10,20,40 mg/kg i.g	HO-1↑, GPX4↑, NQO1↑, SOD↑ MDA↓ Keap-1/Nrf2/HO-1 pathway	Liu et al. (2023b)
15,16 Dihydrotansh-inone I	PC12 OGD/R SD MCAO	10 μM 7.5,15,30 mg/kg i.g	GPX4↑, Fe^{2+}↓, MDA↓, ROS↓ HO-1↑, Nrf2↑	Wu et al. (2023)
Ecdysterone	PC12 OGD/R SD MCAO	20,40,80 μM 20,40 mg/kg i.p	ROS↓, MDA↓, Fe^{2+}↓, ACSL4↓ NCOA4 ↓, FITH↑	Sun et al. (2024a)
Flavonols				
Quercetin	HT-22 OGD/R SD MCAO	30 μM 25,50,70 mg/kg i.g	MDA↓, ROS↓, GSH↑, SOD↑ ACSL4↓, GPX4↑, Fe^{2+}↓ Nrf2/HO-1 signaling pathway	Peng et al. (2024)
Baicalein	HT-22 OGD/R C57 tMCAO	0.8,4 μM 10,80 mg/kg i.g	ROS↓ GPX4↑, Fe^{2+}↓, ACSL4↓ FTFH1↑, xCT↑, MDA↓ GPX4/ACSL4/ACSL3 pathway	Li et al. (2022)
Baicalin	PC12 Hemin C57 ICH	5,10,20 μM 24 mg/kg i. v	ACSL4↓, GPX4↑, SLC7A11↑ SLC3A2↓, TFRC↑, DMT1↓	Duan et al. (2021)
Calycosin	PC12 OGD/R SD MCAO	15,30,60 μM 5,10,20 mg/kg i.g	Fe^{2+}↓, ACSL4↓, GPX4↑, FTFH1↑ TfR1↓ ACSL4-dependent ferroptosis	Liu et al. (2023c)
Vitexin	Primary neurons SD MCAO	0.5, 2.5, 10 nM 50 mg/kg i.p	ROS↓ Keap1/Nrf2/HO-1 signaling pathway	Guo and Shi (2023)
Kaempferol	Primary cortical neurons OGD/R	10 μM		Yuan et al. (2021)

(Continued on following page)

TABLE 3 (*Continued*) Natural products associated with ferroptosis after stroke.

Name	Model	Dose	Influence index/pathway	Ref
			GPX4↑, SLC7A11↑, MDA↓, ROS↓ Fe^{2+}↓,4-HNE↓ Nrf2/SLC7A11/GPX4 pathway	
Oroxin A	HT22 Hemin C57 SAH	0.5,1,5,10 μM 5 mg/kg i.p	MDA↓, GSH↑, Fe^{2+}↓, ROS↓, TNF-α↓, GPX4↑, FTFH1↑, SLC7A11↑ IL-1β↓, IL-6↓, IL-10↑, CD32↓ CD206↑, HO-1↑, FSP1↑	Chen et al. (2024)
Icariside II	Primary astrocytes C57 MCAO	6.25,12.5,25 μM 5,10,20 mg/kg i.p	Fe^{2+}↓, GPX4↑ OXPHOS/NF-κB pathway	Gao et al. (2023)
Curcumin	HT22 cells C57 ICH	20 mg/kg p.o	GPX4↑, HMOX1↑, NFE2L2↑	Yang et al. (2021)
(−)-Epicatechin	C57 ICH	5,15,45 mg/kg p.o	ROS↓, Nrf2↑, SOD-1 ↑, AP-1↓ MMP-9↓	Chang et al. (2014)
Carthamin yellow	SD MCAO	20,40 mg/kg p.o	ROS↓, SOD↑, GSH↑, ACSL4↓, TFR1↓ FTH1↑	Guo et al. (2021)
Phenolic				
Rosmarinic acid	C57 ($TfR1^{-/-}$) MCAO	20 mg/kg p.o	GPX4↑, FPN1↑, TfR1↑ ACSL4/LPCAT3/Lox pathway	Jia et al. (2024)
Caffeic acid	SK-N-SH OGD/R SD pMCAO	30 μM 0.4,2,10 mg/kg i.g	ROS↓, SOD↓, GPX↑, MDA ↓ 8-OHdG↓ Nrf2 signaling pathway	Li et al. (2024a)
Salvianolic acid A	Primary cortical neuronal Hemin SD ICH	50 μM 10 mg/kg i.v	GPX4↑, ROS↓, xCT↑ Akt/GSK-3β/Nrf2 pathway	Shi et al. (2024)
Paeonol	HT22 cells Hemin C57 ICH	5,10,20,40 μM 10,20,30 mg/kg i.p	GSH↑, MDA↓, ROS↓, SLC7A11↑ ACSL4↓ HOTAIR/UPF1/ACSL4 pathway	Jin et al. (2021)
Carvacrol	Gerbils mice MCAO	25,50,100 mg/kg p.o	GSH↑, MDA↓, ROS↓, Fe^{2+}↓ TFR-1↓, FPN1↑	Guan et al. (2019)
Resveratrol	Primary neurons SD MCAO	5,10,15,20 μM 30 mg/kg i.p	GSH↑, Fe^{2+}↓, ACSL4↓, GPX4↑ ROS↓	Zhu et al. (2022a)
Rhein	HT22 OGD/R SD MCAO	10,20,40 μM 20,50,100 mg/kg i.p	Fe^{2+}↓, ROS↓, GPX4↑, GSH↑ NRF2/SLC7A11/GPX4 pathway	Liu et al. (2023d)
Alkaloids				
Berberine	C57 MCAO	Fecal microbiota transplantation	GSH↑, MDA↓, SLC7A11↑, GPX1↑ ACSL4↓	Wang et al. (2023c)
Dauricine	SH-SY5Y RSL3 C57 ICH	0.03 μM–3 μM 5 mg/kg i.g	GPX4↑, GSR↑, ROS↓, MDA↓ Fe^{2+}↓	Peng et al. (2022)
Traditional Chinese medicine formulations				
Angong Niuhuang Wan	SD MCAO SD ICH	257,514 mg/kg p.o	LPO↓, Fe^{2+}↓, GPX4↑ PPARγ/AKT/GPX4 pathway	Bai et al. (2024)
DiHuang YinZi	SD MCAO	0.2,0.4,0.8 mg/kg p.o	MDA↓, ROS↓, GSH↑, Fe^{2+}↓, GPX4↑ P53/SLC7A11 pathway	Yang et al. (2024b)
Naodesheng Pills	SH-SY5Y OGD/R SD MCAO	3.125, 6.25, 12.5, 25 μg 0.54,1.08 mg/kg p.o	TFR1↓, GPX4↑, DMT1↓, GSH↑ MDA↓, SOD↓ ERK1/2 Pathway	Yang et al. (2024c)
Naotaifang	BV2 OGD/R SD MCAO SD MCAO	9,18,27 g/kg 9,18,36 mg/kg p.o 27 g/kg p.o	Fe^{2+}↓, ROS↓, GPX4↑, MDA↓ BMP6/SMADs Pathway Fe^{2+}↓, ROS↓, GPX4↑, MDA↓ TFR1↑, GSH↑, DMT1↓, SLC7A11↑	Liao et al. (2023) Lan et al. (2020)
Tongluo Decoction	HUVECs OGD/R SD MCAO		ROS↓GPX4↑, MDA↓, Fe^{2+}↓ Nrf2/ARE/SLC7A11 Pathway	Li et al. (2024b)

(Continued on following page)

TABLE 3 (*Continued*) Natural products associated with ferroptosis after stroke.

Name	Model	Dose	Influence index/pathway	Ref
	PC12 OGD/R SD MCAO	24 g/kg 6,12,24 mg/kg p.o 6,24 mg/kg p.o	ROS↓, SOD↑, MDA↓, GPX4↑ ACSL4↓	Hui et al. (2022)
Xingnaojing injection	SH-SY5Y OGD/R SD MCAO	0.25%,0.5%, 1% 0.18 mL/100g i.p	GPX4↑, FPN↑, HO-1↑, COX-2↓, TFR ↓, DMT1↓	Liu et al. (2023e)
Tongqiao Huoxue Decoction	PC12 OGD/R SD MCAO	10,20,40 μM 3,6,12 g/kg i.p	SOD↑, ROS↓, MDA↓, GPX4↑ FTH1↑, ACSL4↓	Ou et al. (2024)
Salvia miltiorrhiza	C57 MCAO	200 mg/kg p.o	4-HNE↓, MDA↓, FPN1↓, GPX4↓ ACSL4↓	Ko et al. (2023)
Paeoniae Radix Rubra extract	HT22 OGD/R SD MCAO	100 μg/mL 0.27,0.54,1.08 g/kg p.o	GPX4↑, FTH1↑, Beclin1↑, ROS↓ LC3 II↑, MDA↓, GSH↑	Zhao et al. (2023b)
Danlou tablet	hy926 cell OGD/R C57 MCAO	1400,2800 mg/kg p.o	GPX4↑, COX2↑, SLC7A11↑	Liu et al. (2024)
Danhong Injection	HT22 OGD/R C57 MCAO	5,10,20 μL/mL 2.5,5 mL/kg i.p	SOD↑, MDA↓, GSH↑ SATB1/SLC7A11/HO-1 pathway	Zhan et al. (2023)
Salvia miltiorrhiza Bge. (process with porcine cardiac blood)	SH-SY5Y OGD/R SD MCAO	2.5,5,10 μg/mL 3,6,12 g/kg p.o	GSH↑, MDA↓, SOD↑, ROS↓, GLRX5↓ SLC7A11/GPX4 pathway	Zhou et al. (2024a)
Roots of *Astragalus propinquus* schischkin	Granule Injection SD MCAO	2.5 g/kg p.o 2 mL/kg i.p	HO-1↑, Nrf2↑, GPX4↓, XCT↑ SLC3A2↑, FPN1↑, TRPC6↓ DMT1↓, TFR↓	Chen et al. (2022a)
Polysaccharide				
Neutral polysaccharide from Gastrodia elata	HT22 OGD/R C57 MCAO	100,250,500 μg 0.5,1,2 mg/kg	MDA↓, ROS↓, GSH↑, Fe^{2+}↓ GPX4↓, SLC7A11↑ NRF2/HO-1 pathway	Zhang et al. (2024)
Polysaccharide from Salvia miltiorrhiza Bunge	PC12 OGD/R	20,40,60 μg/mL	ROS↓, Fe^{2+}↓, SLC7A11↑, GSH↑ GPX4↓, MDA↓ Nrf2/HO-1 pathway	Meng et al. (2022)
Other Compounds				
Cottonseed oil	SD MCAO	1.3 mL/kg	GPX4↑, XCT↑, HO-1↑, FTH1↑, SOD↑, ACSL4↓, GSH↑, MDA↓	Sun et al. (2023)
Mastoparan M	HT22 OGD/R C57 MCAO	17, 34, 68 nM 20,40,80 μg/kg i.p	ROS↓, Fe^{2+}↓, Nrf2↑, XCT↑, GPX4↓ NRF2-GPX4 pathway	Wang et al. (2024a)
Melatonin	HT22 OGD/R C57 MCAO HT22 OGD/R C57 MCAO	20,40 μM 5,10 mg/kg i.p 20,40 μM 5,10 mg/kg i.p	ROS↓, GSH↑ ACSL4/CYP1B1 pathway ROS↓, ACSL4↓, MDA↓, SOD↑ MDM2↑, GSH↑	Sun et al. (2024b) Ji et al. (2024)
Dl-3-n-butylphthalide	SD MCAO	10,20,30 mg/kg i.p	SOD↑, ROS↓ MDA↓, TFRC↓, GSH↑, GPX4↑, SLC7A11↑	Xu et al. (2023b)
Taurine	HT22 Hemin C57 SAH	20 μM 400 mg/kg i.p	MDA↓, ROS↓, SOD↑, GPX4↑ SLC7A11↑, Fe^{2+}↓ GABAB/AKT/GSK3β/β-catenin	Liu et al. (2022b)
Crocin	C57 ICH	40 mg/kg p.o	GSH-px↑, SOD↑, MDA↓, Nrf2↑ GPX4↑, FTH1↑, SLC7A11↑	Wang et al. (2022b)

Astragalus membranaceus. It is a white powder with the molecular formula $C_{41}H_{68}O_{14}$. Studies indicate that astragaloside IV exerts a neuroprotective effect through multiple mechanisms, including its anti-inflammatory, anti-oxidative, and anti-apoptotic properties that safeguard nerve cells (Yao et al., 2023). Additionally, it regulates nerve growth factor, inhibits neurodegeneration, and facilitates neuron regeneration (Yao et al., 2023). In MCAO rats, astragaloside IV can inhibit the expression of inflammatory factors TNF-α, IL-1β, IL-6, and NF-κB, increasing the levels of SLC7A11 and GPX4 (Zhang et al., 2023a). Mechanism studies indicate that astragaloside IV triggered the Nrf2/HO-1 signaling pathway and alleviated ferroptosis due to ischemic stroke induction (Zhang et al., 2023a). Notably, ML385 inhibited these effects, and astragaloside IV increased P62 and Nrf2 levels while decreasing Keap1 levels. P62 silencing decreased astragaloside IV's effects on the P62/Keap1/Nrf2 pathway and ferroptosis (Wang et al., 2023b). Another study showed that astragaloside IV promoted the transcription of Fto by regulating Atf3, resulting in a decrease of

Acsl4 levels, thus improving neuronal injury in ischemic stroke by inhibiting ferroptosis (Jin et al., 2023). Interestingly, in the SAH model, astragaloside IV also exerts its anti-ferroptosis effect through the Nrf2/HO-1 pathway (Liu et al., 2022a). Although many studies have shown that it can treat central nervous system (CNS) diseases, previous pharmacokinetic studies have shown that AS-IV has a poor oral bioavailability and membrane permeability (Li et al., 2023b). There are also studies that suggest that it may affect CNS diseases through intestinal bacteria (Li et al., 2023b). If it is to be developed as a drug, further mechanism studies are needed.

β-Caryophyllene (PubChem CID: 26318) is a class of bicyclic sesquiterpenes found in lemon, grapefruit, nutmeg, pepper, raspberry, blackcurrant, cinnamon leaf oil, and clover leaf oil, whose molecular formula is $C_{15}H_{24}$ (Sharma et al., 2016). The *in vivo* study demonstrated that BCP enhanced neurological scores, infarct volume, and pathological features following MCAO/R. They found that BCP significantly increased the nuclear translocation of Nrf2 and activated the Nrf2/HO-1 pathway, thereby safeguarding against ferroptosis (Hu et al., 2022). β-Caryophyllene decreased OGD/R-induced ROS generation and iron accumulation (Hu et al., 2022). Furthermore, the neuroprotective effects of β-Caryophyllene were reversed by the Nrf2 inhibitor ML385. β-Caryophyllene is a food additive that has been cleared by the Food and Drug Administration and is generally thought to be safe. β-Caryophyllene is also highly available orally and has been studied in RCT in vascular diseases, which I think is a good candidate for the treatment of stroke (Yamada et al., 2023).

Ginsenoside Rd (PubChem CID: 11679800) exhibits diverse pharmacological properties including cardiovascular protection, neuroprotection, anti-aging, anti-tumor, and more (Chen et al., 2022c). The chemical formula of the compound is $C_{48}H_{82}O_{18}$. Ginsenoside Rd could increase the expression of ZO-1, occluding-1, and claudin-5 in cerebral micro vessels and bEnd.3 cells on the same side of the brain, leading to a decrease in the loss of endothelial cells and leakage of Evans blue dye. As a result, ginsenoside Rd eventually enhances the integrity of the BBB following cerebral I/R injury (Hu et al., 2024). Ginsenoside Rd can mitigate the breakdown of the BBB generated by ischemic stroke by reducing ferroptosis in endothelial cells (Hu et al., 2024). Functionally, ginsenoside Rd protected against tight junction loss and leakage of the BBB by increasing the expression of NRG1, which in turn activated the tyrosine kinase ErbB4 receptor. This activation subsequently triggered the PI3K/Akt/mTOR signaling pathway, ultimately preventing ischemic stroke-induced ferroptosis in endothelial cells (Hu et al., 2024). Despite its potent pharmacological efficacy, ginsenoside Rd is present in plants in relatively low quantities, making its production from ginseng an expensive process.

Withaferin A (PubChem CID: 265237) is a steroid ester plant metabolite with a molecular formula of $C_{28}H_{38}O_6$. Withaferin A treatment was found to suppress ferroptosis and reduce oxidative stress (OS)-related damage in both an *in vivo* model and an *in vitro* model of ICH (Zhou et al., 2023a). These effects were attributed, at least in part, to the augmented translocation of Nrf2 and increased expression of HO-1 (Zhou et al., 2023a). More precisely, withaferin A can enhance the production of HO-1, SOD, GSH-Px, and Nrf2 while suppressing the production of MDA (Zhou et al., 2023a). However, the sample size of this study is too small, so more pharmacodynamics studies should be carried out in the future, and then mechanism studies should be carried out.

Rehmanioside A (PubChem CID: 6325881) is an iridoid glycoside that promotes immunity, replenishes blood, and lowers blood sugar (Liu et al., 2017). The chemical formula is $C_{21}H_{32}O_{15}$. Furthermore, current research has shown that rehmannioside A could decrease OS, enhance cognitive function, and provide a safeguarding impact on neurons (Sun et al., 2019a). Rehmannioside A exhibits neuroprotective properties and enhances cognitive function following cerebral ischemia by suppressing ferroptosis and activating the PI3K/AKT/Nrf2 and SLC7A11/GPX4 signaling pathways (Fu et al., 2022). Specifically, the rehmannioside A group significantly improved the cognitive impairment and neurological deficits compared to the model group and reduced cerebral infarction in MCAO rats (Fu et al., 2022). Furthermore, the rehmannioside A group demonstrated a noticeable increase in cell viability and a reduction in H_2O_2-induced toxicity (Fu et al., 2022). Further research revealed a significant increase in the expression of p-PI3K, p-Akt, nuclear Nrf2, HO-1, and SLC7A11 in the rehmannioside A group compared to the model group (Fu et al., 2022). In addition, rehmannioside A inhibits the release of pro-inflammatory mediators from microglia and promotes M2 polarization *in vitro*, thereby protecting co-cultured neurons from apoptosis by inhibiting NF-κB and MAPK signaling pathways (Xiao et al., 2021).

Artesunate (PubChem CID: 6917864) possesses the attributes of cost-effectiveness, rapid onset, little toxicity, and resistance development challenges. Recent research has discovered that artesunate could trigger ferroptosis without relying on xCT (Sun et al., 2019b). Chao Qin's research found that artesunate caused LPO and the formation of ROS in BV2 cells stimulated by LPS (Xie et al., 2023). However, the use of the ferroptosis inhibitor Fer-1 effectively restored these effects (Xie et al., 2023). Furthermore, artesunate induced ferroptosis in ICH M1-polarised BV2 cells primarily through the AMPK/mTOR/GPX4 axis and partially through Akt phosphorylation inhibition, but not by severing the link between mTORC1 and lysosomes (Xie et al., 2023). However, the *in vitro* data cannot be considered pharmacological or clinically relevant. More animal experiments should be carried out in the future to verify the anti-ferroptosis effect of artesunate.

15,16-Dihydrotanshinone I (PubChem CID: 11425923, DHT), a lipophilic tanshinone with the chemical formula $C_{18}H_{14}O_3$, is isolated from the root of *Salvia miltiorrhiza Bunge* [Lamiaceae; Salviae miltiorrhizae radix et rhizoma]. The *in vitro* investigation on ferroptosis was reduced by DHT, as evidenced by a decrease in lipid ROS generation, an increase in GPX4 expression and the ratio of GSH/GSSG, and an improvement in mitochondrial function (Wu et al., 2023). The inhibitory effect of DHT on ferroptosis was decreased after Nrf2 silencing. Compared with the pMACO group, DHT significantly increased the expression of GPX4 and reduced the GSH-Px activity. The inhibitory effect of DHT on ferroptosis and its underlying mechanism in pMCAO rats were examined *in vivo*. However, relying on a few indicators of ferroptosis to prove the anti-ferroptosis effect of DHT is far from sufficient. More in-depth mechanism research is needed in the future.

Kellerin (PubChem CID: 40580807) inhibits microglial activation, thereby exerting a potent anti-inflammatory effect. Its molecular formula is $C_{32}H_{26}O_{12}$. Kellerin inhibited the production

of mitochondrial ROS *in vitro*, thereby improving the neuronal injury caused by OGD/R and suppressing ferroptosis (Mi et al., 2024). Kellerin directly contacted Akt and raised its phosphorylation, which resulted in an increase in Nrf2 nuclear translocation and the production of its downstream antioxidant genes (Mi et al., 2024). Kellerin provided protection against IS and prevented ferroptosis *in vivo*, evidenced by its ability to enhance the expression of GSH and GPX4 while decreasing MDA and Fe^{2+} levels.

Additionally, molecular docking, in conjunction with drug affinity responsive target stability assay (DARTS) and cellular thermal shift assay (CETSA), was conducted to evaluate the possible target proteins for kellerin. The findings indicate that kellerin significantly raised the level of Akt phosphorylation, indicating that it can bind to Akt and encourage phosphorylation. In conclusion, we assert that kellerin may serve as a candidate therapeutic agent for the ongoing management of ferroptosis in stroke.

Loureirin C (PubChem CID: 14157896) is a type of dihydrochalcone obtained from resin extracted from the stem of *Chinese Dragon's Blood* (Dracaena cochinchinensis S.C. Chen), with the molecular formula $C_{16}H_{16}O_4$. In mice following MCAO/R, loureirin C not only significantly reduced brain damage and prevented neurons from ferroptosis, but it also reduced ROS accumulation in ferroptosis in a dose-dependent manner following OGD/R (Mi et al., 2024). In addition, loureirin C raises the amount of NQO1, GPX4, and HO-1 following ischemic stroke (Mi et al., 2024). To further prove the anti-ferroptosis effect of Loureirin C, they employed ferroptosis activator (Erastin), aptoptosis inhibitors and pyroptosis inhibitors (Z-VAD-FMK) in OGD/R induced SH-SY5Y cells. The findings suggested Loureirin C could increase the cell survival rate in OGD/R cell model in the presence of Z-VAD-FMK (10 μM) with or without erastin (10 μM). Co-Immunoprecipitation assay suggested that Loureirin C could reduce the level of Nrf2 binding to Keap1. All things considered, we think loureirin C is a candidate medication acting as an antioxidant in ferroptosis stroke treatment.

Ecdysterone (PubChem CID: 5459840), found in *Achyranthes bidentata Blume*, is a crucial active plant metabolite that has enhanced its medical potential by virtue of its antioxidant and neuroprotective properties. The chemical formula of the compound is $C_{27}H_{44}O_7$. In MCAO rats, ecdysterone improves ischemic stroke by inhibiting ferroptosis and OS, resulting from increased GSH levels and decreased MDA, ROS, LPO, and Fe^{2+} levels (Sun et al., 2024a). Further research has demonstrated that ecdysterone inhibits ferroptosis in MCAO rats via ACSL4/NCOA4/FTH1pathway (Sun et al., 2024a). To verify the binding between ecdysterone and ACSL4, CETSA was carried out. Ecdysterone enhanced the thermal stability of ACSL4, suggesting that ecdysterone directly interacts with ACSL4 within the cellular environment. This data indicates that ACSL4 is a direct target of ecdysterone. Nevertheless, there is a paucity of research regarding stroke in relation to ecdysterone. Consequently, we assert that this is not a viable candidate drug. Future investigations of the efficacy of mechanisms are essential.

5.2 Flavonoids

Flavonoids were originally referred to as a class of compounds derived from the backbone of 2-phenylchromenone. It now refers to a series of compounds formed by two benzene rings connected to each other through three carbon atoms, i.e., a general term for a class of compounds with a C6-C3-C6 structure. Plants widely distribute flavonoids, most of which exist as glycosides or carbon glycosides produced by sugar synthesis, while others exist in their free form. Flavonoids, particularly quercetin, have demonstrated significant anti-ferroptosis effects *in vitro* and *in vivo* (Wendlocha et al., 2024). The favorable effects of this group of chemicals are mainly attributed to their antiatherogenic, antithrombotic, and antioxidant properties (Wendlocha et al., 2024).

Quercetin (PubChem CID: 5280343), a prevalent flavonoid present in medicinal plants, demonstrates therapeutic properties against a range of illnesses, such as alcoholic hepatitis, renal IR injury, and cancer (Chen et al., 2021c). Pharmacokinetic investigations have shown that quercetin can penetrate the BBB, indicating its potential to protect against neurodegenerative diseases (Pavlović et al., 2023; Javadinia et al., 2022). Quercetin enhances neurological function, reduces the size of brain tissue damage caused by MCAO in rats, and mitigates pathological characteristics (Peng et al., 2024). Additionally, quercetin promotes the survival of HT-22 cells when exposed to H_2O_2 and erastin (Peng et al., 2024). Specifically, quercetin can inhibit MDA, ROS, and Fe^{2+} expression while increasing SOD and GSH expression (Peng et al., 2024). It was discovered that quercetin inhibited ferroptosis both *in vitro* and *in vivo* by up-regulating GPX4 and FTH1 and down-regulating ACSL4 (Peng et al., 2024). Previous detailed publications on quercetin's research with stroke suggest that it may be a viable candidate for stroke treatment (Zhang et al., 2022b).

Baicalein (PubChem CID: 5281605) is known for its various pharmacological properties, including antibacterial, antiviral, anti-inflammatory, antioxidant, and anti-tumor effects (Srivastava et al., 2021). Pharmacokinetic investigations have demonstrated that baicalein can cross BBB and spread throughout the cerebral nuclei (Zhu et al., 2012). Because of its low toxicity and therapeutic properties, baicalein, a natural bioactive molecule, has been extensively studied for its potential in treating stroke. Baicalein reduces iron levels, LPO generation, and morphological characteristics associated with ferroptosis in the brain tissues of MCAO mice (Li et al., 2022). Furthermore, baicalein suppressed ferroptosis by modulating the expression levels of GPX4, ACSL4, and ACSL3 in OGD/R cells, MCAO mice, and RSL3-stimulated HT22 cells (Li et al., 2022). A preliminary study of baicalin in the preparation of astragali decoction conducted by Zheng et al. reported that after oral administration to rats, baicalin was almost undetectable in serum because *Escherichia coli* hydrolyzed baicalin to baicalein and oroxylin A (Jung et al., 2012). In contrast, a large amount of metabolic jaundice was detected in the gut contents and a small amount of baicalin was detected in the blood of sterile rats 2 h after oral baicalin administration at the same dose. In normal rats, baicalin but not baicalein was detected in the blood soon after oral administration. This indicates that baicalin was not directly absorbed into the blood and that only its transformation into baicalein by the gut microbiota allowed it to enter the blood circulation.

Baicalin (PubChem CID: 64982) is a flavonoid compound with the molecular formula $C_{21}H_{18}O_{11}$. It has pharmacological characteristics, including antioxidant, antiapoptotic, and neuroprotective actions, in several illnesses (Zhou et al., 2019;

Chen et al., 2018). Baicalin also can inhibit the development of ferroptosis in ICH (Duan et al., 2021). It has been reported to exhibit neuroprotective effects against ICH-induced brain injury as well as reduce iron deposition in multiple tissues (Duan et al., 2021). Baicalin enhanced cell viability and suppressed ferroptosis in rat pheochromocytoma PC12 cells treated with hemin, erastin and RSL3 (Duan et al., 2021). Specifically, baicalin can promote the expression of GPX4 and SLC7A while inhibiting SLC3A2 and DMT1 (Duan et al., 2021). However, this experiment only detected a few indicators of ferroptosis and did not perform more in-depth research on the mechanisms involved. More and deeper studies are needed in the future.

Calycosin (PubChem CID: 5280448) is a typical phytoestrogen and possesses a variety of pharmacological activities, including anticancer, anti-cardiotoxicity, anti-diabetic nephropathy, and anti-cerebral ischemic activities, its molecular formula is $C_{16}H_{12}O_5$ (Deng et al., 2021). The administration of calycosin resulted in a reduction in ferroptosis, as shown by the measurement of iron accumulation, MDA, SOD, ceramide, and ROS levels, as well as the expression of ferroptosis-related proteins (ACSL4, TfR1, FTH1, and GPX4) (Liu et al., 2023c). However, the sample size is too small, and more basic research should be needed in the future.

Vitexin (PubChem CID: 5280441) is a biologically active flavonoid chemical obtained from many culinary and medicinal plants. The chemical formula of the compound is $C_{21}H_{20}O_{10}$. Vitexin reduced ferroptosis and protects brain tissue through the Keap1/Nrf2/HO-1 pathway (Guo and Shi, 2023). In comparison to the Model group, treatment with Vitexin (0.5, 2.5, and 10 nM) significantly reduced the levels of HO-1, SLC7A11, and GPX-4, while increasing the levels of Keap1 and Tfr1. However, the oral bioavailability of vitexin is low and high doses of vitexin may lead to elevated liver enzymes, so we do not consider it a very good candidate drug.

Kaempferol (PubChem CID: 5280863), with a chemical formula of $C_{15}H_{10}O_6$, is a prominent bioflavonoid that can be found in a variety of fruits, vegetables, and medicinal plants. Kaempferol exhibits neuroprotective, antioxidant, and anti-cancer attributes against several disorders linked to lipid oxidation, such as stroke, Alzheimer's disease, and cancer (Nezhad Salari et al., 2024). Kaempferol increased SLC7A11, GPX4, and Nrf2 in OGD/R-treated neurons (Yuan et al., 2021). Kaempferol can also alleviate the accumulation of Fe^{2+} in OGD/R cells (Yuan et al., 2021). Kaempferol offers defiance against OGD/R-induced ferroptosis by activating the Nrf2/SLC7A11/GPX4 signaling pathway, at least to some extent (Yuan et al., 2021). Nonetheless, the entire article relies on data derived from *in vitro* experiments, which are inadequate for evaluating pharmacological effects if it is a pan-assay interfering drug. Animal experiments should be carried out in the future to fully verify its pharmacological effects.

Oroxin A (PubChem CID: 5320313), also known as baicalein-7-O-glucoside, is a highly effective flavonoid with the molecular formula $C_{21}H_{20}O_{10}$. Oroxin A against ferroptosis after SAH *in vivo* (Chen et al., 2024). Following SAH, the levels of FTH1, GPX4, and SLC7A11 exhibited a decline, which was subsequently reversed by oroxin A treatment (Chen et al., 2024). Oroxin A can control ferroptosis through the Nrf2/GPX4 pathway and the CoQ10-FSP1 pathway (Chen et al., 2024). The neuroprotective properties of oroxin A are mediated through the Nrf2/GPX4 pathway, and the inhibitory effects of Oroxin A on ferroptosis and neuroinflammation depend on the transcriptional response of Nrf2. However, oroxin A is poorly utilized when taken orally, and it cannot cross the BBB. We consider it not a promising candidate drug.

Icariside II (PubChem CID: 5488822), a naturally occurring flavonoid companies derived from traditional Chinese medicinal *Epimedium sagittatum* (Siebold & Zucc.) Maxim. Its molecular formula is $C_{27}H_{30}O_{10}$, and it possesses several pharmacological activities, such as antioxidant stress, anti-neuroinflammatory, anti-osteoporotic, and anti-cancer properties (Zhang et al., 2023b). Icariside II preconditioning exerts neuroprotective effects by activating the astrocytic Nrf2-mediated OXPHOS/NF-κB/ferroptosis axis (Gao et al., 2023). To be more specific, icariside II preconditioning greatly lowered the production of ROS, MDA, GPX4 levels, SOD2 activity, and SIRT5 activity in MCAO mice (Gao et al., 2023). Compared with the MCAO group, the icariside II precoding group increased the expression of HO-1, NQO-1, SIRT5, and GPX4 (Gao et al., 2023). Surface plasmon resonance assay investigates demonstrate that ICS II interacts with the Nrf2 protein, exhibiting an equilibrium constant (K_D) of 1.033×10^{-4} M. Furthermore, Electrophoretic mobility shift assay confirmed that ICS II preconditioning enhanced the binding of Nrf2 to ARE at 2, 4, and 24 h. A competitive assay verified that the cold probe impeded the DNA-binding activity of Nrf2, hence further substantiating the specificity of the binding. All of these results suggest that ICS II probably activates a Nrf2–ARE signaling pathway by targeting Nrf2. LC-MS analysis demonstrated that the quantities of ICS II in the brain homogenates of mice following MCAO injury were elevated compared to those in the sham group, indicating that ICS II can cross the BBB. Therefore, we state that Icariside II is a viable pharmacological candidate for stroke treatment.

Curcumin (PubChem CID: 969516) is a commonly found phenolic molecule derived from the rhizome of *Curcuma longa L.* with a chemical formula of $C_{21}H_{20}O_6$. It is known to possess strong antioxidants, anti-inflammatory, and neuroprotective properties when used in pharmaceutical applications (Zhang et al., 2018). Curcumin has been observed to have the capacity to modulate ferroptosis processes associated with cancer, tissue injury, and other diseases, as evidenced by the *in vitro* and *in vivo* findings described above (Foroutan et al., 2024). Nevertheless, the effectiveness of curcumin is significantly hindered by its undesired water solubility, inadequate oral bioavailability, inefficiency in crossing the BBB, and other physiological barriers (Tran and Tran, 2020). In the experiments of Cong Yang et al., they encapsulated curcumin in Polymer-based nanoparticles (Cur-NPS) and explored the effect of these Cur-NPs to enhance Cur delivery both *in vitro* and *in vivo* (Yang et al., 2021). The findings demonstrated that Cur-NPs effectively inhibited erastin-induced ferroptosis in HT22 murine hippocampus cells (Yang et al., 2021). However, all the indicators related to ferroptosis are cell experiments. More animal experiments should be carried out in the future. Moreover, due to the characteristics of curcumin, we believe that curcumin cannot be a candidate drug for the treatment of ICH.

(−)-Epicatechin (PubChem CID: 72276) is a naturally derived flavanol molecule, represented by the molecular formula $C_{15}H_{14}O_6$, which is abundantly present in tea, chocolate, and many botanical

drugs. The molecule has a crucial antioxidant role by binding phenolic hydroxyl groups and free radicals, hence achieving free radical scavenging activities. (−)-Epicatechin was found to have the ability to decrease ferroptosis *in vivo*, as evidenced by its reversal of the rising levels of ROS and Fe^{2+} (Chang et al., 2014). Furthermore, the levels of lipid ROS and LC3 in H9C2 cells were reduced with (−)-epicatechin treatment (Chang et al., 2014). Additionally, the processes of autophagy and ferroptosis were also mitigated in a manner that depended on the dosage, as shown *in vitro* (Chang et al., 2014). The co-cultivation of the USP14 inhibitor IU1 and (−)-epicatechin demonstrated that (−)-epicatechin controls ferroptosis via influencing the USP14-autophagy pathway (Chang et al., 2014). However, quantitative experimental methods detected the expressions of several ferroptosis-related genes, including SOD, NQO1, and MDA; these methods are inadequate for evaluating pharmacological effects if the drug is a pan-assay interfering drug.

Carthamin yellow (PubChem CID: 12305280) is derived from the *Carthamus tinctorius L.*, and its chemical formula is $C_{21}H_{22}O_{11}$. Carthamin yellow mitigated the effects of MCAO-induced ferroptosis by reducing iron and ROS accumulation, decreasing LPO, and restoring the expression levels of proteins linked to ferroptosis (Guo et al., 2021). In addition, carthamin yellow therapy suppressed the accumulation of Fe^{2+} and ROS and restored the levels of ACSL4, TFR1, GPX4, and FTH1 proteins in the brain (Guo et al., 2021). Compared with the model group, carthamin yellow group also increased SOD and GSH and decreased MDA levels (Guo et al., 2021). Carthamin yellow can cross the BBB; however, its oral bioavailability is limited, and the precise target mechanisms remain ambiguous. Further comprehensive research on the mechanism is necessary for its potential development into medication in the future.

5.3 Polyphenols

Polyphenols are formed by direct connection between hydroxyl (-OH) groups and aromatic nuclei (benzene rings or condensed benzene rings). Polyphenols are widely present in nature and can be classified into volatile phenols and non-volatile phenols based on their volatility. Polyphenols were long believed to be primarily anti-nutrients and not particularly essential to human nutrition (Rana et al., 2022). However, recent research studies have now shown that polyphenolic substances do many biologically important things, including protecting against metabolic disorders and chronic diseases and acting as an antioxidant (Ganesan and Xu, 2017). Consuming polyphenol-rich foods has been associated with a range of health benefits, including optimizing cardiometabolic health and to a lesser extent positively impacting brain functioning in humans.

Rosmarinic acid (PubChem CID: 5281792) is a water-soluble compound with the chemical formula $C_{18}H_{16}O_8$. Rosmarinic acid is a naturally occurring water-soluble phenolic acid compound characterized by its unstable properties, low lipid solubility, and limited cell membrane permeability (Noor et al., 2022). To overcome this drawback, Cui ling Jia et al. used rosmarinic acid encapsulated in liposomes (RosA-LIP) (Jia et al., 2024). The administration of RosA-LIP improved the structural defects of mitochondria and reinstated the integrity of mitochondrial cristae (Jia et al., 2024). The RosA-LIP treatment resulted in an increase in the activity of SOD and CAT, as well as the levels of Nrf2, HO-1, and GSH (Jia et al., 2024). Compared with the model group, the RosA-LIP group significantly reduced the expression of Ptgs2, ACSL4, LPCAT3, 12-Lox, MDA, and 4-HNE (Jia et al., 2024). In addition, RosA-LIP has shown the capability to specifically inhibit the expression of TfR1 in BMECs, resulting in a decrease in the uptake of iron in the brain and minimizing the occurrence of ferroptosis, which is dependent on ACSL4/LPCAT3/Lox, in the ischemic brain (Jia et al., 2024). The specific target of rosmarinic acid remains undetermined, necessitating future investigation for accurate identification.

Caffeic acid (PubChem CID: 689043) is a polyphenolic compound present in a vast array of dietary plant metabolites. Its molecular formula is $C_9H_8O_4$. In MCAO rat brain and in OGD/R-treated SK-N-SH cells *in vitro*, caffeic acid decreased the expression of TFR1 and ACSL4, and increased the synthesis of glutathione via the Nrf2 signaling pathway to inhibit ferroptosis (Li et al., 2024a). Application of ML385, an Nrf2 inhibitor, blocked the neuroprotective effects of caffeic acid in both *vivo* and *in vitro* models, evidenced by excessive accumulation of iron ions and inactivation of the ferroptosis defense system. This is the initial study on the investigation of caffeic acid in relation to stroke. We suggest that more research should be conducted in the future to validate the efficacy of Caffeic acid on stroke.

Salvianolic acid A (PubChem CID: 5281793) has been identified as a particularly effective agent that possesses anti-inflammatory and antioxidant properties, as well as the ability to modulate the integrity and functionality of the BBB (Liu et al., 2021). Salvianolic acid A reduced MDA and Fe^{2+} production and reversed the downregulation of GSH, XCT, and GPX4 (Shi et al., 2024). Further research shows that salvianolic acid A inhibits ferroptosis after ICH through Akt/GSK-3β/Nrf2 signaling pathway (Shi et al., 2024). A pharmacokinetic investigation demonstrated that circulatory system exposure to Salvianolic acid A was comparable between sham controls and I/R rats; however, brain exposure to Salvianolic acid A was markedly elevated in I/R rats compared to sham controls (fold change of 9.17), indicating that the increased exposure to Salvianolic acid A facilitated its neuroprotective effect (Feng et al., 2017). In conclusion, we conclude that Salvianolic A is a potential therapeutic agent for stroke treatment.

Carvacrol (PubChem CID: 10364) is a natural compound that occurs in the leaves of several plants and botanical drug including wild bergamot, thyme and pepperwort, but which is most abundant in *Origanum vulgare L.* The molecular formula of carvacrol is $C_{10}H_{14}O$. Compare with the model group, carvacrol could increase SOD, GSH-Px, and CAT in MCAO mice and reduce the expression of MDA and Fe^{2+} (Guan et al., 2019). *In vitro* experiments indicated that carvacrol significantly decreased the levels of MDA, H2AX protein expression, and hippocampal neuron impairment compared to those in the anoxia/reoxygenation group, but these effects were reversed by silencing GPx4. This study performed *in vivo* and *in vitro* experiments to illustrate the protective impact of carvacrol on stroke; nonetheless, there is an insufficient amount of research on carvacrol in the context of stroke, necessitating further pharmacological and mechanistic investigations in the future.

Resveratrol (PubChem CID: 445154), a nonflavonoid polyphenol molecule, possesses potent anti-inflammatory, anti-

OS, and anti-apoptotic properties. Pretreatment with resveratrol can effectively prevent iron overload, enhance neuronal survival, increase levels of GSH, reduce levels of ROS, decrease the expression of ACSL4 protein, increase the expression of Ferritin and GPX4 proteins, and mitigate damage to mitochondrial structure following OGD/R injury *in vitro* (Zhu et al., 2022a). Additionally, resveratrol pretreatment may improve the rate of neuronal survival *in vitro* and lessen the effects of erastin and RSL3-induced ferroptosis (Zhu et al., 2022a). Resveratrol has been the subject of extensive research on stroke, but the bioavailability is only 1%. Future research should address this drawback. To sum up, we believe that resveratrol is a candidate drug for the treatment of stroke.

Rhein (PubChem CID: 10168) is the primary plant metabolite in several traditional Chinese herbal medicine. It has a variety of pharmacological properties, including antioxidant, antitumor, antifibrosis, and anti-inflammation properties (Wu et al., 2020). The molecular formula of a is $C_{15}H_8O_6$. Rhein effectively inhibited OS, intracellular ROS production, and protein expression associated to ferroptosis in both *in vivo* and *in vitro* MCAO models. Mechanistically, rhein counteracted OGD/R-induced damage in HT22 cells by modulating the NRF2/SLC7A11/GPX4 signaling pathway (Liu et al., 2023d). At present, there are relatively few studies on stroke from rhein, and the liver and kidney toxicity of rhein is also very high. More studies should be carried out in the future. We don't think this is a promising candidate drug.

5.4 Alkaloids

Alkaloids are a kind of nitrogen-containing alkaline organic compounds that exist in nature (mainly plants, but some also exist in animals). Most of them have complex ring structures. The ring primarily contains nitrogen, which exhibits significant biological activity. It is one of the important effective plant metabolites in Chinese herbal medicine. Alkaloids are an important plant metabolite of natural products with structural diversity. Currently, researchers have reported over 60 types of alkaloids.

Berberine (PubChem CID: 2353), a bioactive alkaloid extracted from many herbal plant species, has several pharmacological properties such as antibacterial, antidiabetic, and anticancer actions (Song et al., 2020). Berberine mitigates MCAO-induced ferroptosis, as evidenced by the upregulated expression of SLC7A11 but the reduced expression of ACSL4, TFR1, and COX2 (Wang et al., 2023c). Numerous pharmacological investigations on Berberine currently exist, and it is utilized in therapeutic practice. Consequently, we believe that Berberine is an excellent candidate for medication for the treatment of stroke.

Dauricine (PubChem CID: 73400) the molecular formula is $C_{38}H_{44}N_2O_6$. It was demonstrated in the ferroptosis model of SH-SY5Y cells that the increase of GPX4 expression by dauricine suppressed ferroptosis in SH-SY5Y cells caused by RSL3 and enhanced cell survival (Peng et al., 2022). In the C57 mouse ICH model, it was demonstrated that dauricine increased the expression of GPX4 (Peng et al., 2022). This experiment validated the therapeutic effect of dauricine on stroke both *in vivo* and *in vitro*; nonetheless, there is a deficiency of data about dauricine's impact on stroke, necessitating further pharmacological and mechanistic investigations in the future.

5.5 Traditional Chinese medicine formulations

Angong Niuhuang pill (AGNHP) originated from *Wenbing Tiaobian* written by the febrile disease expert Wu Jutong in the Qing Dynasty. *In vivo* experiments indicated that AGNHP inhibits ROS, LPO, and Fe^{2+} accumulations in MCAO and ICH rats (Bai et al., 2024). Evidence from *in vitro* experiments shown that AGNHP mitigated ferroptosis damage caused by erastin in PC12 cells, enhanced cell survival, decreased LPO and Fe^{2+} concentrations, and boosted mRNA expressions of PPARγ, AKT, and GPX4 (Bai et al., 2024). Subsequent investigation demonstrates that AGNHP reduced the damage caused by MCAO and ICH by inhibiting ferroptosis through the activation of the PPARγ/ATK/GPX4 pathway (Bai et al., 2024).

Di-Huang-Yin-Zi (DHYZ) is a traditional herbal medicine employed for the prevention and treatment of neurological disorders since the Song Dynasty. The treatment with DHYZ reduced levels of ROS and MDA, thereby suppressing the expression of markers associated to ferroptosis, including Fe^{2+}, SLC7A11, and GPX4 (Yang et al., 202b). The mechanism by which DHYZ reduces symptoms and improves the functional capacity of rats with poststroke depression is mostly through the suppression of ferroptosis via the P53/SLC7A11/GPX4 pathway (Yang et al., 2024b).

Naodesheng pills (NDSP) are commonly prescribed traditional Chinese medicines consisting of *Panax notoginseng* (Burk.) F. H. Chen, *C. tinctorius L.*, *Crataegus pinnatifida* Bunge and *Puerariae Lobatae Radix*, and *Ligusticum chuanxiong* Hort. Both *in vivo* and *in vitro* experiments have shown that NDSP can increase the expression of GPX4, SLC7A11, and SOD while inhibiting the expression of MDA, TFR1, DMT1, ROS, and Fe^{2+} (Yang et al., 2024b). This mechanism is associated with the regulation of ferroptosis via the ERK1/2 signaling pathway (Yang et al., 2024b).

Naotai formula (NTF) is composed of *A. membranaceus*, *Rhizoma Chuanxiong*, *Lumbricus* and *Bombyx Batryticatus*. NTF could prevent MCAO-induced neuronal ferroptosis in rats by increasing the levels of SCL7A11, GPX4, and GSH (Lan et al., 2020). Hepcidin, BMP6, and SMADs levels were also lowered by NTF treatment, while SLC40A1 and GPX4 levels were raised (Liao et al., 2023).

Tongluo Decoction (TLD), a traditional Chinese medicine prescription, has been extensively employed for the management of ischemic stroke. TLD reduced MDA, ROS and Fe^{2+} related activity and increased SOD levels (Li et al., 2024b). Compared with the MCAO group, TLD treatment significantly upregulated Nrf2, SLC7A11, FTH1, GPX4 and HO-1 levels (Li et al., 2024b). Mechanistically, TLD rescued endoplasmic reticulum stress and ferroptosis but promoted Sonic Hedgehog signaling in rats with MCAO (Hui et al., 2022).

Xingnaojing (XNJ) is a traditional Chinese medicinal agent used clinically for treating stroke. XNJ has been approved by the Chinese National Drug Administration. XNJ reportedly increases BBB permeability, lowers inflammation, and boosts circulation (Qu et al., 2019). Compared with the MCAO group, XNJ can reduce the expression of COX-2, TFR, and DMT1 and increase the expression of GPX4, FPN, and HO-1 (Liu et al., 2023e). Moreover, XNJ increased GPX4 levels and inhibited COX-2 and TFR protein expression after SH-SY5Y cell hypoxia (Liu et al., 2023e).

Tongqiao Huoxue Decoction (TQHX) mainly composed of eight botanical drugs, including *Paeoniae Radix Rubra*, *Rhizoma Chuanxiong*, *Prunus persica (L.)* Batsch, *Ziziphus jujuba Mill.*, *Zingiberis Rhizoma Recens*. TQHX is employed to improve blood circulation and eradicate blood stasis. In comparison to the MCAO group, the TQHX treatment effectively reduced the levels of MDA, Fe^{2+}, ROS and ACSL4 while simultaneously increasing the expression of SOD, FTH1, and GPX4 (Ou et al., 2024).

***Salvia miltiorrhiza* (SM)** has a long history of application in China. It was first recorded in the Shennong Herbal's classic of the Eastern Han Dynasty. SM is a commonly employed treatment for vascular diseases, particularly ischemic cardiovascular diseases. The levels of 4-HNE, ACLS4 and MDA in the penumbra of the MCAO mouse brain could be reduced by SM, which was induced by OS upregulation (Ko et al., 2023). In MCAO mice, SM treatment upregulated GPX4 and GSH and reduced FPN1 and ferritin (Ko et al., 2023).

Paeoniae Radix Rubra (PRR), the root of *Paeonia lactiflora Pall.* or *Paeonia veitchii Lynch*, is extensively used in Chinese clinical practice to enhanjce blood circulation and alleviate blood stagnation. An *in vivo* study revealed that PRR enhanced the expression of GPX4, FTH1, Beclin1, LC3 II, and p-Akt in the rat of MCAO (Zhao et al., 2023b). Additionally, the *in vitro* study showed that PRR can reduce H_2O_2-induced HT22 cell damage by regulating cytokines like MDA, reduced GSH, and ROS, as well as by elevating the expressions of GPX4 and Beclin1 (Zhao et al., 2023b).

Danhong injection (DHI), a standardized injection that contains *S. miltiorrhiza* Bunge and *C. tinctorius L.*, is frequently employed to treat cerebrovascular and cardiovascular diseases. Compared with the model group, DHI increased the expression of SOD, GSH, and SATB1 and decreased the expression of MDA, TfR1, and TF (Zhan et al., 2023). Mechanism research shows that DHI inhibitors ferroptosis in ischemic stroke by regulating the SATB1/SLC7A11/HO-1 pathway (Zhan et al., 2023).

Danlou tablet (DLT) is composed of *Pericarpium Trichosanthis*, *Allium macrostemon* Bunge, *Puerariae Lobatae Radix*, *L. chuanxiong Hort.*, *S. miltiorrhiza Bunge* [Lamiaceae; Salviae miltiorrhizae radix et rhizoma], *Paeoniae Radix Rubra*, *Alisma plantago-aquatica Linn.*, *A. membranaceus, Drynariae Rhizoma* and *Curcumae Radix*. DLT promotes blood circulation, resolves phlegm and stasis, alleviates stagnation, and dispels congestion. Both *vivo* and *vitro* studies showed that DLT markedly decreased the level of COX2 protein, GSSG, and MDA, while simultaneously increasing the levels of SLC7A11, GPX4, and GSH (Liu et al., 2024).

Salvia miltiorrhiza Bge processed with Porcine cardiac blood (PCB-DS) aims to enhance the brain-targeted therapy of DS through the BBB. DS shows great potential as a Chinese herbal medicine for the treatment of vascular disorders, particularly cerebrovascular disorders. PCB is a characteristic of Chinese herbal medicine, and a pharmacological reference frequently used for the treatment of brain disorders. Prior research demonstrated that PCB-DS mitigated MCAO by downregulating OS, which included reducing intracellular ROS and MDA levels (Zhou et al., 2023b). In both *vivo* and *in vitro* studies, PCB-DS modulated Fe^{2+} levels, GSH, MDA, SOD, ROS, and liperfluo to inhibit ferroptosis (Zhou et al., 202a). Further studies reveal that PCB-DS directly activated GLRX5, therefore reducing the iron-starvation response, and downregulated the SLC7A11/GPX4 signaling pathway to prevent ferroptosis (Zhou et al., 2024a).

5.6 Polysaccharide

Polysaccharides are essential plant metabolites of the cell wall of microorganisms, the membrane of animal cells, and higher plants. It is also closely associated with physiological functions. In recent years, there has been a growing focus on polysaccharides as a significant category of bioactive natural products. Various studies have illustrated the bioactivities of natural polysaccharides, which have resulted in their use in the treatment of various diseases.

Neutral polysaccharide (NPGE) is a metabolite extracted from the plant that exhibits immunomodulatory, neuroprotective, and antioxidant properties. In contrast to the MCAO group, the NPGE treatment alleviated neuronal ferroptosis by increasing GPX4 levels, decreasing ROS, MDA, and Fe^{2+} excessive accumulation, and enhancing GSH levels and SOD enzymatic activity (Zhang et al., 2024). Further mechanistic research demonstrates that NPGE reduces cerebral IR injury by inhibiting ferroptosis-mediated neuroinflammation through the NRF2/HO-1 signaling pathway (Zhang et al., 2024). The pharmacodynamic experiments of NPGE were conducted very comprehensively. In the future, small molecule and protein interaction experiments should be carried out to further confirm the target of NPGE.

Roots of *Astragalus propinquus* Schischkin (RAP) can raise yang qi and tonifying the spleen and lung qi, thereby facilitating urination and reducing edema. Compared with the MCAO group, RAP could increase the expression of XCT, SLC3A2, IREB2, Nrf2, HO-1, and GPX4 (Chen et al., 2022a). Both granules and injections have the same effect, but granules have the best effect. The Marker for ferroptosis in the article is too simple, with Western blot experiments being the majority. Other experiments (such as immunofluorescence) should be carried out to prove the therapeutic effect of RAP on stroke from multiple perspectives.

5.7 Other compounds

Cottonseed oil (CSO) is a vegetable oil frequently intended for the dissolution of lipid-soluble medications. Prior studies have demonstrated that CSO provides protection against intestinal inflammation, tumor metastasis, OS, and atherosclerosis (Araujo et al., 2019). Notably, current research has demonstrated its effectiveness in treating disorders of the nervous system (Sun et al., 2023). Compared to the MCAO group, CSO reduced the influx of Fe^{2+}, TF, and TF receptors; upregulated anti-ferroptosis proteins (GPX4, xCT, HO1, and FTH1) while down-regulating ferroptosis-related protein ACSL4; raised GSH and SOD activity; and decreased MDA and LPO levels (Sun et al., 2023). Nevertheless, an excessively small sample size may result in erroneous conclusions. Consequently, we suggest that CSO is not an optimal candidate medication for the treatment of stroke. Nevertheless, an excessively small sample size may result in erroneous conclusions. Consequently, we suggest that CSO is not an optimal candidate medication for the treatment of stroke.

Mastoparan M (MM) is a peptide derived from bee venom that has biological activity and mainly affects intracellular signal transduction by acting on the cell membrane. After receiving MM therapy for 24 or 48 h, the ischemic hemisphere of MCAO mice showed a decrease in Fe^{2+} and MDA levels, but a rise in the expression of the proteins LC3B, x-CT, NRF2, and GPX4 (Sun et al., 2023). In addition, these results were confirmed in three models: OGD/R, peroxidation mediated by H_2O_2, and ferroptosis triggered by erastin (Sun et al., 2023). However, MM has conducted relatively few studies on stroke, and more fundamental research needs to be carried out in the future.

Melatonin is one of the hormones secreted by the pineal gland, which is an amine hormone produced by the pineal gland. Within the HT-22 cell model, melatonin enhanced cell proliferative capacity, decreased apoptosis, and lowered ROS generation (Sun et al., 2024b). Melatonin's protective benefits are mediated by its inhibition of ferroptosis, an iron-dependent form of controlled cell death, via the regulation of the ACSL4/CYP1B1 pathway, according to further mechanistic investigations (Sun et al., 2024b). Melatonin lower LPO, ROS generation, and ACSL4 protein expression in MCAO mice (Sun et al., 2024b). Another study found that melatonin regulates ACSl4 ubiquitination and impacts ferroptosis by increasing MDM2 expression, which contributes to its therapeutic efficacy in stroke treatment (Ji et al., 2024). Melatonin is an over-the-counter medication in the United States and Canada, and a dietary supplement in mainland China; hence, it is not regarded as a viable pharmaceutical option for stroke treatment.

Dl-3-n-butylphthalide (PubChem CID: 61361, DL-NBP) is a synthetic compound extracted from celery seeds. The effectiveness and safety of the treatment have been assessed in multiple clinical trials conducted in China (Fan et al., 2021). Compared with the MCAO group, DL-NBP could significantly reduce the levels of MDA, ROS, and Fe^{2+} and increase the expression of GSH, GPx4, and SLC7A11 (Xu et al., 2023b). Further research into the mechanism revealed that DL-NBP presumably acts as a mediator in the SLC7A11/GSH/GPX4 signaling pathway to mitigate ferroptosis (Xu et al., 2023b). There have been many pharmacological studies on DL-NBP, and DL-NBP has also been applied in clinical practice. Therefore, we believe that DL-NBP is an effective candidate drug for the treatment of stroke.

Taurine (PubChem CID: 1123) is an amino acid converted from sulfur-containing amino acids that is widely distributed in various tissues and organs in the body. It mainly exists in the interstitial fluid and intracellular fluid in a free state. Its molecular formula is $C_2H_7NO_3S$. The current investigation revealed a significant reduction in taurine levels in cerebrospinal fluid among patients with SAH (Liu et al., 2022b). This finding implies that administering taurine treatment after SAH may enhance neurological impairment, reduce OS, regulate iron accumulation, maintain BBB integrity, and prevent neuronal ferroptosis in the SAH model *in vivo* (Liu et al., 2022b). Thorough investigations suggest that taurine may modulate MDA levels and ROS accumulation, as well as control the expression of SLC7A11 and GPX4, and the AKT/GSK3β pathway *in vitro* (Liu et al., 2022b). Nonetheless, there exists an absence of research about the treatment of stroke by taurine. Future research should focus on more mechanism and efficacy investigations. Consequently, we do not regard taurine as a viable candidate medication for the treatment of stroke.

Crocin (PubChem CID: 5281233) is a hydrophilic carotenoid that is synthesized in the *Crocus sativus L*. Compared to the ICH group, the crocin-treated group significantly increased the activities of SOD and GSH-px (Wang et al., 2022b). On the other hand, crocin therapy significantly reduced the concentration of MDA (Wang et al., 2022b). The observed elevation in Fe^{2+} concentration and the upregulation of GPX4, FTH1, and SLC7A11 genes demonstrated the inhibition of ferroptosis in neuron cells by crocin (Wang et al., 2022b). Mechanistically, crocin alleviates ICH-induced neuronal ferroptosis by facilitating Nrf2 nuclear translocation (Wang et al., 2022b). While there are limited studies on the treatment of ICH by crocin, pertinent research exists regarding the treatment of ischemic stroke by crocin (Shahbaz et al., 2022). Future efficacy studies should be conducted to validate the treatment of ICH by crocin.

6 Clinical translation discussion

Compared with the previous review published by Zhou et al., we have summarized the mechanism of ferroptosis in stroke and summarized its natural products, rather than only focusing on ischemic stroke (Wang et al., 2024b). Another review systematically summarizes the status and progress of TCM in regulating various CNS diseases through the ferroptosis pathway (Zhou et al., 2024b). Its emphasis is on TCM rather than natural products. This article assesses traditional Chinese medical treatment methods, including Chinese herbal medicine, acupuncture, and moxibustion, as well as their mechanisms of action in regulating the ferroptosis pathway (Zhou et al., 2024b). In this review, we systematically summarized the mechanisms and targets of ferroptosis after stroke and summarized the related natural products (Figure 5). We aimed to provide insights into more feasible treatment schemes based on the currently proven effective natural products and Chinese medicine. Although natural products perform well as anti-ferroptosis in stroke animal experiments, there are few clinical applications. Thus, we summarized the clinical trial of the 56 natural products aimed at stroke treatment (Table 4, www.clinicaltrials.gov.). We discovered that clinical trials on stroke only used 7 natural products (Table 4).

From the translation of preclinical evaluation to clinical trial, model animals for stroke are relatively simple and cannot fully represent the more complex pathological features of human beings. This phenomenon may have multiple causes: 1. Regarding natural active plant metabolites, while the pharmacodynamic effects and targets are rather well-defined, research on their remains is inadequate. The study on the *in vivo* processes (absorption, distribution, metabolism, excretion, toxicokinetic) and safety assessment is insufficient, prolonging the research and development cycle of new pharmaceuticals from laboratory to clinical use; 2. The difficulty of standardizing traditional Chinese medicine. The investigations on herbal extracts and formulations reveal a complex composition, resulting in challenges in target identification, and the mechanisms involved may not be readily or thoroughly clarified. Moreover, the many technologies employed in the manufacturing of herbal extracts and formulations pose a challenge to the quality control of their active plant metabolites. 3. Disease model. Animal models struggle to accurately replicate the pathogenesis of patients, a fundamental issue that hinders the development of laboratory drugs.

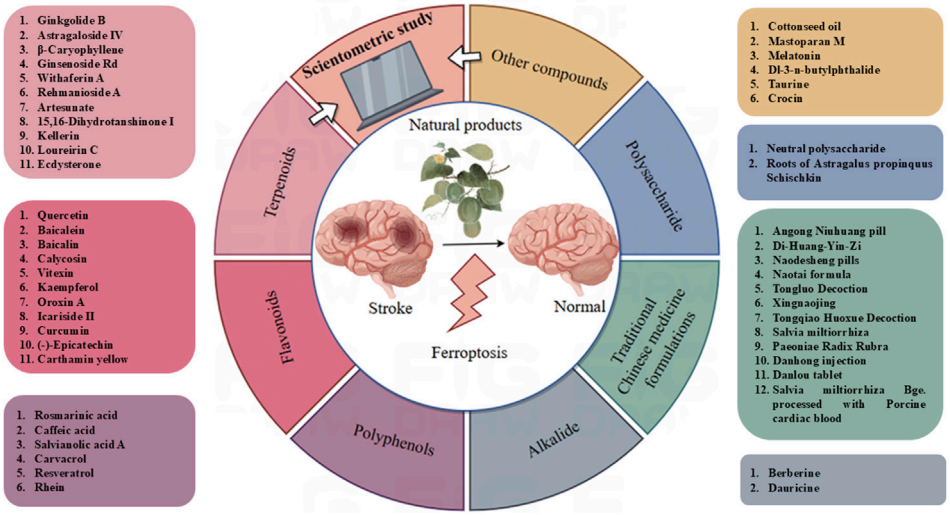


FIGURE 5
Application of natural products and herbal medicine in ferroptosis after stroke.

TABLE 4 Clinical trials on the treatment of stroke using the above natural products.

Name	Dose	Sample size	Trial phase	Clinical trial Identifier
Ginsenoside Rd	10, 20 mg/14 days	199	Completed	NCT00591084
	20 mg/14 days	390	Completed	NCT00815763
Quercetin	—	106	Completed	NCT01376011
Rhein	—	12,000	Recruiting	NCT03157934
Salvia miltiorrhiza		46	Completed	NCT02176395
		1,900	Observational	NCT04359589
		2,200	Completed	NCT02334969
		1,503	Completed	NCT01677208
Danhong Injection	40 mL, 10 days	320	Unknown status	NCT02152280
		46	Completed	NCT02176395
		1,503	Completed	NCT01677208
Melatonin	14 mg, 90 days	140	Unknown status	NCT01863277
	10 mg, 90 days	100	Recruiting	NCT05857046
	3 mg, 14 days	80	Recruiting	NCT05247125
Dl-3-n-butylphthalide	5–10 mg, 24weeks	3,200	Recruiting	NCT05976152
	100 mL, 10 days	84	Completed	NCT02149875

Enhanced exploitation of natural products for illness treatment necessitates the development of specific methods and feasible methodologies. 1. Natural products with well-defined targets can improve mechanistic comprehension in fundamental research and promote their use in clinical applications. Biotechnological techniques, such as the cellular thermal shift assay (CETSA) or target-responsive accessibility profiling (TRAP) can be employed to identify the targets of various natural products. 2. The *in vivo* pharmacokinetic and pharmacological properties should be investigated (Tu et al., 2023). 3. Through pre-clinical research or illness prediction models, the dosage of a particular natural substance must correspond with the optimal type, stage, and timing of the disease. 4. Certain natural products encounter obstacles such as restricted water solubility, diminished bioavailability, or insufficient stability. Structural alteration, nano-delivery, or co-administration may be utilized to enhance efficacy. Utilizing multi-omics and artificial intelligence facilitates a more thorough exploration of the mechanisms behind natural products (Zhu et al., 2022b).

Preclinical models may be utilized to evaluate the efficiency of prospective therapies or procedures, acknowledging that the significance of harm and protective pathways, along with the administration of therapy, may vary between the preclinical model and people. Preclinical models can yield critical insights into the pharmacokinetics and pharmacodynamics of prospective treatments, along with potential toxicity. Critical factors must be evaluated when determining whether to forward a medication or technique from preclinical testing in stroke models to clinical trials. Effective translation necessitates diverse expertise, including basic scientists, clinical trialists, neuroimaging specialists, neurosurgeons, and rehabilitation specialists. Clinically significant outcome metrics are essential in preclinical modelling, while recognizing the intrinsic limitations of these models. Conversely, the prospective influence of a target or outcome measure within a model (together with its constraints) must be conveyed to those implementing it in clinical trials. Preliminary collaboration throughout the translational pipeline is expected to produce more resilient preclinical modelling and clinical trial design. At present, neurological impairments serve as the principal outcome measurement to assess preclinical efficacy in clinical trials. Nonetheless, possessing supplementary biomarkers or surrogate measures (e.g., imaging, inflammatory markers, brain oedema, and atrophy), particularly in relation to specific therapeutic target engagement, can be highly beneficial for comparative analysis across species and in clinical trials.

Currently, research on ferroptosis concentrates on four primary aspects: morphological feature assessment, gene expression analysis, protein level evaluation, and biochemical characteristic index measurement. Thus, the absence of definitive and authoritative standards for ferroptosis detection has emerged as a significant concern in preclinical and clinical research. Finally, both the development of natural drugs or traditional Chinese medicine and the exploration of the mechanism of stroke disease need a long way to go.

7 Conclusion

In this review, we summarize the natural products and herbal medicine currently employed in ferroptosis along with their mechanisms of action, highlighting the potential and challenges of clinical translation. This review is to accelerate the development of novel natural and herbal medicine treatments and to offer novel perspectives on how to treat ferroptosis in stroke.

Author contributions

YZ: Conceptualization, Supervision, Writing – original draft, Writing – review and editing. XT: Conceptualization, Investigation, Supervision, Writing – original draft. XW: Conceptualization, Supervision, Writing – review and editing. RM: Conceptualization, Investigation, Supervision, Writing – review and editing. JG: Funding acquisition, Supervision, Writing – review and editing.

Funding

The author(s) declare that financial support was received for the research and/or publication of this article. This work was supported by the National Natural Science Foundation of China (No. 8247447), National Science and Technology Major Project (No. 2023ZD0505600), State Key Laboratory of Dampness Syndrome of Traditional Chinese Medicine (No. SZ2021ZZ06), State Key Laboratory of Traditional Chinese Medicine Syndrome Projects (Grant No. SKLKY2025C0003) and Guangzhou Municipal Science and Technology Bureau (No. 2024B01J1352).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declare that no Generative AI was used in the creation of this manuscript.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

Alvarez, S. W., Sviderskiy, V. O., Terzi, E. M., Papagiannakopoulos, T., Moreira, A. L., Adams, S., et al. (2017). NFS1 undergoes positive selection in lung tumours and protects cells from ferroptosis. *Nature* 551 (7682), 639–643. doi:10.1038/nature24637

Araujo, R. S., Oliveira, A. C., Sousa, F. C. B., Dourado, L. R. B., Guimarães, S. E. F., Silva, W., et al. (2019). Effects of cottonseed oil and ferrous sulfate on the performance and expression of antioxidant enzymes in broilers. *Poult. Sci.* 98 (9), 3860–3869. doi:10.3382/ps/pez103

Bai, X., Zheng, E., Tong, L., Liu, Y., Li, X., Yang, H., et al. (2024). Angong Niuhuang Wan inhibit ferroptosis on ischemic and hemorrhagic stroke by activating PPARγ/AKT/GPX4 pathway. *J. Ethnopharmacol.* 321, 117438. doi:10.1016/j.jep.2023.117438

Bai, Y., Meng, L., Han, L., Jia, Y., Zhao, Y., Gao, H., et al. (2019). Lipid storage and lipophagy regulates ferroptosis. *Biochem. Biophys. Res. Commun.* 508 (4), 997–1003. doi:10.1016/j.bbrc.2018.12.039

Bao, W. D., Zhou, X. T., Zhou, L. T., Wang, F., Yin, X., Lu, Y., et al. (2020). Targeting miR-124/Ferroportin signaling ameliorated neuronal cell death through inhibiting apoptosis and ferroptosis in aged intracerebral hemorrhage murine model. *Aging Cell* 19 (11), e13235. doi:10.1111/acel.13235

Bu, Z. Q., Yu, H. Y., Wang, J., He, X., Cui, Y. R., Feng, J. C., et al. (2021). Emerging role of ferroptosis in the pathogenesis of ischemic stroke: a new therapeutic target? *ASN Neuro* 13, 17590914211037505. doi:10.1177/17590914211037505

Chang, C. F., Cho, S., and Wang, J. (2014). (-)-Epicatechin protects hemorrhagic brain via synergistic Nrf2 pathways. *Ann. Clin. Transl. Neurol.* 1 (4), 258–271. doi:10.1002/acn3.54

Chen, B., Wang, H., Lv, C., Mao, C., and Cui, Y. (2021a). Long non-coding RNA H19 protects against intracerebral hemorrhage injuries via regulating microRNA-106b-5p/acyl-CoA synthetase long chain family member 4 axis. *Bioengineered* 12 (1), 4004–4015. doi:10.1080/21655979.2021.1951070

Chen, G., Chen, X., Niu, C., Huang, X., An, N., Sun, J., et al. (2018). Baicalin alleviates hyperglycemia-induced endothelial impairment 1 via Nrf2. *J. Endocrinol.* doi:10.1530/JOE-18-0457

Chen, H., Ren, L., and Ma, W. (2023). Mechanism of SOX10 in ferroptosis of hippocampal neurons after intracerebral hemorrhage via the miR-29a-3p/ACSL4 axis. *J. Neurophysiol.* 129 (4), 862–871. doi:10.1152/jn.00374.2022

Chen, J., Ma, D., Bao, J., Zhang, Y., and Deng, G. (2022a). Roots of Astragalus propinquus Schischkin regulate transmembrane iron transport and ferroptosis to improve cerebral ischemia-reperfusion injury. *Evid. Based Complement. Altern. Med.* 2022, 7410865. doi:10.1155/2022/7410865

Chen, J., Ou, Z., Gao, T., Yang, Y., Shu, A., Xu, H., et al. (2022b). Ginkgolide B alleviates oxidative stress and ferroptosis by inhibiting GPX4 ubiquitination to improve diabetic nephropathy. *Biomed. Pharmacother.* 156, 113953. doi:10.1016/j.biopha.2022.113953

Chen, J., Shi, Z., Zhang, C., Xiong, K., Zhao, W., and Wang, Y. (2024). Oroxin A alleviates early brain injury after subarachnoid hemorrhage by regulating ferroptosis and neuroinflammation. *J. Neuroinflammation* 21 (1), 116. doi:10.1186/s12974-024-03099-3

Chen, J., Wang, Y., Wu, J., Yang, J., Li, M., and Chen, Q. (2020). The potential value of targeting ferroptosis in early brain injury after acute CNS disease. *Front. Mol. Neurosci.* 13, 110. doi:10.3389/fnmol.2020.00110

Chen, L., Liu, J., Mei, G., Chen, H., Peng, S., Zhao, Y., et al. (2021c). Quercetin and non-alcoholic fatty liver disease: a review based on experimental data and bioinformatic analysis. *Food Chem. Toxicol.* 154, 112314. doi:10.1016/j.fct.2021.112314

Chen, W., Jiang, L., Hu, Y., Tang, N., Liang, N., Li, X. F., et al. (2021b). Ferritin reduction is essential for cerebral ischemia-induced hippocampal neuronal death through p53/SLC7A11-mediated ferroptosis. *Brain Res.* 1752, 147216. doi:10.1016/j.brainres.2020.147216

Chen, Y.-Y., Liu, Q.-P., An, P., Jia, M., Luan, X., and Tang, J.-Y. (2022c). A promising natural neuroprotective agent. *Phytomedicine* 95, 153883. doi:10.1016/j.phymed.2021.153883

DeGregorio-Rocasolano, N., Martí-Sistac, O., Ponce, J., Castelló-Ruiz, M., Millán, M., Guirao, V., et al. (2018). Iron-loaded transferrin (Tf) is detrimental whereas iron-free Tf confers protection against brain ischemia by modifying blood Tf saturation and subsequent neuronal damage. *Redox Biol.* 15, 143–158. doi:10.1016/j.redox.2017.11.026

Deng, M., Chen, H., Long, J., Song, J., Xie, L., and Li, X. (2021). Calycosin: a review of its pharmacological effects and application prospects. *Expert Rev. Anti Infect. Ther.* 19 (7), 911–925. doi:10.1080/14787210.2021.1863145

Dietrich, R. B., and Bradley, W. G., Jr. (1988). Iron accumulation in the basal ganglia following severe ischemic-anoxic insults in children. *Radiology* 168 (1), 203–206. doi:10.1148/radiology.168.1.3380958

Dixon, S. J., Lemberg, K. M., Lamprecht, M. R., Skouta, R., Zaitsev, E. M., Gleason, C. E., et al. (2012). Ferroptosis: an iron-dependent form of nonapoptotic cell death. *Cell* 149 (5), 1060–1072. doi:10.1016/j.cell.2012.03.042

Duan, C., Jiao, D., Wang, H., Wu, Q., Men, W., Yan, H., et al. (2022). Activation of the PPARγ prevents ferroptosis-induced neuronal loss in response to intracerebral hemorrhage through synergistic actions with the Nrf2. *Front. Pharmacol.* 13, 869300. doi:10.3389/fphar.2022.869300

Duan, L., Zhang, Y., Yang, Y., Su, S., Zhou, L., Lo, P. C., et al. (2021). Baicalin inhibits ferroptosis in intracerebral hemorrhage. *Front. Pharmacol.* 12, 629379. doi:10.3389/fphar.2021.629379

Fan, X., Shen, W., Wang, L., and Zhang, Y. (2021). Efficacy and safety of DL-3-n-butylphthalide in the treatment of poststroke cognitive impairment: a systematic review and meta-analysis. *Front. Pharmacol.* 12, 810297. doi:10.3389/fphar.2021.810297

Fang, Y., Chen, X., Tan, Q., Zhou, H., Xu, J., and Gu, Q. (2021). Inhibiting ferroptosis through disrupting the NCOA4-FTH1 interaction: a new mechanism of action. *ACS Cent. Sci.* 7 (6), 980–989. doi:10.1021/acscentsci.0c01592

Feng, S. Q., Aa, N., Geng, J. L., Huang, J. Q., Sun, R. B., Ge, C., et al. (2017). Pharmacokinetic and metabolomic analyses of the neuroprotective effects of salvianolic acid A in a rat ischemic stroke model. *Acta Pharmacol. Sin.* 38 (11), 1435–1444. doi:10.1038/aps.2017.114

Foroutan, Z., Butler, A. E., Zengin, G., and Sahebkar, A. (2024). Curcumin and ferroptosis: a promising target for disease prevention and treatment. *Cell Biochem. Biophys.* 82 (2), 343–349. doi:10.1007/s12013-023-01212-6

Fu, C., Wu, Y., Liu, S., Luo, C., Lu, Y., Liu, M., et al. (2022). Rehmannioside A improves cognitive impairment and alleviates ferroptosis via activating PI3K/AKT/Nrf2 and SLC7A11/GPX4 signaling pathway after ischemia. *J. Ethnopharmacol.* 289, 115021. doi:10.1016/j.jep.2022.115021

Ganesan, K., and Xu, B. (2017). A critical review on polyphenols and health benefits of black soybeans. *Nutrients* 9 (5), 455. doi:10.3390/nu9050455

Gao, J., Ma, C., Xia, D., Chen, N., Zhang, J., and Xu, F. (2023). Icariside II preconditioning evokes robust neuroprotection against ischaemic stroke, by targeting Nrf2 and the OXPHOS/NF-κB/ferroptosis pathway. *Br. J. Pharmacol.* 180 (3), 308–329. doi:10.1111/bph.15961

Gao, M., Monian, P., Quadri, N., Ramasamy, R., and Jiang, X. (2015). Glutaminolysis and transferrin regulate ferroptosis. *Mol. Cell* 59 (2), 298–308. doi:10.1016/j.molcel.2015.06.011

Gao, M., Yi, J., Zhu, J., Minikes, A. M., Monian, P., Thompson, C. B., et al. (2019). Role of mitochondria in ferroptosis. *Mol. Cell* 73 (2), 354–363. doi:10.1016/j.molcel.2018.10.042

GBD 2021 Diseases and Injuries Collaborators (2024). Global incidence, prevalence, years lived with disability (YLDs), disability-adjusted life-years (DALYs), and healthy life expectancy (HALE) for 371 diseases and injuries in 204 countries and territories and 811 subnational locations, 1990-2021: a systematic analysis for the Global Burden of Disease Study 2021. *Lancet* 403 (10440), 2133–2161. doi:10.1016/S0140-6736(24)00757-8

Guan, X., Li, X., Yang, X., Yan, J., Shi, P., Ba, L., et al. (2019). The neuroprotective effects of carvacrol on ischemia/reperfusion-induced hippocampal neuronal impairment by ferroptosis mitigation. *Life Sci.* 235, 116795. doi:10.1016/j.lfs.2019.116795

Guo, H., Zhu, L., Tang, P., Chen, D., Li, Y., Li, J., et al. (2021). Carthamin yellow improves cerebral ischemia-reperfusion injury by attenuating inflammation and ferroptosis in rats. *Int. J. Mol. Med.* 47 (4), 52. doi:10.3892/ijmm.2021.4885

Guo, L., and Shi, L. (2023). Vitexin improves cerebral ischemia-reperfusion injury by attenuating oxidative injury and ferroptosis via keap1/nrf2/HO-1signaling. *Neurochem. Res.* 48 (3), 980–995. doi:10.1007/s11064-022-03829-0

Hilkens, N. A., Casolla, B., Leung, T. W., and de Leeuw, F. E. (2024). Stroke. *Lancet* 403 (10446), 2820–2836. doi:10.1016/S0140-6736(24)00642-1

Hou, W., Xie, Y., Song, X., Sun, X., Lotze, M. T., Zeh, H. J., et al. (2016). Autophagy promotes ferroptosis by degradation of ferritin. *Autophagy* 12 (8), 1425–1428. doi:10.1080/15548627.2016.1187366

Hu, Q., Zuo, T., Deng, L., Chen, S., Yu, W., Liu, S., et al. (2022). β-Caryophyllene suppresses ferroptosis induced by cerebral ischemia reperfusion via activation of the NRF2/HO-1 signaling pathway in MCAO/R rats. *Phytomedicine* 102, 154112. doi:10.1016/j.phymed.2022.154112

Hu, S., Fei, Y., Jin, C., Yao, J., Ding, H., Wang, J., et al. (2024). Ginsenoside Rd enhances blood-brain barrier integrity after cerebral ischemia/reperfusion by alleviating endothelial cells ferroptosis via activation of NRG1/ErbB4-mediated PI3K/Akt/mTOR signaling pathway. *Neuropharmacology* 251, 109929. doi:10.1016/j.neuropharm.2024.109929

Huang, Y., Liu, J., He, J., Hu, Z., Tan, F., Zhu, X., et al. (2022). UBIAD1 alleviates ferroptotic neuronal death by enhancing antioxidative capacity by cooperatively restoring impaired mitochondria and Golgi apparatus upon cerebral ischemic/reperfusion insult. *Cell Biosci.* 12 (1), 42. doi:10.1186/s13578-022-00776-9

Hui, Z., Wang, S., Li, J., Wang, J., and Zhang, Z. (2022). Compound Tongluo Decoction inhibits endoplasmic reticulum stress-induced ferroptosis and promoted angiogenesis by activating the Sonic Hedgehog pathway in cerebral infarction. *J. Ethnopharmacol.* 283, 114634. doi:10.1016/j.jep.2021.114634

Hvidberg, V., Maniecki, M. B., Jacobsen, C., Højrup, P., Møller, H. J., and Moestrup, S. K. (2005). Identification of the receptor scavenging hemopexin-heme complexes. *Blood* 106 (7), 2572–2579. doi:10.1182/blood-2005-03-1185

Javadinia, S. S., Abbaszadeh-Goudarzi, K., Mahdian, D., Hosseini, A., Ghalenovi, M., and Javan, R. (2022). A review of the protective effects of quercetin-rich natural compounds for treating ischemia-reperfusion injury. *Biotech. Histochem* 97 (4), 237–246. doi:10.1080/10520295.2021.1937701

Ji, Q., Zhang, L., and Ye, H. (2024). Melatonin improves stroke through MDM2-mediated ubiquitination of ACSL4. *Aging (Albany NY)* 16 (2), 1925–1937. doi:10.18632/aging.205469

Jia, C. L., Gou, Y., Gao, Y., Pei, X., Jin, X., Li, B. L., et al. (2024). Rosmarinic acid liposomes suppress ferroptosis in ischemic brain via inhibition of TfR1 in BMECs. *Phytomedicine* 132, 155835. doi:10.1016/j.phymed.2024.155835

Jiang, Z., Yang, H., Ni, W., Gao, X., Pei, X., Jiang, H., et al. (2024). Attenuation of neuronal ferroptosis in intracerebral hemorrhage by inhibiting HDAC1/2: microglial heterogenization via the Nrf2/HO1 pathway. *CNS Neurosci. Ther.* 30 (3), e14646. doi:10.1111/cns.14646

Jin, Z., Gao, W., Guo, F., Liao, S., Hu, M., Yu, T., et al. (2023). Astragaloside IV alleviates neuronal ferroptosis in ischemic stroke by regulating fat mass and obesity-associated-N6-methyladenosine-acyl-CoA synthetase long-chain family member 4 axis. *J. Neurochem.* 166 (2), 328–345. doi:10.1111/jnc.15871

Jin, Z. L., Gao, W. Y., Liao, S. J., Yu, T., Shi, Q., Yu, S. Z., et al. (2021). Paeonol inhibits the progression of intracerebral haemorrhage by mediating the HOTAIR/UPF1/ACSL4 axis. *ASN Neuro* 13, 17590914211010647. doi:10.1177/17590914211010647

Jung, M. A., Jang, S. E., Hong, S. W., Hana, M. J., and Kim, D. H. (2012). The role of intestinal microflora in anti-inflammatory effect of baicalin in mice. *Biomol. Ther. Seoul.* 20 (1), 36–42. doi:10.4062/biomolther.2012.20.1.036

Kagan, V. E., Mao, G., Qu, F., Angeli, J. P. F., Doll, S., Croix, C. S., et al. (2017). Oxidized arachidonic and adrenic PEs navigate cells to ferroptosis. *Nat. Chem. Biol.* 13 (1), 81–90. doi:10.1038/nchembio.2238

Ko, G., Kim, J., Jeon, Y. J., Lee, D., Baek, H. M., and Chang, K. A. (2023). Salvia miltiorrhiza alleviates memory deficit induced by ischemic brain injury in a transient MCAO mouse model by inhibiting ferroptosis. *Antioxidants (Basel)* 12 (4), 785. doi:10.3390/antiox12040785

Kondo, Y., Ogawa, N., Asanuma, M., Ota, Z., and Mori, A. (1995). Regional differences in late-onset iron deposition, ferritin, transferrin, astrocyte proliferation, and microglial activation after transient forebrain ischemia in rat brain. *J. Cereb. Blood Flow. Metab.* 15 (2), 216–226. doi:10.1038/jcbfm.1995.27

Lan, B., Ge, J.-W., Cheng, S.-W., Zheng, X.-L., Liao, J., and He, C. (2020). Extract of Naotaifang, a compound Chinese herbal medicine, protects neuron ferroptosis induced by acute cerebral ischemia in rats. *J. Integr. Med.* 18 (4), 344–350. doi:10.1016/j.joim.2020.01.008

Lan, T., Hu, L., Sun, T., Wang, X., Xiao, Z., Shen, D., et al. (2023). H3K9 trimethylation dictates neuronal ferroptosis through repressing Tfr1. *J. Cereb. Blood Flow. Metab.* 43 (8), 1365–1381. doi:10.1177/0271678X231165653

Lang, J., Luo, J., Wang, L., Xu, W., Jia, J., Zhao, Z., et al. (2024). Electroacupuncture suppresses oxidative stress and ferroptosis by activating the mTOR/SREBP1 pathway in ischemic stroke. *Crit. Rev. Immunol.* 44 (6), 99–110. doi:10.1615/CritRevImmunol.2024051934

Li, C., Sun, G., Chen, B., Xu, L., Ye, Y., He, J., et al. (2021). Nuclear receptor coactivator 4-mediated ferritinophagy contributes to cerebral ischemia-induced ferroptosis in ischemic stroke. *Pharmacol. Res.* 174, 105933. doi:10.1016/j.phrs.2021.105933

Li, M., Meng, Z., Yu, S., Li, J., Wang, Y., Yang, W., et al. (2022). Baicalein ameliorates cerebral ischemia-reperfusion injury by inhibiting ferroptosis via regulating GPX4/ACSL4/ACSL3 axis. *Chem. Biol. Interact.* 366, 110137. doi:10.1016/j.cbi.2022.110137

Li, P., Wang, Z., Zhao, T., Cheng, X., Zhang, Z., Wang, J., et al. (2024b). Protective effect of compound tongluo decoction on brain vascular endothelial cells after ischemia-reperfusion by inhibition of ferroptosis through regulating Nrf2/ARE/SLC7A11 signaling pathway. *Adv. Biol. (Weinh)* 8 (3), e2300416. doi:10.1002/adbi.202300416

Li, R., Zhang, X., Gu, L., Yuan, Y., Luo, X., Shen, W., et al. (2023a). CDGSH iron sulfur domain 2 over-expression alleviates neuronal ferroptosis and brain injury by inhibiting lipid peroxidation via AKT/mTOR pathway following intracerebral hemorrhage in mice. *J. Neurochem.* 165 (3), 426–444. doi:10.1111/jnc.15785

Li, X. N., Shang, N. Y., Kang, Y. Y., Sheng, N., Lan, J. Q., Tang, J. S., et al. (2024a). Caffeic acid alleviates cerebral ischemic injury in rats by resisting ferroptosis via Nrf2 signaling pathway. *Acta Pharmacol. Sin.* 45 (2), 248–267. doi:10.1038/s41401-023-01177-5

Li, Z., Hu, E., Zheng, F., Wang, S., Zhang, W., Luo, J., et al. (2023b). The effects of astragaloside IV on gut microbiota and serum metabolism in a mice model of intracerebral hemorrhage. *Phytomedicine* 121, 155086. doi:10.1016/j.phymed.2023.155086

Liang, Z., Currais, A., Soriano-Castell, D., Schubert, D., and Maher, P. (2021). Natural products targeting mitochondria: emerging therapeutics for age-associated neurological disorders. *Pharmacol. Ther.* 221, 107749. doi:10.1016/j.pharmthera.2020.107749

Liao, J., Wei, M., Wang, J., Zeng, J., Liu, D., Du, Q., et al. (2023). Naotaifang formula attenuates OGD/R-induced inflammation and ferroptosis by regulating microglial M1/M2 polarization through BMP6/SMADs signaling pathway. *Biomed. Pharmacother.* 167, 115465. doi:10.1016/j.biopha.2023.115465

Liu, A., Hu, J., Yeh, T. S., Wang, C., Tang, J., Huang, X., et al. (2023a). Neuroprotective strategies for stroke by natural products: advances and perspectives. *Curr. Neuropharmacol.* 21 (11), 2283–2309. doi:10.2174/1570159X21666230717144752

Liu, C., He, P., Guo, Y., Tian, Q., Wang, J., Wang, G., et al. (2022b). Taurine attenuates neuronal ferroptosis by regulating GABA(B)/AKT/GSK3β/β-catenin pathway after subarachnoid hemorrhage. *Free Radic. Biol. Med.* 193 (Pt 2), 795–807. doi:10.1016/j.freeradbiomed.2022.11.003

Liu, C., Liu, E., Li, Z., Li, W., Jin, J., Sui, H., et al. (2024). Danlou tablet attenuates ischemic stroke injury and blood–brain barrier damage by inhibiting ferroptosis. *J. Ethnopharmacol.* 322, 117657. doi:10.1016/j.jep.2023.117657

Liu, C., Ma, R., Wang, L., Zhu, R., Liu, H., and Guo, Y. (2017). Rehmanniae Radix in osteoporosis: a review of traditional Chinese medicinal uses, phytochemistry, pharmacokinetics and pharmacology. *J. Ethnopharmacol.* 198, 351–362. doi:10.1016/j.jep.2017.01.021

Liu, C. D., Liu, N. N., Zhang, S., Ma, G. D., Yang, H. G., Kong, L. L., et al. (2021). Salvianolic acid A prevented cerebrovascular endothelial injury caused by acute ischemic stroke through inhibiting the Src signaling pathway. *Acta Pharmacol. Sin.* 42 (3), 370–381. doi:10.1038/s41401-020-00568-2

Liu, H., An, N., Wang, L., Li, Y., Song, K., Sun, Y., et al. (2023e). Protective effect of Xingnaojing injection on ferroptosis after cerebral ischemia injury in MCAO rats and SH-SY5Y cells. *J. Ethnopharmacol.* 301, 115836. doi:10.1016/j.jep.2022.115836

Liu, H., Zhang, T. A., Zhang, W. Y., Huang, S. R., Hu, Y., and Sun, J. (2023d). Rhein attenuates cerebral ischemia-reperfusion injury via inhibition of ferroptosis through NRF2/SLC7A11/GPX4 pathway. *Exp. Neurol.* 369, 114541. doi:10.1016/j.expneurol.2023.114541

Liu, H., Zhao, Z., Yan, M., Zhang, Q., Jiang, T., and Xue, J. (2023c). Calycosin decreases cerebral ischemia/reperfusion injury by suppressing ACSL4-dependent ferroptosis. *Arch. Biochem. Biophys.* 734, 109488. doi:10.1016/j.abb.2022.109488

Liu, X. (2021). Changes and significance of serum CXCL-16, GDF-15, PLA-2 levels in patients with cerebral infarction. *Am. J. Transl. Res.* 13 (5), 5617–5622.

Liu, Y., Mi, Y., Wang, Y., Meng, Q., Xu, L., and Liu, Y. (2023b). Loureirin C inhibits ferroptosis after cerebral ischemia reperfusion through regulation of the Nrf2 pathway in mice. *Phytomedicine* 113, 154729. doi:10.1016/j.phymed.2023.154729

Liu, Z., Zhou, Z., Ai, P., Zhang, C., Chen, J., and Wang, Y. (2022a). Astragaloside IV attenuates ferroptosis after subarachnoid hemorrhage via Nrf2/HO-1 signaling pathway. *Front. Pharmacol.* 13, 924826. doi:10.3389/fphar.2022.924826

Lu, J., Xu, F., and Lu, H. (2020). LncRNA PVT1 regulates ferroptosis through miR-214-mediated TFR1 and p53. *Life Sci.* 260, 118305. doi:10.1016/j.lfs.2020.118305

Lv, B., Fu, P., Wang, M., Cui, L., Bao, L., Wang, X., et al. (2024). DMT1 ubiquitination by Nedd4 protects against ferroptosis after intracerebral hemorrhage. *CNS Neurosci. Ther.* 30 (4), e14685. doi:10.1111/cns.14685

Medina-Inojosa, J. R., Somers, V. K., Garcia, M., Thomas, R. J., Allison, T., Chaudry, R., et al. (2023). Performance of the ACC/AHA pooled cohort cardiovascular risk equations in clinical practice. *J. Am. Coll. Cardiol.* 82 (15), 1499–1508. doi:10.1016/j.jacc.2023.07.018

Meng, H., Wu, J., Shen, L., Chen, G., Jin, L., Yan, M., et al. (2022). Microwave assisted extraction, characterization of a polysaccharide from Salvia miltiorrhiza Bunge and its antioxidant effects via ferroptosis-mediated activation of the Nrf2/HO-1 pathway. *Int. J. Biol. Macromol.* 215, 398–412. doi:10.1016/j.ijbiomac.2022.06.064

Mi, Y., Wang, Y., Liu, Y., Dang, W., Xu, L., Tan, S., et al. (2024). Kellerin alleviates cerebral ischemic injury by inhibiting ferroptosis via targeting Akt-mediated transcriptional activation of Nrf2. *Phytomedicine* 128, 155406. doi:10.1016/j.phymed.2024.155406

Nezhad Salari, A. M., Rasoulizadeh, Z., Shabgah, A. G., Vakili-Ghartavol, R., Sargazi, G., and Gholizadeh Navashenaq, J. (2024). Exploring the mechanisms of kaempferol in neuroprotection: implications for neurological disorders. *Cell Biochem. Funct.* 42 (2), e3964. doi:10.1002/cbf.3964

Noor, S., Mohammad, T., Rub, M. A., Raza, A., Azum, N., Yadav, D. K., et al. (2022). Biomedical features and therapeutic potential of rosmarinic acid. *Arch. Pharm. Res.* 45 (4), 205–228. doi:10.1007/s12272-022-01378-2

Ou, Z., Deng, Y., Wu, Y., Wang, Y., Zhao, Y., Liu, C., et al. (2024). Tongqiao Huoxue Decoction inhibits ferroptosis by facilitating ACSL4 ubiquitination degradation for neuroprotection against cerebral ischemia-reperfusion injury. *Phytomedicine* 130, 155701. doi:10.1016/j.phymed.2024.155701

Pavlović, N., Milošević Sopta, N., Mitrović, D., Zaklan, D., Tomas Petrović, A., Stilinović, N., et al. (2023). Principal component analysis (PCA) of molecular descriptors for improving permeation through the blood-brain barrier of quercetin analogues. *Int. J. Mol. Sci.* 25 (1), 192. doi:10.3390/ijms25010192

Peng, C., Ai, Q., Zhao, F., Li, H., Sun, Y., Tang, K., et al. (2024). Quercetin attenuates cerebral ischemic injury by inhibiting ferroptosis via Nrf2/HO-1 signaling pathway. *Eur. J. Pharmacol.* 963, 176264. doi:10.1016/j.ejphar.2023.176264

Peng, C., Fu, X., Wang, K., Chen, L., Luo, B., Huang, N., et al. (2022). Dauricine alleviated secondary brain injury after intracerebral hemorrhage by upregulating GPX4 expression and inhibiting ferroptosis of nerve cells. *Eur. J. Pharmacol.* 914, 174461. doi:10.1016/j.ejphar.2021.174461

Qin, Z., Zhu, G., Luo, H., and Deng, Y. (2022). Regulation of FOXO3 on neuronal ferroptosis after intracerebral hemorrhage via modulating NOX4 transcription. *Eur. Surg. Res.* doi:10.1159/000527617

Qu, X. Y., Zhang, Y. M., Tao, L. N., Gao, H., Zhai, J. H., Sun, J. M., et al. (2019). XingNaoJing injections protect against cerebral ischemia/reperfusion injury and alleviate blood-brain barrier disruption in rats, through an underlying mechanism of NLRP3 inflammasomes suppression. *Chin. J. Nat. Med.* 17 (7), 498–505. doi:10.1016/S1875-5364(19)30071-8

Rana, A., Samtiya, M., Dhewa, T., Mishra, V., and Aluko, R. E. (2022). Health benefits of polyphenols: a concise review. *J. Food Biochem.* 46 (10), e14264. doi:10.1111/jfbc.14264

Salvador, G. A. (2010). Iron in neuronal function and dysfunction. *Biofactors* 36 (2), 103–110. doi:10.1002/biof.80

Selim, M. H., and Ratan, R. R. (2004). The role of iron neurotoxicity in ischemic stroke. *Ageing Res. Rev.* 3 (3), 345–353. doi:10.1016/j.arr.2004.04.001

Shahbaz, K., Chang, D., Zhou, X., Low, M., Seto, S. W., and Li, C. G. (2022). Crocins for ischemic stroke: a review of current evidence. *Front. Pharmacol.* 13, 825842. doi:10.3389/fphar.2022.825842

Sharma, C., Al Kaabi, J. M., Nurulain, S. M., Goyal, S. N., Kamal, M. A., and Ojha, S. (2016). Polypharmacological properties and therapeutic potential of β-caryophyllene: a

dietary phytocannabinoid of pharmaceutical promise. *Curr. Pharm. Des.* 22 (21), 3237–3264. doi:10.2174/1381612822666160311115226

Shi, Y., Yan, D., Nan, C., Sun, Z., Zhuo, Y., Huo, H., et al. (2024). Salvianolic acid A inhibits ferroptosis and protects against intracerebral hemorrhage. *Sci. Rep.* 14 (1), 12427. doi:10.1038/s41598-024-63277-4

Song, D., Hao, J., and Fan, D. (2020). Biological properties and clinical applications of berberine. *Front. Med.* 14 (5), 564–582. doi:10.1007/s11684-019-0724-6

Srivastava, S., Kumar, A., Yadav, P. K., Kumar, M., Mathew, J., Pandey, A. C., et al. (2021). Formulation and performance evaluation of polymeric mixed micelles encapsulated with baicalein for breast cancer treatment. *Drug Dev. Ind. Pharm.* 47 (9), 1512–1522. doi:10.1080/03639045.2021.2007394

Sun, J., Zhao, K., Zhang, W., Guo, C., and Liu, H. (2024a). Ecdysterone improves oxidative damage induced by acute ischemic stroke via inhibiting ferroptosis in neurons through ACSL4. *J. Ethnopharmacol.* 331, 118204. doi:10.1016/j.jep.2024.118204

Sun, M., Liu, M., Li, Q., Zhang, X., Liu, S., Yang, H., et al. (2023). Cottonseed oil alleviates ischemic stroke injury by inhibiting ferroptosis. *Brain Behav.* 13 (10), e3179. doi:10.1002/brb3.3179

Sun, M., Shen, X., and Ma, Y. (2019a). Rehmannioside A attenuates cognitive deficits in rats with vascular dementia (VD) through suppressing oxidative stress, inflammation and apoptosis. *Biomed. Pharmacother.* 120, 109492. doi:10.1016/j.biopha.2019.109492

Sun, X., Ou, Z., Xie, M., Kang, R., Fan, Y., Niu, X., et al. (2015). HSPB1 as a novel regulator of ferroptotic cancer cell death. *Oncogene* 34 (45), 5617–5625. doi:10.1038/onc.2015.32

Sun, X., Yan, P., Zou, C., Wong, Y. K., Shu, Y., Lee, Y. M., et al. (2019b). Targeting autophagy enhances the anticancer effect of artemisinin and its derivatives. *Med. Res. Rev.* 39 (6), 2172–2193. doi:10.1002/med.21580

Sun, Y., Jin, H., He, J., Lai, J., Lin, H., and Liu, X. (2024b). Melatonin alleviates ischemic stroke by inhibiting ferroptosis through the CYP1B1/ACSL4 pathway. *Environ. Toxicol.* 39 (5), 2623–2633. doi:10.1002/tox.24136

Sun, Y., Li, Q., Guo, H., and He, Q. (2022). Ferroptosis and iron metabolism after intracerebral hemorrhage. *Cells* 12 (1), 90. doi:10.3390/cells12010090

Tran, P. H. L., and Tran, T. T. D. (2020). Developmental strategies of curcumin solid dispersions for enhancing bioavailability. *Anticancer Agents Med. Chem.* 20 (16), 1874–1882. doi:10.2174/1871520620666200708103845

Tsao, C. W., Aday, A. W., Almarzooq, Z. I., Anderson, C. A. M., Arora, P., Avery, C. L., et al. (2023). Heart disease and stroke statistics-2023 update: a report from the American Heart association. *Circulation* 147 (8), e93–e621. doi:10.1161/CIR.0000000000001123

Tu, Y., Tan, L., Tao, H., Li, Y., and Liu, H. (2023). CETSA and thermal proteome profiling strategies for target identification and drug discovery of natural products. *Phytomedicine* 116, 154862. doi:10.1016/j.phymed.2023.154862

Tuo, Q. Z., Lei, P., Jackman, K. A., Li, X. L., Xiong, H., Li, X. L., et al. (2017). Tau-mediated iron export prevents ferroptotic damage after ischemic stroke. *Mol. Psychiatry* 22 (11), 1520–1530. doi:10.1038/mp.2017.171

Tuo, Q. Z., Liu, Y., Xiang, Z., Yan, H. F., Zou, T., Shu, Y., et al. (2022). Thrombin induces ACSL4-dependent ferroptosis during cerebral ischemia/reperfusion. *Signal Transduct. Target Ther.* 7 (1), 59. doi:10.1038/s41392-022-00917-z

Venketasubramanian, N., Agustin, S. J., Padilla, J. L., Yumul, M. P., Sum, C., Lee, S. H., et al. (2022). Comparison of different laboratory tests to identify "aspirin resistance" and risk of vascular events among ischaemic stroke patients: a double-blind study. *J. Cardiovasc Dev. Dis.* 9 (5), 156. doi:10.3390/jcdd9050156

Wan, J., Ren, H., and Wang, J. (2019). Iron toxicity, lipid peroxidation and ferroptosis after intracerebral haemorrhage. *Stroke Vasc. Neurol.* 4 (2), 93–95. doi:10.1136/svn-2018-000205

Wang, B., Zhang, X., Zhong, J., Wang, S., Zhang, C., Li, M., et al. (2022a). Dexpramipexole attenuates white matter injury to facilitate locomotion and motor coordination recovery via reducing ferroptosis after intracerebral hemorrhage. *Oxid. Med. Cell Longev.* 2022, 6160701. doi:10.1155/2022/6160701

Wang, F., Li, W. L., Shen, L. J., Jiang, T. T., Xia, J. J., You, D. L., et al. (2022b). Crocin alleviates intracerebral hemorrhage-induced neuronal ferroptosis by facilitating Nrf2 nuclear translocation. *Neurotox. Res.* 40 (2), 596–604. doi:10.1007/s12640-022-00500-y

Wang, L., Liu, C., and Tang, B. (2023b). Astragaloside IV mitigates cerebral ischaemia-reperfusion injury via inhibition of P62/Keap1/Nrf2 pathway-mediated ferroptosis. *Eur. J. Pharmacol.* 944, 175516. doi:10.1016/j.ejphar.2023.175516

Wang, P., Cui, Y., Ren, Q., Yan, B., Zhao, Y., Yu, P., et al. (2021). Mitochondrial ferritin attenuates cerebral ischaemia/reperfusion injury by inhibiting ferroptosis. *Cell Death Dis.* 12 (5), 447. doi:10.1038/s41419-021-03725-5

Wang, Q., Liu, C., Chen, M., Zhao, J., Wang, D., Gao, P., et al. (2024a). Mastoparan M promotes functional recovery in stroke mice by activating autophagy and inhibiting ferroptosis. *Biomed. Pharmacother.* 174, 116560. doi:10.1016/j.biopha.2024.116560

Wang, X., Zhang, J., Wang, S., Song, Z., Sun, H., Wu, F., et al. (2023c). Berberine modulates gut microbiota to attenuate cerebral ferroptosis induced by ischemia-reperfusion in mice. *Eur. J. Pharmacol.* 953, 175782. doi:10.1016/j.ejphar.2023.175782

Wang, Y., Lan, X., Liu, N., Ma, L., DU, J., Wei, W., et al. (2024b). Traditional Chinese medicines derived natural inhibitors of ferroptosis on ischemic stroke. *Chin. J. Nat. Med.* 22 (8), 746–755. doi:10.1016/S1875-5364(24)60603-5

Wang, Y., Wu, S., Li, Q., Sun, H., and Wang, H. (2023a). Pharmacological inhibition of ferroptosis as a therapeutic target for neurodegenerative diseases and strokes. *Adv. Sci. (Weinh)* 10 (24), e2300325. doi:10.1002/advs.202300325

Wendlocha, D., Kubina, R., Krzykawski, K., and Mielczarek-Palacz, A. (2024). Selected flavonols targeting cell death pathways in cancer therapy: the latest achievements in research on apoptosis, autophagy, necroptosis, pyroptosis, ferroptosis, and cuproptosis. *Nutrients* 16 (8), 1201. doi:10.3390/nu16081201

Wu, C., Duan, F., Yang, R., Dai, Y., Chen, X., and Li, S. (2023). 15, 16-Dihydrotanshinone I protects against ischemic stroke by inhibiting ferroptosis via the activation of nuclear factor erythroid 2-related factor 2. *Phytomedicine* 114, 154790. doi:10.1016/j.phymed.2023.154790

Wu, J., Wei, Z., Cheng, P., Qian, C., Xu, F., Yang, Y., et al. (2020). Rhein modulates host purine metabolism in intestine through gut microbiota and ameliorates experimental colitis. *Theranostics* 10 (23), 10665–10679. doi:10.7150/thno.43528

Xiao, S., Wang, C., Yang, Q., Xu, H., Lu, J., and Xu, K. (2021). Rea regulates microglial polarization and attenuates neuronal apoptosis via inhibition of the NF-κB and MAPK signalings for spinal cord injury repair. *J. Cell Mol. Med.* 25 (3), 1371–1382. doi:10.1111/jcmm.16220

Xie, G., Liang, Y., Gao, W., Wu, L., Zhang, Y., Ye, Z., et al. (2023). Artesunate alleviates intracerebral haemorrhage secondary injury by inducing ferroptosis in M1-polarized microglia and suppressing inflammation through AMPK/mTORC1/GPX4 pathway. *Basic Clin. Pharmacol. Toxicol.* 132 (5), 369–383. doi:10.1111/bcpt.13848

Xing, P., Zhong, Y., Cui, X., Liu, Z., and Wu, X. (2023). Natural products in digestive tract tumors metabolism: functional and application prospects. *Pharmacol. Res.* 191, 106766. doi:10.1016/j.phrs.2023.106766

Xu, S., Li, X., Li, Y., Li, X., Lv, E., Zhang, X., et al. (2023b). Neuroprotective effect of Dl-3-n-butylphthalide against ischemia-reperfusion injury is mediated by ferroptosis regulation via the SLC7A11/GSH/GPX4 pathway and the attenuation of blood-brain barrier disruption. *Front. Aging Neurosci.* 15, 1028178. doi:10.3389/fnagi.2023.1028178

Xu, Y., Li, K., Zhao, Y., Zhou, L., Liu, Y., and Zhao, J. (2023a). Role of ferroptosis in stroke. *Cell Mol. Neurobiol.* 43 (1), 205–222. doi:10.1007/s10571-022-01196-6

Xu, Y., Liu, Y., Li, K., Yuan, D., Yang, S., Zhou, L., et al. (2022). COX-2/PGE2 pathway inhibits the ferroptosis induced by cerebral ischemia reperfusion. *Mol. Neurobiol.* 59 (3), 1619–1631. doi:10.1007/s12035-021-02706-1

Yamada, K., Toyota, K., Tsunoda, Y., Matahira, Y., Matsumura, S., Yoshioka, Y., et al. (2023). Effects of inhaled β-caryophyllene on vascular stiffness in smokers: a randomized, double-blind, placebo-controlled trial. *Exp. Ther. Med.* 25 (1), 57. doi:10.3892/etm.2022.11756

Yang, C., Han, M., Li, R., Zhou, L., Zhang, Y., Duan, L., et al. (2021). Curcumin nanoparticles inhibiting ferroptosis for the enhanced treatment of intracerebral hemorrhage. *Int. J. Nanomedicine* 16, 8049–8065. doi:10.2147/IJN.S334965

Yang, W. S., Kim, K. J., Gaschler, M. M., Patel, M., Shchepinov, M. S., and Stockwell, B. R. (2016). Peroxidation of polyunsaturated fatty acids by lipoxygenases drives ferroptosis. *Proc. Natl. Acad. Sci. U. S. A.* 113 (34), E4966–E4975. doi:10.1073/pnas.1603244113

Yang, Y., Wu, Q., Shan, X., Zhou, H., Wang, J., Hu, Y., et al. (2024a). Ginkgolide B attenuates cerebral ischemia-reperfusion injury via inhibition of ferroptosis through disrupting NCOA4-FTH1 interaction. *J. Ethnopharmacol.* 318 (Pt B)), 116982. doi:10.1016/j.jep.2023.116982

Yang, Y. Y., Deng, R. R., and Xiang, D. X. (2024c). Naodesheng pills ameliorate cerebral ischemia reperfusion-induced ferroptosis via inhibition of the ERK1/2 signaling pathway. *Drug Des. Devel Ther.* 18, 1499–1514. doi:10.2147/DDDT.S443479

Yang, Z., Jiang, Y., Xiao, Y., Qian, L., Jiang, Y., Hu, Y., et al. (2024b). Di-Huang-Yin-Zi regulates P53/SLC7A11 signaling pathway to improve the mechanism of post-stroke depression. *J. Ethnopharmacol.* 319 (Pt 2), 117226. doi:10.1016/j.jep.2023.117226

Yao, M., Zhang, L., and Wang, L. (2023). Astragaloside IV: a promising natural neuroprotective agent for neurological disorders. *Biomed. Pharmacother.* 159, 114229. doi:10.1016/j.biopha.2023.114229

Yao, M. Y., Liu, T., Zhang, L., Wang, M. J., Yang, Y., and Gao, J. (2021). Role of ferroptosis in neurological diseases. *Neurosci. Lett.* 747, 135614. doi:10.1016/j.neulet.2020.135614

Yi, X., and Tang, X. (2021). Exosomes from miR-19b-3p-modified ADSCs inhibit ferroptosis in intracerebral hemorrhage mice. *Front. Cell Dev. Biol.* 9, 661317. doi:10.3389/fcell.2021.661317

Yin, M., Chen, W., Li, M., Wang, K., Hu, N., and Li, Z. (2022). circAFF1 enhances intracerebral hemorrhage induced neuronal ferroptosis by targeting miR-140-5p to regulate GSK-3β mediated Wnt/β-catenin signal pathway. *Brain Res. Bull.* 189, 11–21. doi:10.1016/j.brainresbull.2022.08.005

Yu, X., Wang, S., Wang, X., Li, Y., and Dai, Z. (2024). Melatonin improves stroke by inhibiting autophagy-dependent ferroptosis mediated by NCOA4 binding to FTH1. *Exp. Neurol.* 379, 114868. doi:10.1016/j.expneurol.2024.114868

Yu, Y., Li, X., Wu, X., Li, X., Wei, J., Chen, X., et al. (2023). Sodium hydrosulfide inhibits hemin-induced ferroptosis and lipid peroxidation in BV2 cells via the CBS/H(2)S system. *Cell Signal* 104, 110594. doi:10.1016/j.cellsig.2023.110594

Yuan, Y., Zhai, Y., Chen, J., Xu, X., and Wang, H. (2021). Kaempferol ameliorates oxygen-glucose deprivation/reoxygenation-induced neuronal ferroptosis by activating nrf2/slc7a11/GPX4 Axis. *Biomolecules* 11 (7), 923. doi:10.3390/biom11070923

Zhan, S., Liang, J., Lin, H., Cai, J., Yang, X., Wu, H., et al. (2023). SATB1/SLC7A11/HO-1 Axis ameliorates ferroptosis in neuron cells after ischemic stroke by danhong injection. *Mol. Neurobiol.* 60 (1), 413–427. doi:10.1007/s12035-022-03075-z

Zhang, C., Shi, Z., Xu, Q., He, J., Chen, L., Lu, Z., et al. (2023a). Astragaloside IV alleviates stroke-triggered early brain injury by modulating neuroinflammation and ferroptosis via the Nrf2/HO-1 signaling pathway. *Acta Cir. Bras.* 38, e380723. doi:10.1590/acb380723

Zhang, J., Tang, L., Li, G. S., and Wang, J. (2018). The anti-inflammatory effects of curcumin on renal ischemia-reperfusion injury in rats. *Ren. Fail* 40 (1), 680–686. doi:10.1080/0886022X.2018.1544565

Zhang, L., Ma, J., Yang, F., Li, S., Ma, W., Chang, X., et al. (2022b). Neuroprotective effects of quercetin on ischemic stroke: a literature review. *Front. Pharmacol.* 13, 854249. doi:10.3389/fphar.2022.854249

Zhang, L., Wang, X., Che, W., Yi, Y., Zhou, S., and Feng, Y. (2022a). Methyltransferase-like 3 silenced inhibited the ferroptosis development via regulating the glutathione peroxidase 4 levels in the intracerebral hemorrhage progression. *Bioengineered* 13 (6), 14215–14226. doi:10.1080/21655979.2022.2084494

Zhang, X., Tang, B., Wen, S., Wang, Y., Pan, C., Qu, L., et al. (2023b). Advancements in the biotransformation and biosynthesis of the primary active flavonoids derived from Epimedium. *Molecules* 28 (20), 7173. doi:10.3390/molecules28207173

Zhang, Y., Ye, P., Zhu, H., Gu, L., Li, Y., Feng, S., et al. (2024). Neutral polysaccharide from Gastrodia elata alleviates cerebral ischemia-reperfusion injury by inhibiting ferroptosis-mediated neuroinflammation via the NRF2/HO-1 signaling pathway. *CNS Neurosci. Ther.* 30 (3), e14456. doi:10.1111/cns.14456

Zhang, Z., Pang, Y., Wang, W., Zhu, H., Jin, S., Yu, Z., et al. (2021). Neuroprotection of heme oxygenase-2 in mice AfterIntracerebral hemorrhage. *J. Neuropathol. Exp. Neurol.* 80 (5), 457–466. doi:10.1093/jnen/nlab025

Zhao, F., Peng, C., Li, H., Chen, H., Yang, Y., Ai, Q., et al. (2023b). Paeoniae Radix Rubra extract attenuates cerebral ischemia injury by inhibiting ferroptosis and activating autophagy through the PI3K/Akt signalling pathway. *J. Ethnopharmacol.* 315, 116567. doi:10.1016/j.jep.2023.116567

Zhao, H., Li, X., Yang, L., Zhang, L., Jiang, X., Gao, W., et al. (2021b). Isorhynchophylline relieves ferroptosis-induced nerve damage after intracerebral hemorrhage via miR-122-5p/TP53/slc7a11 pathway. *Neurochem. Res.* 46 (8), 1981–1994. doi:10.1007/s11064-021-03320-2

Zhao, J., Wu, Y., Liang, S., and Piao, X. (2022). Activation of SSAT1/ALOX15 Axis aggravates cerebral ischemia/reperfusion injury via triggering neuronal ferroptosis. *Neuroscience* 485, 78–90. doi:10.1016/j.neuroscience.2022.01.017

Zhao, X., Kruzel, M., and Aronowski, J. (2021a). Lactoferrin and hematoma detoxification after intracerebral hemorrhage. *Biochem. Cell Biol.* 99 (1), 97–101. doi:10.1139/bcb-2020-0116

Zhao, Y., Liu, Y., Xu, Y., Li, K., Zhou, L., Qiao, H., et al. (2023a). The role of ferroptosis in blood-brain barrier injury. *Cell Mol. Neurobiol.* 43 (1), 223–236. doi:10.1007/s10571-022-01197-5

Zhou, R., Wang, J., Han, X., Ma, B., Yuan, H., and Song, Y. (2019). Baicalin regulates the dopamine system to control the core symptoms of ADHD. *Mol. Brain* 12 (1), 11. doi:10.1186/s13041-019-0428-5

Zhou, S., Gao, X., Chen, C., Zhang, J., Zhang, Y., Zhang, L., et al. (2023b). Porcine cardiac blood - Salvia miltiorrhiza root alleviates cerebral ischemia reperfusion injury by inhibiting oxidative stress induced apoptosis through PI3K/AKT/Bcl-2/Bax signaling pathway. *J. Ethnopharmacol.* 316, 116698. doi:10.1016/j.jep.2023.116698

Zhou, S., Wang, Z., Wang, T., Peng, C., Zhang, J., Liu, C., et al. (2024a). Salvia miltiorrhiza Bge. processed with porcine cardiac blood inhibited GLRX5-mediated ferroptosis alleviating cerebral ischemia-reperfusion injury. *Phytomedicine* 129, 155622. doi:10.1016/j.phymed.2024.155622

Zhou, S. Y., Cui, G. Z., Yan, X. L., Wang, X., Qu, Y., Guo, Z. N., et al. (2020). Mechanism of ferroptosis and its relationships with other types of programmed cell death: insights for potential interventions after intracerebral hemorrhage. *Front. Neurosci.* 14, 589042. doi:10.3389/fnins.2020.589042

Zhou, Z., Yu, Y., Miao, J., Wang, G., Wang, Y., Wang, T., et al. (2024b). Research progress of traditional Chinese medicine in treating central nervous system diseases by modulating ferroptosis. *Am. J. Chin. Med.* 52 (7), 1989–2019. doi:10.1142/S0192415X24500770

Zhou, Z. X., Cui, Q., Zhang, Y. M., Yang, J. X., Xiang, W. J., Tian, N., et al. (2023a). Withaferin A inhibits ferroptosis and protects against intracerebral hemorrhage. *Neural Regen. Res.* 18 (6), 1308–1315. doi:10.4103/1673-5374.355822

Zhu, H., Huang, J., Chen, Y., Li, X., Wen, J., Tian, M., et al. (2022a). Resveratrol pretreatment protects neurons from oxygen-glucose deprivation/reoxygenation and ischemic injury through inhibiting ferroptosis. *Biosci. Biotechnol. Biochem.* 86 (6), 704–716. doi:10.1093/bbb/zbac048

Zhu, H., Wang, Z., Xing, Y., Gao, Y., Ma, T., Lou, L., et al. (2012). Baicalin reduces the permeability of the blood-brain barrier during hypoxia *in vitro* by increasing the expression of tight junction proteins in brain microvascular endothelial cells. *J. Ethnopharmacol.* 141 (2), 714–720. doi:10.1016/j.jep.2011.08.063

Zhu, Y., Ouyang, Z., Du, H., Wang, M., Wang, J., Sun, H., et al. (2022b). New opportunities and challenges of natural products research: when target identification meets single-cell multiomics. *Acta Pharm. Sin. B* 12 (11), 4011–4039. doi:10.1016/j.apsb.2022.08.022

Glossary

ICH	Intracerebral hemorrhage
SAH	Subarachnoid hemorrhage
BBB	Blood-brain barrier
RBC	Red blood cells
HO-1	Heme oxygenase-1
CO	Carbon monoxide
TFR1	Transferrin receptor 1
STEAP3	Six-Transmembrane Epithelial Antigen of Prostate 3
LIP	Labile iron pool
SLC11A2	Solute Carrier Family 11 Member 2
LOXs	Lipoxygenases
ROS	Reactive oxygen species
NCOA4	Nuclear receptor coactivator 4
ATG5	Autophagy-related 5
ATG7	Autophagy-related 7
NFS1	Nitrogen fixation 1
HSPB1	Heat shock factor-binding Protein 1
PUFAs	Polyunsaturated fatty acids
I/R	Ischemia-reperfusion
GSH	Glutathione
MCAO	Middle Cerebral Artery Occlusion
RosA-LIP	Osmarinic acid encapsulated in liposomes
SOD	Superoxide Dismutase
MDA	Malondialdehyde
GPX4	Glutathione Peroxidase 4
ACSL4	Acyl-Co A synthetase long-chain family member 4
FTH1	Ferritin Heavy Chain 1
LDH	Lactate Dehydrogenase
Nrf2	Nuclear factor erythroid 2 related factor 2
Fto	Fat mass and obesity-associated protein
Atf3	Activation transcription factor 3
ZO-1	Zonula Occludens 1
xCT	Cystine/Glutamate Antiporter
COX2	Cyclooxygenase-2
4-HNE	4-Hydroxynonenal
GSH-Px	Glutathione peroxidase
MMP-9	Matrix Metalloprotein 9
OS	Oxidative stress
RNS	Reactive nitrogen species
CNS	Central Nervous System
HDAC1/2	Histone deacetylases 1/2
H3K9me3	Histone H3 Lysine 9 Trimethylation
Nedd4	Neural precursor cell expressed, developmentally downregulated 4
FOXO3	Forkhead box O3
CISD2	CDGSH iron sulfur domain 2
H2S	H2S-producing enzymes
circAFF1	circular RNA AFF1
SLC7A11	Solute Carrier Family 7 Member 11
METTL3	Methyltransferase like 3
FSP1	Ferroptosis-suppressor-protein 1
Fpn	Ferroportin
IRP	Iron Regulatory Protein
Tau	microtubule-associated protein tau
LncRNA PVT1	plasmacytoma variant translocation 1
SSAT1	Spermidine/spermine N1-acetyltransferase 1
UBIAD1	UbiA prenyltransferase domain containing 1
PGE2	ProstaglandinE2
FtMt	Ferritin Mitochondrial
USP14	ubiquitin specific peptidase 14
SIRT6	Sirtuin 6
mTOR	Mechanistic Target of Rapamycin

frontiers | Frontiers in Pharmacology

TYPE Review
PUBLISHED 30 May 2025
DOI 10.3389/fphar.2025.1561688

Check for updates

OPEN ACCESS

EDITED BY
Huazheng Liang,
Monash University - Southeast University Joint Research Institute (Suzhou), China

REVIEWED BY
Xin Guan,
Anhui Medical University, China
Zhijun Pu,
Army Medical University, China

*CORRESPONDENCE
Rukaiah Fatma Begum,
rukaiah786@gmail.com
Ankul Singh S,
ankulsingh57@gmail.com

RECEIVED 16 January 2025
ACCEPTED 12 May 2025
PUBLISHED 30 May 2025

CITATION
Sowmiya S, Begum RF, Dhivya LS, Rajendran P, Harikrishnan N and Singh S A (2025) Traditional, complementary, and integrative medicine in the management of ischemic stroke: a narrative review.
Front. Pharmacol. 16:1561688.
doi: 10.3389/fphar.2025.1561688

COPYRIGHT
© 2025 Sowmiya, Begum, Dhivya, Rajendran, Harikrishnan and Singh S. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

Traditional, complementary, and integrative medicine in the management of ischemic stroke: a narrative review

S. Sowmiya[1], Rukaiah Fatma Begum[2]*, L. S. Dhivya[3], Praveen Rajendran[1], N. Harikrishnan[4] and Ankul Singh S[1]*

[1]Department of Pharmacology, Dr M.G.R. Educational and Research Institute, Chennai, Tamil Nadu, India, [2]Institute of Pharmaceutical Research, GLA University, Mathura, Uttar Pradesh, India, [3]Department of Pharmaceutical Chemistry, Dr M.G.R. Educational and Research Institute, Chennai, Tamil Nadu, India, [4]Department of pharmaceutical analysis, Dr M.G.R. Educational and Research Institute, Chennai, Tamil Nadu, India

Ischemic stroke remains a leading cause of mortality and long-term disability worldwide, despite advancements in acute intervention and rehabilitation strategies. Traditional, Complementary, and Integrative Medicine (TCIM) systems; including herbal medicine, acupuncture, and mind-body interventions are increasingly being explored as adjunct therapies in stroke management. This narrative review evaluates the current evidence supporting TCIM approaches for ischemic stroke, highlighting their potential neuroprotective, anti-inflammatory, antioxidant, and cerebrovascular effects. Particular emphasis is placed on well-studied botanical interventions such as *Salvia miltiorrhiza* Bunge [Lamiaceae; Salviae miltiorrhizae radix et rhizoma], *Ginkgo biloba* L. [Ginkgoaceae; Ginkgo folium], and *Panax ginseng* C.A. Mey. [Araliaceae; Ginseng radix]. The review discusses the mechanisms of action, clinical trial outcomes, and integration challenges, while underscoring the need for standardization, quality control, and rigorous scientific validation. This work aims to support informed decisions regarding the integration of evidence-based TCIM practices into conventional stroke care protocols.

KEYWORDS

ischemic stroke, traditional, complementary, and integrative medicine, neuroinflammation, oxidative stress, complementary alternative medicine

1 Introduction

Ischemic stroke is a sudden neurological impairment triggered by an interruption in the blood supply to a section of the brain. At the molecular level, cerebral blood perfusion causes an acute loss of oxygen and glucose, which lowers the generation of adenosine triphosphate (ATP), causes lactic acidosis, and disrupts cellular homeostasis (Haupt et al., 2023). Ischemic stroke includes an imbalance of ions, abnormal activation of immune cells, and neuroinflammation, which can lead to neuron death (Zhu et al., 2022). It accounts for the second leading cause of death worldwide, accounting for 5.9 million deaths and 102 million disability-adjusted life years lost (Qin et al., 2022a). Several risk factors have been associated with the development of stroke, including diabetes mellitus, smoking, hyperlipidemia, and hypertension (McFarlane et al., 2005). The underlying causes of ischemic stroke primarily involve *in situ* small vessel disease, artery-to-artery

embolism, and cardioembolic events (Chen et al., 2019). Clinically, ischemic stroke often presents with sudden-onset symptoms such as non-orthostatic vertigo, slurred speech, diplopia, sensory disturbances (numbness), and unilateral motor weakness or paralysis (Qin et al., 2022b). Interrupting the blood supply causes several pathophysiological alterations that lead to irreversible neural disruption, such as the production of oxygen free radicals and cerebral edema, reactive oxygen species, neuroinflammation, blood-brain barrier (BBB) destruction, and local inflammatory cell infiltration (Wang et al., 2021).

Herbal medicines are being explored as potential neuroprotective agents for ischemic stroke, based on their long history of use in traditional medicine. Plants naturally produce chemical compounds to protect against diseases, predators, and environmental stress. Many of these phytochemicals have anti-inflammatory, antioxidant, and anti-apoptotic properties, which may help in understanding and managing the underlying causes of ischemic stroke (Ranjan, 2023). Acute ischemic stroke can currently be effectively treated with thrombectomy and thrombolysis. However, only a small percentage of patients can benefit from these techniques due to time frame restrictions (Tong et al., 2021; Hurd et al., 2021). Neuroprotective drugs have not worked well in clinical trials because most treatment targets are discovered in animal studies, not in humans. Therefore, there is an urgent need for effective treatments that can be widely used for ischemic stroke (Zhu et al., 2021). Traditional Chinese, Malay, and Indian (Ayurvedic/Siddha/Unani) medicines, as well as homoeopathic remedies, are part of alternative medicine. Acupuncture, homoeopathy, Chinese medicine, Ayurveda, herbal therapies, mind-body practices, and physiotherapy are all included in Complementary alternative medicine (CAM) for stroke (Rajahthurai et al., 2022).

Integrative medicine was established in the 1980s. Using the complementary advantages of macro and micro, global and local, structure and function, traditional and modern, disease differentiation, and syndrome differentiation in Western medicine and Traditional Chisnese Medicine, integrative medicine pioneers have been developing new theories of medicine and pharmacology based on the tenet of "system learning, comprehensively improving mastering, and sorting" (Wang and Xiong, 2012). As integrative medicine continues to evolve, a growing number of stroke centres in China are incorporating TCM to address the increasing demands of the patient population. More standardised and holistic stroke treatment protocols are being developed nationwide. This comprehensive strategy, which combines conventional and traditional therapies, is referred to as integrative medicine rehabilitation (Saceleanu et al., 2023).

An imperative feature of TCM is acupuncture, which involves putting tiny needles into the skin or deep tissues of particular body areas (acupoints). In addition to being safe, effective, cost-effective, and easy to use, acupuncture can be administered by hand, electric stimulation, or warmth. Acupuncture has been shown in numerous clinical and experimental studies to ameliorate neurological impairments caused by ischemic stroke, particularly those related to stroke outcomes. To produce stronger proof, researchers should keep refining the design of clinical trials, expanding the sample size, standardising and quantifying acupuncture procedures, and utilising interdisciplinary approaches (Qin et al., 2022a). The aim of this review is to critically evaluate the current scientific evidence supporting the use of TCIM approaches in ischemic stroke, identify key pharmacological mechanisms and therapeutic targets, highlight limitations in existing studies, and propose directions for future research. This review also assesses the feasibility of integrating evidence-based TCIM practices into conventional stroke management frameworks.

2 Literature search strategy

A comprehensive literature search was conducted using four major databases: PubMed/MEDLINE, Scopus, Web of Science, and Google Scholar (for supplementary and grey literature). The search was limited to articles published between January 2000 and January 2025 to capture recent and relevant advances in the field of traditional, complementary, and integrative medicine (TCIM) for ischemic stroke. We used a combination of Medical Subject Headings (MeSH) and free-text terms with Boolean operators such as ("Ischemic Stroke" OR "Cerebral Infarction" OR "Brain Ischemia") AND ("Traditional Medicine" OR "Complementary Medicine" OR "Integrative Medicine" OR "Ayurveda" OR "Herbal Medicine" OR "Chinese Medicine" OR "Acupuncture" OR "Yoga") AND ("Treatment" OR "Therapy" OR "Intervention" OR "Management" OR "Neuroprotection"). Only peer-reviewed English-language articles were included, covering *in vitro*, *in vivo*, or human studies that evaluated TCIM interventions specifically for ischemic stroke and discussed mechanisms of action, pharmacological effects, or clinical outcomes. We excluded non-English publications, case reports, editorials, conference abstracts, non-peer-reviewed sources, studies unrelated to ischemic stroke, and those focused on hemorrhagic stroke unless ischemic models were explicitly addressed. The screening process involved an initial review of titles and abstracts by two independent reviewers, followed by full-text screening for eligible studies. Any disagreements during selection were resolved through discussion or consultation with a third reviewer.

3 Pathophysiology of ischemia stroke

Ischemic occlusions account for approximately 85% of stroke-related deaths, while the remaining cases are due to intracerebral hemorrhage. Ischemic strokes are primarily caused by embolism or brain thrombosis. Beyond the initial vascular occlusion, several secondary pathophysiological mechanisms contribute to the progression of stroke injury. These include inflammation, energy failure, loss of cellular homeostasis, acidosis, elevated intracellular calcium levels, excitotoxicity, cytokine- and free radical-mediated cytotoxicity, complement system activation, disruption of the blood-brain barrier, activation of glial cells, oxidative stress, and leukocyte infiltration (Figure 1) (Kuriakose and Xiao, 2020).

3.1 Oxidative stress

Oxidative stress occurs when the production of reactive oxygen species (ROS) exceeds the body's ability to neutralize and eliminate

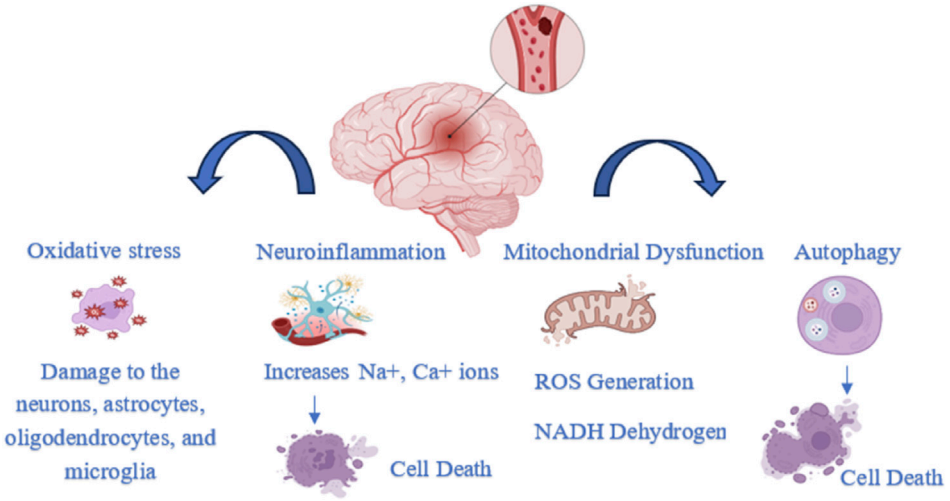


FIGURE 1
Pathophysiology of ischemia Stroke.

them (Juan et al., 2021). The brain contains low levels of antioxidant enzymes and cytochrome c oxidase, making it prone to superoxide generation during ATP production. Its high membrane surface-to-volume ratio and lipid-rich plasmalemma increase vulnerability to oxidative damage. Neurotransmitter metabolism leads to calcium overload and ROS formation, while iron released from damaged tissue further promotes free radical production (Jurcau and Ardelean, 2022). ROS are primarily generated as byproducts of mitochondrial electron transport and metal-catalyzed oxidation. Both internal and external factors contribute to their production. Elevated ROS levels are strongly associated with risk factors for ischemic stroke, especially in older adults. (Koutsaliaris et al., 2021). When blood flow is restored to the brain, the return of oxygen can trigger the "oxygen paradox," leading to a surge in reactive oxygen species that further damages neurons, oligodendrocytes, microglia, and astrocytes (Granger and Kvietys, 2015). Ischemia-reperfusion injury (IRI) causes oxidative stress that leads to endothelial damage, inflammation, and disruption of the blood-brain barrier. This triggers microglial activation, lipid peroxidation, and cell death through processes like ferroptosis, pyroptosis, necroptosis, autophagy, and apoptosis. Long-term damage results from glial scar formation, poor axonal regrowth, ongoing inflammation, and reduced new blood vessel formation and remyelination (Briones-Valdivieso et al., 2024).

Reactive nitrogen species (RNS) are highly reactive molecules produced during nitrogen metabolism. They play key roles in both normal and disease processes, like regulating blood vessel function. Nitrite (NO_2^-), a compound made of nitrogen and oxygen, can form salts such as sodium nitrite (NaNO2) or potassium nitrite (KNO2). These nitrite salts, along with SNO-Hb, serve as reservoirs for nitric oxide (NO), which can be released to promote vasodilation and increase blood flow to distant tissues (Salvagno et al., 2024). During ischemia-reperfusion damage in the brain, an increase in both nitric oxide (NO) and superoxide (O2) leads to the rapid formation of peroxynitrite (ONOO) through their direct reaction. Peroxynitrite is more toxic than its precursors. It can nitrify protein tyrosine, forming 3-nitrotyrosine (3-NT), which is believed to carry the signature of ONOO damage (Chen et al., 2018). The generation of reactive compounds like NO_2^+, NO2, and OH leads to a series of redox reactions, contributing to secondary nitroxidative stress. Compared to eNOS and nNOS, iNOS produces much higher levels of NO•. This makes iNOS, often called the 'pathological' type of NO• synthase, capable of generating $ONOO^-$ and highly reactive hydroxyl radicals. NO• plays a critical role in the brain, affecting the neurovascular unit. NO signaling, mediated by constitutive NOS isoforms (nNOS and eNOS), is essential for regulating blood vessel dilation, neuronal excitability, and glial cell function (Wierońska et al., 2021).

3.2 Neuroinflammation

Inflammation is another critical factor in ischemic stroke. It worsens nerve tissue damage and cell death through an inflammatory cascade triggered by cerebral ischemia and subsequent reperfusion. This cascade involves oxidative stress, excitotoxicity, inflammatory cell infiltration, and the production of harmful inflammatory mediators (Mo et al., 2020). Consequently, the buildup of Na^+ and Ca^{2+} ions leads to cell death, membrane damage, and organelle dysfunction. Impaired ATP production also reduces glutamate uptake, causing its excessive accumulation outside cells, which contributes to neuronal death in the ischemic penumbra. The overactivation of glutamate receptors further amplifies excitotoxicity and calcium overload, ultimately leading to mitochondrial failure and cell death (Pawluk et al., 2020). During ischemia, glial cells (microglia, astrocytes), blood cells (like leukocytes), and endothelial cells release various inflammatory mediators, including pro-inflammatory enzymes, cytokines, and chemokines. Alongside these cellular responses, genetic factors that regulate inflammation also significantly contribute to the progression of stroke-related inflammatory processes (Pawluk et al., 2020). During a stroke, astrocytes are rapidly activated by damage-associated molecular pattern molecules (DAMPs) released from injured neurons and glial cells. This activation contributes to

blood–brain barrier (BBB) disruption and the recruitment of peripheral leukocytes, driven by proinflammatory cytokines, chemokines, and matrix metalloproteinases such as MMP-9 secreted by reactive astrocytes. These events collectively lead to secondary brain tissue damage (Xu et al., 2020). Following a haemorrhagic stroke, two types of damage occur: primary and secondary. The immune response is believed to play a major role in the secondary phase. Several immunological and inflammatory mechanisms contribute to this stage. For instance, thrombin and blood clots can activate the protease and complement systems, leading to receptor activation and cell lysis. Inflammation during secondary injury can also disrupt the BBB through matrix metalloproteinases (MMPs) and cytokines, increasing capillary permeability and worsening brain edema (Zhao et al., 2022).

3.3 Mitochondrial dysfunction

Intracellular organelles like mitochondria have a double membrane and play a crucial role in energy production, cell cycle regulation, and apoptosis. Under low oxygen conditions, the mitochondrial respiratory chain is disrupted, halting ATP synthesis. Electrons accumulate at complexes I and III because they enter complex I more rapidly than they move through complex IV. This imbalance slows the electron transport chain and proton pumping across the inner mitochondrial membrane, ultimately reducing the mitochondrial membrane potential (Jurcau and Ardelean, 2021). Mitochondrial complex I (NADH dehydrogenase), the first component of the mitochondrial respiratory chain (MRC), is strongly implicated in many neurodegenerative diseases due to its role in excessive ROS production and membrane polarization regulation. While complex II (succinate dehydrogenase) also contributes to ROS generation and apoptotic cell death, its involvement in ischemia-induced MRC dysfunction is comparatively limited. Following ischemic injury, both intrinsic and extrinsic apoptotic pathways are activated, involving mitochondrial membrane potential loss, MRC alterations, cytochrome c release, disrupted redox balance, and impaired antioxidant defenses (Reverse Electron Transport at Mitochondrial, 2024).

3.4 Autophagy

Controlled cell death is vital for maintaining host defense and tissue homeostasis. PANoptosis, a newly identified form of regulated cell death, integrates features of pyroptosis, apoptosis, and necroptosis. A key link between these pathways is the Caspase family, which plays roles in both pyroptosis and apoptosis, indicating a shared evolutionary origin. Caspases are broadly classified into inflammatory caspases, such as caspase-1, -4, -5, and -11, and apoptotic caspases, such as caspase-3, -6, -7, -8, -9, and -10, reflecting their distinct but interconnected functions (Tian et al., 2025). Pyroptosis is a form of programmed inflammatory cell death driven by caspase-1 activation, characterized by cell swelling and membrane rupture. This process leads to the release of pro-inflammatory cytokines like interleukin-1β and interleukin-18. A key mediator of pyroptosis is the Gasdermin D (GSDMD) protein, which forms pores in the cell membrane, triggering inflammation and cell death. These mechanisms are closely associated with the progression of inflammation-related conditions, including stroke, neurodegenerative diseases, and brain injury (Sun and Zhu, 2023). Apoptosis, triggered through both intrinsic and extrinsic pathways, contributes significantly to neuronal death following cerebral ischemia/reperfusion (I/R). In parallel, necroptosis, a regulated form of necrotic cell death, is primarily controlled by RIPK1, RIPK3, and MLKL. Emerging evidence suggests that necroptosis plays a crucial role in the development of ischemic stroke and related conditions. In experimental models, inhibiting necroptosis has been shown to reduce brain infarct size and improve motor and cognitive outcomes, indicating its potential neuroprotective value. Another form of programmed cell death, pyroptosis, is characterized by rapid plasma membrane rupture and the release of proinflammatory contents, further contributing to post-ischemic inflammation (Shu et al., 2022).

Autophagy is a cellular process in which cytoplasmic proteins or organelles are engulfed into vesicles that fuse with lysosomes to form autolysosomes, where the contents are subsequently degraded (Mo et al., 2020). Autophagy acts as a double-edged sword, capable of either repairing or degrading damaged neurons following an ischemic insult. However, excessive or prolonged activation of autophagy can lead to neuron damage and trigger cell death (Ajoolabady et al., 2021).

3.5 Ferroptosis

Iron is involved in several mechanisms of neuronal damage following ischemic stroke. It plays a key role in major molecular processes like free radical production, excitotoxicity, and neuroinflammation. Additionally, its accumulation can lead to ferroptosis (Guo et al., 2023). Existing research shows that ferroptosis regulation is primarily governed by the control of iron, lipid peroxidation, and several antioxidant systems, including the GSH/GPX4 axis, CoQ10/FSP1 axis, and others (Xu et al., 2023). Glutathione (Glu-Cyc-Gly), a tripeptide, helps protect cells from oxidative damage by interacting with free radicals. When glutathione (GSH) is converted to oxidized glutathione (GSSG), the antioxidant enzyme Gpx4 reduces harmful lipid peroxides to alcohols. During ferroptosis, redox-active iron accumulates through the Fenton reaction, depleting GSH reserves, inhibiting Gpx4, and triggering an excessive antioxidant response (Liu et al., 2020). Ferroptosis, a unique form of programmed cell death (PCD), is characterized by extreme iron overload and lipid peroxidation driven by ROS production. This pathway plays a crucial role in neuron death. Unlike other forms of cell death, ferroptosis is marked by the reduction or loss of mitochondrial cristae and a condensed mitochondrial membrane structure (Wang et al., 2020). Ferroptosis is characterized by distinct morphological changes in mitochondria, such as fragmentation, reduced size, rupture of the outer mitochondrial membrane (OMM), and the disappearance of mitochondrial cristae (She et al., 2023). Ferritin-rich brain tissue and iron-rich blood are the result of direct compression and stimulation of the hematoma, which weakens the blood-brain barrier and causes primary brain injury (Mo et al., 2020). The release of iron from the hematoma after intracerebral hemorrhage

TABLE 1 Chinese herbal medicines and their outcomes.

S.no	Herbal medicines	Key metabolite(s)	Mechanism of action	Reference
1	Bu-yang-huan-wu-tang	*Angelica sinensis* (Oliv.) Diels (Apiaceae), *Astragalus mongholicus* Bunge Fabaceae), *Conioselinum anthriscoides* 'Chuanxiong' (Apiaceae), *Carthamus tinctorius* L. (Asteraceae), *Carthamus tinctorius* L., Prunus persica (L) Batsch (Rosaceae), and *Paeonia lactiflora* Pall (Paeoniaceae)	Neuroprotection: BYHWT supports neuronal survival by reducing oxidative stress, preventing apoptosis, and improving mitochondrial function. Anti-inflammatory Effects: It also modulates inflammatory pathways, lowering pro-inflammatory cytokine levels and promoting neurovascular repair	Hung et al. (2015)
2	Danhong injection	*Salvia miltiorrhiza* Bunge [Lamiaceae] and *Carthamus tinctorius* L. [Asteraceae]	These processes help upregulate the expression of growth-associated protein 43 (GAP-43), promote axonal regeneration, and accelerate neural functional recovery	Han et al. (2017)
3	Sanhua	*Rheum officinale* Baill. [Polygonaceae], *Magnolia officinalis Rehder* & E.H.Wilson [Magnoliaceae], *Citrus aurantium* L. [Rutaceae], and *Hansenia weberbaueriana* [Apiaceae]	SHD significantly improved rheological parameters, including blood viscosity, fibrinogen levels, and haematocrit, thereby enhancing blood flow and reducing the risk of vascular complications	Zheng et al. (2023)
4	Zhi-gan-cao-tang	*Glycyrrhiza glabra* L. [Fabaceae],*Panax ginseng* C.A.Mey. [Araliaceae], *Neolitsea cassia* (L.) Kosterm. [Lauraceae], *Zingiber officinale* Roscoe [Zingiberaceae], *Ophiopogon japonicus* (Thunb.) Ker Gawl. [Asparagaceae], *Ziziphus jujuba* Mill. [Rhamnaceae], *Rehmannia glutinosa* (Gaertn.) Libosch. Ex DC. [Orobanchaceae], Cannabis sativa L. [Cannabaceae]	ZGCT may assist individuals with premature ventricular contractions by alleviating associated symptoms such as heart palpitations, shortness of breath (dyspnea), insomnia, and fatigue	Hung et al. (2016)
5	Ginkgo biloba leaf	*Ginkgo biloba* L. [Ginkgoaceae]	*Ginkgo biloba* extract (GBE) is developed to optimize the therapeutic potential of its leaves, with EGb761 being the standardized formulation. In traditional Chinese medicine, Ginkgo biloba leaves are believed to astringe the lungs, regulate the intestines, promote blood circulation, and nourish the heart	Zhao et al. (2021a)
6	Huanglian Jie Du decoction	*Coptis chinensis* Franch. [Ranunculaceae], *Scutellaria baicalensis* Georgi [Lamiaceae], *Phellodendron amurense* Rupr. [Rutaceae], *Gardenia jasminoides* J. Ellis [Rubiaceae]	Both extrinsic pathways, like death receptor-mediated apoptosis, and intrinsic pathways, such as those mediated by mitochondria, calpain, p53, p38, ERK, JNK, and the endoplasmic reticulum, are crucial for activating downstream apoptotic molecules that lead to cell death	Yu et al. (2022)
7	Buyang Huanwu	*Astragalus mongholicus* Bunge [Fabaceae] *Prunus persica* (L.) Batsch [Rosaceae], *Carthamus tinctorius* L. [Asteraceae] *Paeonia lactiflora* Pall. [Paeoniaceae], *Angelica sinensis* (Oliv.) Diels [Apiaceae] *Conioselinum anthriscoides* 'Chuanxiong' [Apiaceae]	By activating the PI3K-Akt signaling pathway, BHD may disrupt the progression of Ischemic stroke (IS) and induce cuproptosis, thereby exerting anti-apoptotic effects	Zhong et al. (2023)
8	Angong Niuhuang pill	*Bos taurus domesticus* Curcuma aromatica Salisb. [Zingiberaceae] *Rhinoceros unicornis* Gardenia jasminoides J. Ellis [Rubiaceae] Coptis chinensis Franch. [Ranunculaceae] *Scutellaria baicalensis* Georgi [Lamiaceae]	In brain tissue, *Angong Niuhuang* Pill has been shown to significantly reduce NO levels and NOS activity. Additionally, it can increase the erythrocyte aggregation index and platelet aggregation rate, while enhancing both blood and plasma viscosity	Xu et al. (2022)
9	Qingkailing (QKL) injection	*Isatis tinctoria* L. [Brassicaceae] *Lonicera japonica* Thunb. [Caprifoliaceae] *Concha Margaritifera* Usta, *Betula pubescens* var. pubescens [Betulaceae] *Gardenia jasminoides* J.Ellis [Rubiaceae], and *Cornus mas* L. [Cornaceae]	In a mouse model of cerebral ischemia/reperfusion, QKL injection has been shown to reduce calcium overload, enhance endothelial nitric oxide synthase expression, regulate matrix metalloproteinase-9 levels, and suppress inflammatory responses	Cheng et al. (2012)
10	Xue-Fu-Zhu-Yu Decoction (XFZYD)	*Angelica sinensis* (Oliv.) Diels (Apiaceae) Root, *Bupleurum chinensis* (Apiaceae), *Carthamus tinctorius* L. (Asteraceae), *Citrus limon (L.) Osbeck* (Rutaceae), *Cyathula officinalis* K.C. Kuan (Amaranthaceae), *Glycyrrhiza glabra L.* (Leguminosae), *Conioselinum anthriscoides* 'Chuanxiong' (Apiaceae), *Paeonia lactiflora* Pall.	XFZYD enhanced rt-PA-mediated neuroprotection in rats with thromboembolic stroke. This effect is likely due to the suppression of HIF-1α and TNF-α, which in turn inhibits inflammation (via iNOS) and apoptosis (via active caspase-3). These findings provide scientific support for the therapeutic potential of combining	Lee et al. (2011)

(Continued on following page)

TABLE 1 (*Continued*) Chinese herbal medicines and their outcomes.

S.no	Herbal medicines	Key metabolite(s)	Mechanism of action	Reference
		(Paeoniaceae), *Platycodon grandiflorus* (Jacq.) A. DC. (Campanulaceae), *Prunus persica* (L.) Batsch (Rosaceae), *Rehmannia glutinosa* (Gaertn.) Libosch. Ex DC. (Orobanchaceae)	XFZYD with rt-PA in the treatment of Ischemic stroke	

(ICH) can trigger perihematomal edema, oxidative stress, and increased ROS levels, ultimately leading to ferroptosis (Dong et al., 2023).

4 Traditional medicines in stroke management

4.1 Traditional Chinese medicine

Traditional Chinese medicine has been utilised to prevent various illnesses in Asian nations, particularly China. Chinese Materia medica, TCM preparation, and their active metabolite(s) may help treat brain damage brought on by ischemia/reperfusion (Table 1). Numerous studies on TCM's ability to protect the BBB have been published in recent years (Li et al., 2019).The botanical drug(s) resistance to platelets, such as *Toxicodendron vernicifluum* (Anacardiaceae), *Salvia miltiorrhiza* Bunge (Lamiaceae), *Biancaea sappan*. (Fabaceae), *Curcuma aromatica* Salisb.(Zingiberaceae), *CinnamomumcassiaPresl* (Lauraceae),*Paeonialactiflora* (Paeoniaceae), PanaxginsengC.A.Mey.(Araliaceae),*Carthamus tinctoriusa* (Asteraceae) (Kim and Park, 2019). Traditional Chinese medicine (TCM) offers several notable advantages in treating multisite, multitarget disorders and general regulation. Certain TCMS, including *Gastrodia elata* (Orchidaceae), *Rehmannia glutinosa* (Orobanchaceae), *Ginkgo biloba* (Ginkgoaceae), and *Panax notoginseng* (Araliaceae), show higher therapeutic effects on neurological illnesses than on other conditions. NBP, or dl-3-n-butylphthalide, has been shown to possess neuroprotective properties (Zhu et al., 2021). Phenolic glycosides are characteristic of Salidroside, which is obtained from the traditional Tibetan medicinal plant *Rhodiola rosea* (Crassulaceae). Traditional Chinese medicine is commonly used to increase the body's resistance to weariness. Numerous studies have demonstrated that it has various biological effects, including antioxidant properties (Zuo et al., 2018).

Composition of the traditional Chinese medicine of some remedies used for ischemic stroke. The botanical herbs used for the preparation of Xue-Fu-Zhu-Yu decoction and the Amount used are *Angelica sinensis* (Oliv) Diels (Apiaceae) Root 4.5 g, *Bupleurum chinensis* (Apiaceae) Root 1.5 g, *Carthamus tinctorius* L. (Asteraceae) Flower 4.5 g, *Citrus* × *limon (L.) Osbeck* (Rutaceae) Fruit 3.0 g, *Cyathula officinalis* K.C.Kuan (Amaranthaceae) Root 4.5 g, *Glycyrrhiza glabra L.* (Leguminosae) Root 1.5 g, *Conioselinum anthriscoides* 'Chuanxiong' (Apiaceae) Root 2.3 g, *Paeonia lactiflora* Pall. (Paeoniaceae) Root 3.0 g, *Platycodon grandiflorus* (Jacq.) A. DC. (Campanulaceae) Root 2.3 g, *Prunus persica* (L.). Batsch (Rosaceae) Seed 6.0 g, *R. glutinosa* (Gaertn.) Libosch. ex DC. (Orobanchaceae) Root 4 g (Lee et al., 2011). *Buyang Huanwu* decoction consist of seven botanical herbs such as *A. sinensis* (Oliv.) Diels (Apiaceae), *Astragalus mongholicus* Bunge (Fabaceae) (120 g) dried roots, *C. anthriscoides* "Chuanxiong:" (Apiaceae) 3 g dried rhizomes, *C. tinctorius* L. (Asteraceae) 3 g dried flowers *of C. tinctorius* L., Prunus persica (L) Batsch (Rosaceae) 3 g dried seeds, and *P. lactiflora* Pall (Paeoniaceae) 4.5 g dried roots (Zhang et al., 2018).

Notably, Table 2 summarizes several *in vivo* studies showcasing the molecular and functional outcomes of these TCMs, supporting their potential as adjunct therapies in ischemic stroke treatment.

4.2 Herbal medicines

"In the last 30 years, polyphenols and micronutrients in foods have gained attention for their antioxidant effects and potential to prevent diseases like cancer, heart problems, and brain disorders linked to oxidative stress. Polyphenols, made by plants, help protect against UV rays and infections. People consume about 1 g of polyphenols daily, much more than vitamin E or C, making them a major source of dietary antioxidants (Pacifici et al., 2021). Polyphenols are plant compounds classified into five groups: lignans, curcumins, stilbenes, phenolic acids, and flavonoids. In stroke models, they show neuroprotective effects by reducing brain damage and aiding recovery. Their antioxidant properties come from their ability to bind metals and neutralize reactive oxygen species. Key polyphenols like gallic acid, resveratrol, and quercetin help protect against ischemic stroke by targeting multiple pathways involved in damage and repair (Abdelsalam et al., 2023). Polyphenols have gained attention for their health benefits, especially their antioxidant and neuroprotective effects after ischemic stroke, as shown in recent studies (Zhou et al., 2021).

4.2.1 Resveratrol

Resveratrol (3,5,4′-trihydroxy-trans-diphenyl-ethylene) is a natural antioxidant that helps combat inflammation and neutralize free radicals in the body (Fodor et al., 2018). Resveratrol promotes neuroprotection in ischemic stroke by activating SIRT1 and NRF2 pathways. This enhances mitochondrial function, antioxidant defense, and stress response, helping cells survive and reducing apoptosis (Owjfard et al., 2024).

4.2.2 Gallic acid

Gallic acid is a well-absorbed polyphenol that helps reduce cerebral edema, a common issue after ischemic stroke. It also promotes microglial activation and shifts their phenotype, aiding in stroke recovery (Qu et al., 2022). Mechanistically, GA has been demonstrated to control M1-type macrophage polarization processes and, in part, suppress inflammatory responses by preventing M1-type macrophage polarization. Furthermore, it has been demonstrated that GA–chitosan complexes alter inflammatory responses in lipopolysaccharide-stimulated RAW264.7 cells (macrophage line) via affecting the cellular NF-κB, AP-1, and MAPK pathways (Qu et al., 2022).

TABLE 2 *In vivo* studies of traditional Chinese medicines used in Ischemia stroke.

S.no	Background	Methodology	Molecular findings	Conclusion	Ref
1	EGb 761, a standardized extract of Ginkgo biloba, exhibits neuroprotective effects in ischemic stroke. In a rat stroke model, MRI has been used for non-invasive evaluation of its influence on neurovascular reconstruction and axonal regeneration	Male Sprague-Dawley rats underwent permanent right middle cerebral artery occlusion (MCAO) and were treated daily with either EGb 761 (60 mg/kg) or saline for 15 days, starting 6 h post-MCAO. Functional recovery was assessed using beam walking. Structural and vascular changes were evaluated through multi-parametric MRI, histology, and Western blot analysis of remodeling-related proteins	Treatment increased p-AKT and p-GSK-3β levels, decreased p-CRMP2, and upregulated GAP-43. Additionally, it downregulated the axonal growth inhibitors NogoA and NgR	EGb 761 facilitated neurovascular restoration, enhanced endogenous neurogenesis, and supported axonal regeneration, all of which contributed to both structural and functional recovery after a stroke	Li et al. (2018)
2	EGb 761 is a standardized extract of Ginkgo biloba containing 24% ginkgo-flavonol glycosides and 6% terpene lactones. Known for its neuroprotective effects, the mechanisms behind its action, particularly those involving heme oxygenase 1 (HO1), are not well understood. This research aimed to investigate the neuroprotective potential of EGb 761 and its bioactive metabolites—Bilobalide (BB), Ginkgolide A (GA), Ginkgolide B (GB), and Terpene Free Material (TFM)—under ischemic conditions	Mice underwent permanent distal middle cerebral artery occlusion (pMCAO) and were observed for 7 days. Both HO1 knockout and wildtype (WT) mice were used to investigate the role of HO1. Treatment with EGb 761 or compounds was administered 4 h following pMCAO.	Protein levels of HO1, VEGF, and eNOS were increased in the EGb 761 and BB-treated groups, but not in the GA, GB, or TFM-treated groups, highlighting the specific role of these compounds in neurovascular protection	The HO1-induced pathway plays a key role in mediating the neuroprotective effects of EGb 761. Identifying target molecules that are elevated by natural substances provides a novel approach to enhancing stroke treatment	Shah et al. (2011)
3	The protective action of Xue-Fu-Zhu-Yu Decoction (XFZYD), either alone or in combination with recombinant tissue plasminogen activator (rt-PA), was assessed in a rat model of thromboembolic stroke. XFZYD, a traditional cardiovascular disease remedy, has been shown to promote endothelial progenitor cell angiogenesis and may be used as adjunct therapy for ischemic stroke	A rat model of cerebral thromboembolic stroke was used to assess the effects of interventions: rt-PA (8 mg/kg), XFZYD (1.5 or 3.0 g/kg/day), either alone or in combination. The outcomes were evaluated by measuring infarct volume and analyzing protein expression (TNF-α, iNOS, HIF-1, active caspase-3) through immunoblotting	XFZYD enhanced rt-PA-mediated suppression of inflammatory markers (TNF-α, iNOS) and apoptotic markers (HIF-1, caspase-3), indicating a synergistic neuroprotective mechanism	XFZYD significantly enhances rt-PA-mediated neuroprotection in thromboembolic stroke, likely by inhibiting inflammation and apoptosis. These results support the potential of XFZYD as an adjunct therapy to rt-PA for ischemic stroke treatment	(Lee et al., 2011).
4	Investigate the expression of transforming growth factor-β1 (TGF-β1) in rats with cerebral ischemia-reperfusion injury following treatment with Qingkailing injection (QKL)	Three groups of healthy Wistar rats were randomly assigned: QKL-treated, normal, and model (ischemia-reperfusion). Bilateral common carotid artery (CCA) occlusion was performed to induce cerebral ischemia-reperfusion injury. Immunohistochemistry was used to assess the expression of TGF-β1 and glial fibrillary acidic protein (GFAP) in brain regions at various time points	Following ischemia-reperfusion injury, both GFAP and TGF-β1 expression levels were elevated. However, treatment with QKL significantly reduced the expression of GFAP and TGF-β1 at various time points compared to the untreated model group	QKL may exert neuroprotective effects in cerebral ischemia-reperfusion injury by downregulating TGF-β1 expression, potentially through the inhibition of astrocyte (AST) activation	Yan-shu et al. (2008)
5	Investigation of the therapeutic effect and underlying mechanisms of Ruyi Zhenbao pill in promoting neurological recovery following cerebral ischemia/reperfusion injury in rats	Male Sprague-Dawley rats underwent middle cerebral artery occlusion (MCAO) followed by reperfusion. The rats were treated intragastrically with Ruyi Zhenbao pill at doses of 0.2, 0.4, or 0.8 g/kg for 14 days. Neurological function was assessed through cylinder, adhesive removal, and beam-walking tests. Neurogenesis and angiogenesis were evaluated via immunofluorescence staining, while levels of BDNF, NGF, and VEGF were measured using ELISA.	The beneficial effects of Ruyi Zhenbao are linked to the enhanced expression of neurotrophic and angiogenic factors, such as BDNF, NGF, and VEGF, which promote improved neurogenesis and angiogenesis	Ruyi Zhenbao pill aids neurological recovery following cerebral ischemia/reperfusion by stimulating neurogenesis and angiogenesis, primarily through the upregulation of BDNF, NGF, and VEGF.	WANG et al. (2016)
6	Investigation of the neuroprotective mechanisms of Huang-Lian Jie-Du Decoction (HLJDD) on cerebral ischemia involves understanding its	Cerebral ischemia was induced in rats using the middle cerebral artery occlusion (MCAO) model with reperfusion. The pharmacological	HLJDD's therapeutic effects are based on its ability to modulate multiple targets, influencing key neurochemical processes such as	HLJDD exerts neuroprotective effects in cerebral ischemia by modulating metabolic stress, regulating glutamate	Zhu et al. (2018)

(Continued on following page)

TABLE 2 (*Continued*) *In vivo* studies of traditional Chinese medicines used in Ischemia stroke.

S.no	Background	Methodology	Molecular findings	Conclusion	Ref
	metabolic targets and pathways through a systems biology approach	effects of HLJDD were tested, and plasma extracts from control, MCAO, and HLJDD-treated rats were analyzed using UPLC-Q-TOF/MS. Data processing was done with MassLynx and EZinfo 2.0, followed by analysis through PCA, OPLS-DA, and pathway databases (HMDB, KEGG, Metlin, SMPD). Biochemical assays on brain tissue were performed to confirm key pathways	energy metabolism, neurotransmitter balance, and neuroinflammation	metabolism, and enhancing acetylcholine function, thereby highlighting its multi-target, systems-level action	
7	The neuroprotective mechanism of Buyang Huanwu Decoction (BHD) in treating ischemic stroke is investigated, with a specific focus on the S1P/S1PR1/PI3K/Akt signaling pathway	A chemically analyzed lyophilized form of BHD was investigated using UPLC-Q-TOF/MS. A mouse model of permanent middle cerebral artery occlusion (pMCAO) was established. Male KM mice were divided into seven groups: sham, model, FTY720, BHD, BHD + W146 (S1PR1 inhibitor), SEW2871 (S1PR1 agonist), and Calycosin. Treatments were administered for 14 days. Outcomes included neurological scores (mNSS), infarct volume, and protein/mRNA expression of SphK1, SphK2, S1PR1, PI3K, Akt, and p-Akt, which were assessed by Western blot, IHC, and qRT-PCR in brain areas	SEW2871 (agonist) mimicked BHD's effects by enhancing the expression of S1PR1, Akt, and SphK2. The neuroprotective effects of BHD are likely mediated through the activation of the S1P/S1PR1/PI3K/Akt pathway and the inhibition of SphK1	BHD protects against cerebral ischemic injury by modulating the S1P/S1PR1/PI3K/Akt signaling pathway, reducing SphK1 levels, and increasing SphK2 along with downstream protective proteins. This supports BHD as a potential treatment for ischemic stroke	Liu et al. (2023)

4.2.3 Curcumin

One of the primary active metabolite(s) in Chinese botanical drug(s), curcumin, regulates the NLRP3 signalling pathway to promote neuronal repair and neuroprotection (Du et al., 2023).According to a study, pretreatment with curcumin reduces neuronal mortality in the CA1 region of the hippocampus of rats with LPS-induced depression, which is accompanied by an improvement in synaptic function (Fan et al., 2021).Additionally, curcumin therapy improved cell survival, reduced cell apoptosis, and increased Bcl-2 protein levels while decreasing caspase-3 expressions and Bax in mouse N2a cells after OGD/R injury. Curcumin treatment also stopped Bax activation and maintained the integrity of the mitochondrial membrane (Xie et al., 2018).

4.2.4 Flavonoid

Flavonoids, which are abundant in fruits and vegetables and other foods we eat every day, have been proven to have positive therapeutic effects on cerebral ischemia injury by reducing neuroinflammation. Apigenin, baicalein, Naringenin, EGCG, quercetin, rutin, and other flavonoids, for instance, have been found to inhibit and limit neuroinflammation following ischemic stroke by affecting the activation of astrocytes and microglia (Lu et al., 2024).Flavonoids are classified into six groups based on the various structures that link the two benzene rings: flavanols, flavanones, flavonols, isoflavones, flavones, and anthocyanidins (Liu S. et al., 2022).The degree of unsaturation, the location of the benzoid substituent, and the orientation of hydroxylation or methylation are some characteristics that distinguish different flavonoids from one another. Their antiproliferative and antioxidant properties are limited by their structural features (Cebova and Pechanova, 2020).The neuroprotective properties of flavonoids are found in Chinese herbal medicine. By reducing excitotoxicity, oxidative stress, inflammation, thrombin toxicity, and cell deaths, as well as by preserving the blood-brain barrier, Ca_2^+ overloading and neurogenesis, flavonoids, a type of botanical medication, have a neuroprotective effect (Zhou et al., 2024).

4.2.5 Quercetin

Quercetin reduces blood-brain interference and condenses the quantity of MMP-9 in tests for cerebral ischemia, according to antioxidant analyses that are similar to those of green tea polyphenols. By reducing lipid peroxidation and ion channel acid sensing, two factors that contribute to ion channel dysregulation, quercetin helps prevent ischemic injury (Maqbool and Zehravi, 2021). Quercetin prevents platelet aggregation by inhibiting agonists like thrombin, collagen, and ADP. It also blocks calcium signaling and binds to the GPIIb/IIIa receptor to reduce platelet activity. In C57BL/6 rats, this action helps reduce thrombosis and improves blood flow after $FeCl_3$-induced carotid artery injury (Zhang et al., 2022). Table 3 illustrates *In vivo* studies performed in the ischemic stroke using herbal medicines.

4.3 Ayurveda

The oldest and most significant tradition in India, Ayurveda, is founded on experimentation and philosophy. With an emphasis on strengthening the host's immune system, Ayurveda has a database of numerous medicinal botanical drug(s) and is accessible in several regional languages. According to several classical books, Ayurveda mentions 1200 illnesses. The 700 therapeutic botanical drug(s) in the Atharveda (c. 1200 BC), Charak Samhita, and Sushrat Samhita (c.

TABLE 3 *In vivo* studies performed for ischemia stroke using herbal medicines.

Natural compound	Animal model & treatment	Dosage & duration	Outcomes & mechanisms	Reference
Astragalosides	MCAO in rats	10–50 mg/kg, i.p., daily for 7 days	Reduced infarct size, anti-inflammatory, antioxidant, neuroprotection	Shang et al. (2022)
Baicalin	MCAO in rats	50–100 mg/kg, oral, 14 days	Reduced apoptosis, oxidative stress, improved BBB integrity	fan et al. (2025)
Glycyrrhizin	MCAO in rats	10–20 mg/kg, i.p., daily for 5 days	Suppressed HMGB1, reduced neuroinflammation, decreased infarct size	Li et al. (2024)
Ginseng	MCAO in mice	100–200 mg/kg, oral, 7 days	Enhanced neurogenesis, reduced oxidative damage, improved motor function	Liu et al. (2019)
Curcumin	MCAO in rats	80 mg/kg, oral, 10 days	Antioxidant, anti-inflammatory, reduced infarct volume	Gokce et al. (2016)
Resveratrol	MCAO in mice	20 mg/kg, i.p., daily for 14 days	Increased SIRT1 expression, reduced oxidative stress	Lopez et al. (2015)
Quercetin	MCAO in rats	50 mg/kg, oral, 7 days	Anti-inflammatory, modulated Nrf2 pathway, reduced neuronal death	Zhang et al. (2022)
Epigallocatechin Gallate (EGCG)	MCAO in mice	30 mg/kg, oral, 14 days	Reduced neuroinflammation, enhanced antioxidant defense	Sun et al. (2023)
Hesperidin	MCAO in rats	50 mg/kg, oral, 7 days	Attenuated oxidative stress, reduced BBB disruption	Lee et al. (2020a)
Berberine	MCAO in rats	100 mg/kg, oral, 14 days	Anti-inflammatory, improved mitochondrial function	Zhao et al. (2021b)

1000–500 BC) (Ara et al., 2022).*Adoxa moschatellina, Bacopa monnieri, Centella asiatica, Mucuna urens, Phyllanthus emblica, Terminalia arjuna, and Withania somnifera* are the plants that are the basis for the Mentat, also known as BR-16A. Because of its free-radical-scavenging qualities and antioxidant, it functions as a neuroprotective agent and may be utilized to help patients recover from ischemic stroke (Ibáñez et al., 2023). Ashwagandha (*W. somnifera)* is a traditional, well-known Indian medicinal plant that is frequently used as a component in Ayurvedic formulations sold to treat neurological conditions. It is helpful. Inhibition of gelatinases (MMP-9) Therapeutically active in ischemic stroke and hemorrhagic stroke (Zahiruddin et al., 2020).Ayurveda views hemiplegia as a clinical entity, "*Pakshaghata*," grouped under "*Vatavyadhi.*" The central role of Ayurveda lies during the time of stroke rehabilitation. It employs internal medicines along with procedures, including *Panchakarma* therapies. Evidence-based medicine (EBM) requires proof of effectiveness, efficiency, and safety for decision-making in patient care. Integrating individual clinical expertise with the best external clinical evidence is the core of EBM practice. Thus, empirical Ayurvedic knowledge accrued over the years can be tapped for integrated stroke rehabilitation after proper evaluation (M. A et al., 2025).

5 Mechanisms of action involved in TCIM approaches

Studies show that acupuncture combined with the Tianma Gouteng Yin formula is effective in treating acute cerebral infarction. This combination significantly improves vascular endothelial function and cerebral vascular function, including measures like pulsatility index, cerebrovascular reserve, neurological function, platelet activation, and inflammation (IL-6, hs-CRP). It also positively impacts NIHSS and BI scores (Zhang et al., 2023). TCM can treat stroke through multiple targets and pathways with lower side effects. It affects pathways like AKT/PI3K and SLC7A11/GPX4, which promote angiogenesis, neuroprotection, and reduce ferroptosis. This multi-target approach may lead to more effective stroke treatment (Fu et al., 2022). A study found that Astragaloside IV could boost the levels of SLC7A11 by activating the Nrf2/HO-1 signalling pathway. and GPX4 and ROS. In an ICH model with intravascular perforation, this activation resulted in increased antioxidant capacity and lipid peroxidation inhibition (Liu Z. et al., 2022). Through several processes, TCM has been utilized to treat stroke either alone or in conjunction with other treatments. such include promoting neurogenesis and angiogenesis, regulating the blood-brain barrier, inhibiting platelet activation, preventing inflammation, oxidative stress, and apoptosis (Chen and Jin, 2023).

6 Complementary medicines

Complementary alternative medicine encompasses a variety of goods, techniques, and structures that are not typically associated with traditional care. Geographical and cultural disparities may cause different CAMs to be utilized in Asian and African nations to treat stroke. Over the past 10 years, stroke patients' interest in complementary and alternative medicine has rapidly increased (Chang et al., 2016). Integrative medicine addresses patients' complicated demands by combining alternative therapies with traditional medical treatments, especially in cases of ischemic stroke. Nowadays, a lot of people use Chinese medicine in

conjunction with conventional therapy to prevent and cure stroke (Ni et al., 2020).

6.1 Yoga

Yoga therapy is based on the Panchakosha concept from the Taittiriya Upanishad, which views a person as having five layers: physical (Annamaya), energy or life force (Pranamaya), mind (Manomaya), intellect (Vijnanamaya), and bliss (Anandamaya) (Jasti et al., 2022). Yoga combines breathing, stretching, and balance to improve both mental and physical wellbeing. It helps calm the mind and build focus. Breathing is coordinated with various poses, whether standing, sitting, lying, or prone (Pal et al., 2023). Yoga may help control key risk factors for stroke, especially high blood pressure—the most modifiable risk factor. Evidence suggests it can safely and effectively reduce hypertension in stroke patients (Thayabaranathan et al., 2018). Yoga boosts hemoglobin and red blood cell levels, improving oxygen delivery to cells. It also helps lower blood pressure, reducing the risk of stroke and heart failure caused by blood clots (Dubey, 2024). In a pilot study by Arlene et al., 47 chronic stroke patients were randomized to either therapeutic yoga (n = 37) or a control group (n = 10). After 8 weeks, the yoga group showed significant improvements in physical function. This is important, as chronic stroke often leads to disabilities that affect daily activities and social participation (Schmid et al., 2014).

6.2 Acupuncture

Acupuncture, a key part of Traditional Chinese Medicine, involves inserting fine needles into specific body areas (Mu et al., 2023). Acupuncture has been used to treat stroke since ancient times, including body, scalp, eye, and electro-acupuncture methods. Choosing the correct points is crucial for effective treatment (Li et al., 2022). Acupuncture supports neurogenesis after ischemic injury by boosting neural stem cell activity and reducing local inhibitory factors, creating a healing-friendly environment (Mu et al., 2023). Current studies suggest that acupuncture may aid in treating ischemic stroke by reducing oxidative stress, protecting the blood-brain barrier, and regulating exosomes involved in neuroprotection, neuroplasticity, cell growth, apoptosis, immunity, and inflammation (Zhu et al., 2024).

6.2.1 Mechanism of action

Acupuncture may help reduce inflammation in ischemic stroke by lowering proinflammatory cytokines like IL-1β, IL-6, and TNF-α in the brain and blood. This effect is likely linked to inhibition of NF-κB activation, which reduces cytokine production (Kuang et al., 2024). The following sections discuss methods to prevent oxidative stress, including repairing damaged proteins, lipids, or DNA, limiting ROS-induced cell death or autophagy, reducing ROS production, and neutralizing ROS through antioxidant enzymes or other signaling pathways (Poljsak, 2011).

Acupuncture and low-intensity laser (LA) therapy are used to treat various conditions. In rats with right middle cerebral artery blockage, LA at GV20 reduced MDA levels and increased the activity of GPx, CAT in the cerebral cortex, and SOD in mitochondria (Jittiwat, 2017). A randomized controlled trial assessed acupuncture's effectiveness in ischemic stroke rehabilitation. Patients were divided into two acupuncture groups and one control group with only rehabilitative instructions. After 2 weeks, the acupuncture groups showed significant improvements in NIHSS scores, and the second acupuncture group had higher BI scores than the control. These results suggest that acupuncture may aid in functional rehabilitation post-stroke (Li et al., 2022).

6.3 Physical exercise

Exercise promotes neuroplasticity, neurotrophins, and cognitive function, while improving overall health and reducing the risk of hypokinetic diseases associated with a sedentary lifestyle (Sakakima, 2019). After a stroke, physical therapy aids in regaining function and movement. While task-specific training was once the main focus, recent attention has shifted to exercise-based rehabilitation (Pogrebnoy and Dennett, 2020). Exercise after a stroke may enhance the expression of Ang-1 and Tie-2, aiding brain recovery. Ischemic stroke can impair memory by increasing apoptosis and affecting BDNF levels in the hippocampus. Treadmill training helps recover memory by boosting BDNF expression, promoting cell growth, and reducing apoptosis (Xing et al., 2018). Cerebral ischemia triggers a cascade of events that increase cerebrovascular permeability and disrupt the blood-brain barrier (BBB), leading to brain edema. MMP-9 contributes to BBB failure by breaking down the extracellular matrix (Zhang et al., 2019). Exercise preconditioning strengthens the basal lamina and reduces blood-brain barrier (BBB) dysfunction in ischemic stroke by increasing MMP-9. Chronic cerebral hypoperfusion can be countered by treadmill exercise, which boosts MMP-9 levels, lowers occludin, and degrades zonula occludens-1 (ZO-1) (Lee SS. et al., 2020).

6.4 Dietary and nutritional supplementation

Nutrition plays a key role in stroke risk, which can be reduced by up to 80% with a healthy lifestyle, also lowering stroke risk factors (Spence, 2019). The former treatment regimen involves consuming specific nutrients (e.g., metals, fibers, fatty acids, vitamins), while the latter refers to time-restricted feeding, fasting, or nutrient restriction (e.g., carbs, amino acids). Both approaches, used in preclinical or clinical settings, help maintain nutritional balance and may slow the progression of neurological diseases (Mao et al., 2021). The former treatment regimen involves consuming specific nutrients (e.g., metals, fibers, fatty acids, vitamins), while the latter refers to time-restricted feeding, fasting, or nutrient restriction (e.g., carbs, amino acids). Both approaches, used in preclinical or clinical settings, help maintain nutritional balance and may slow the progression of neurological diseases (Ciancarelli et al., 2024). Research shows that omega-3 fatty acids, found in fish, nut oils, and leafy vegetables, improve neurotransmission and prevent membrane fluidity loss caused by cholesterol. These effects help maintain cell signaling and synaptic function. Regular intake of polyphenol-rich foods can enhance cerebral blood flow, cognition,

and promote neurogenesis and synaptogenesis. Flavonoids, present in many edible plants, may support neuroplasticity, neurogenesis, and cerebral blood flow, thanks to their anti-inflammatory and antioxidant properties (Siotto et al., 2022).

6.4.1 Vitamins

Vitamins and minerals can support neuroprotection and recovery by boosting antioxidant capacity and aiding functional recovery in stroke patients. Antioxidants like vitamin E and C help reduce oxidative damage (Lieber et al., 2018). Vitamin C administration improves microcirculation, neurological outcomes, and survival, while reducing oxidative stress, heart damage, and arrhythmias (Spoelstra-de Man et al., 2018). The study showed that supplementing with tetrahydrobiopterin, arginine, and vitamin C improved blood flow during ischemia by reducing oxidative stress and increasing endothelial NO synthase activity (Tang et al., 2022). Vitamin D may have antithrombotic effects by reducing cytokine-induced changes in tissue factor and thrombomodulin, as suggested by several experimental studies (Cui et al., 2024). EPA metabolites from Omega-3 fatty acids reduce inflammation, lower monocyte adhesion and platelet aggregation, and improve endothelial function by inhibiting COX-1/2 enzymes, leading to less production of inflammatory prostaglandins (Ueno et al., 2019).

6.4.2 Minerals

Copper (Cu) and zinc (Zn) are the most common metals in the human body and are found in high concentrations in the brain. While their exact roles in inflammation are still being studied, they are essential for controlling oxidative stress and regulating the production of free radicals (Wessels et al., 2017). Selenium (Se) is an essential mineral with roles in immune function, cancer prevention, and cell regulation. As part of the antioxidant system, selenium and its proteins may also offer neuroprotective benefits (Mirończuk et al., 2021).

6.5 Aromatherapy

Essential oils are used therapeutically in aromatherapy as a supplemental medicine to cure a variety of mental and physical illnesses and to advance patient wellbeing (Reis and Jones, 2017). Essential oil components can enter the bloodstream and cross the blood-brain barrier through various routes such as topical, subcutaneous, oral, intraperitoneal, or inhalation applications. Aromatic oils like lavender, bergamot, and curcuma have shown numerous beneficial effects (Contrada et al., 2021). It has been demonstrated that essential oils contain anti-inflammatory and antioxidant qualities in addition to psychological benefits that might lessen emotional and physical ailments like exhilaration, exhaustion, and delirium (Lim and Kim, 2024). Neurotransmitter systems involved in neuronal survival and plasticity are influenced by specific essential oils. For example, it has been demonstrated that chemicals in sandalwood oil interact with gamma-aminobutyric acid (GABA) receptors, modifying excitatory and inhibitory neurotransmission to promote neuroprotective effects (Younis and Mohamed, 2020). Essential oils like bergamot and lavender, known for their antioxidant properties, help reduce oxidative stress during ischemia. They boost antioxidant enzymes like glutathione peroxidase and superoxide dismutase, which lower ROS production and cellular damage. Additionally, these oils have anti-inflammatory effects by suppressing pro-inflammatory cytokines, reducing inflammation linked to ischemic stroke (Contrada et al., 2021).

6.6 Music therapy

Music therapy uses musical elements to improve mood and neurological function. It has shown positive results in stroke rehabilitation across various studies (Xu et al., 2022). A home-based music therapy program was developed for self-rehabilitation in chronic stroke patients. Music therapy has a significant impact on improving neurological function and can change the brain's structure and function in stroke patients (Huang et al., 2021). Thirty stroke patients received music therapy for 4 weeks. Those in the music group showed significantly better performance on the Wolf Motor Function Test, with improved timing and motion quality, compared to the mute group. Most patients in the music group could complete the task independently (Tong et al., 2015). Music can impact the brain stem, stimulate the cerebral cortex, regulate peripheral nerves, strengthen muscles, and boost physical vitality in stroke patients (Barclay et al., 2020).

7 Clinical trials of ischemia stroke

A randomized clinical trial showed that patients with acute ischemic stroke who received Tongxinluo along with their usual medication within 72 h of symptom onset had a better functional outcome compared to those who received a placebo (Dong et al., 2024). Acupuncture, as a complementary treatment, has shown benefits for ischemic stroke patients in randomized controlled trials (Zhang et al., 2015). A study using Wen Dan Decoction in ischemic stroke patients showed it helps improve and stabilize atherosclerotic plaques (Xu et al., 2015). Table 4 illustrates Clinical studies of ischemic stroke using traditional medicines and complementary medicines.

8 Limitations and future directions

Traditional and herbal formulations often vary in preparation methods, dosages, and phytochemical profiles, leading to inconsistent therapeutic outcomes. Most evidence supporting TCIM approaches is based on preclinical (*in vitro*/*in vivo*) models, while high-quality, large-scale randomized controlled trials (RCTs) in humans remain scarce. Many studies evaluate outcomes over brief periods, failing to assess long-term safety, efficacy, or the risk of relapse. Additionally, most clinical trials involve homogenous populations, limiting the applicability of results across diverse demographics and co-morbid conditions. Potential interactions between herbal remedies and standard stroke medications are seldom explored, raising concerns about safety. Methodological flaws, such as inadequate randomization, lack of placebo controls, and absence of blinding, further compromise the reliability of findings. Studies also vary widely in their outcome measures and definitions of key clinical endpoints like

TABLE 4 Clinical studies of ischemic stroke using traditional medicines and complementary medicines.

S.no	Category	Types of medicines	Study type	Outcome measured	Ref
1	Traditional Medicines	Herbal Medicine	Randomised Clinical Trial	Oral capsules of Tongxinluo improve the Neuroprotection, anti-inflammatory, and antioxidative effects	Dong et al. (2024)
2	Complementary medicines	Acupuncture	Multicenter single-blinded, randomized controlled trial. Eight hundred sixty-two hospitalized patients	Wider use of acupuncture following a stroke could have a significant positive impact on health if the above prospective advantages are verified	Zhang et al. (2015)
3	Traditional Medicines	Traditional Chinese Medicines	Randomised Clinical Trial	The enhancement of hemorrhagic and ischemic strokes demonstrates WDD in this study. WDD-induced Qi-Energy may keep the blood in the cerebral arteries, halting the bleeding and stabilizing the atherosclerotic plaques	Xu et al. (2015)
4	Traditional Medicines	Herbal medicines	Randomised Placebo-controlled clinical study	This study offers proof that Di-Huang-yin-zi DHYZ may be in the ischemic region's blood flow enhancement, anti-apoptotic, and anti-free radical mechanisms. This study will offer valuable insights into the potential efficacy of DHYZ as a patient adjuvant therapy	Yu et al. (2015)
5	Complementary medicines	Yoga	A Non-Inferiority Randomized Controlled Trial conducted using 36 patients	Yoga is beneficial in enhancing chronic post-stroke patients' physical capabilities and mental health	Lenoir dit et al. (2024)
6	Complementary medicines	Yoga	A Pilot Controlled Study	Patients with chronic stroke may benefit from yoga's ability to strengthen hand grips and lower blood pressure when combined with therapy. Age and sex may also have an impact on rehabilitation outcomes	Lai et al. (2023)
7	Traditional medicines	Ayurveda	A prospective study	Self-selected patients who were 1-month post-stroke reported that they tolerated Ayurvedic massage well. These self-selected patients had improved their standing and discharge mobility more quickly	Sankaran et al. (2019)
8	Complementary medicine	Aromatherapy	Randomised clinical trial study using 70 patients	In addition to lowering malondialdehyde and raising antioxidant levels, the use of lavender 10% essential oil helped patients with ischemic stroke symptoms such as confusion, speech difficulties, and muscle weakness	Salehi et al. (2019)
9	Traditional medicine	Traditional Chinese medicine	Randomized Clinical Trial	Xuesaitong soft capsules raised the chance of functional independence at 3 months in ischemic stroke patients in this randomized clinical trial, suggesting that this could be a safe and efficient alternative treatment to enhance prognosis in this population	Wu et al. (2023)
10	Traditional medicine	Herbal medicines	Randomized clinical trial Over the course of the 4-day investigation, the control group received standard stroke care, whereas the saffron group received standard care along with a 400 mg/day (200 mg twice day) saffron capsule	Saffron significantly reduced oxidative stress markers and improved antioxidant levels in patients with acute ischemic stroke. It also led to reduction in stroke severity after 4 days. The improvements were correlated with higher GSH and TAC levels and lower MDA levels. These findings suggest saffron extract may be a promising therapeutic option for ischemic stroke recovery	Gudarzi et al. (2022)
11	Complementary medicine	Physical exercise	A randomized controlled trial	The study findings demonstrate that, compared to standard care, a 1-year exercise intervention gradually enhanced executive functioning. The medical	Deijle et al. (2024)

(Continued on following page)

TABLE 4 (*Continued*) Clinical studies of ischemic stroke using traditional medicines and complementary medicines.

S.no	Category	Types of medicines	Study type	Outcome measured	Ref
				providers to consider incorporating exercise and physical activity into the conventional secondary stroke prevention treatment for individuals with or small stroke	
12	Complementary medicines	Acupuncture	Randomized Controlled Trial (n = 162)	In the subacute stage of an ischemic stroke, acupuncture seems to be safe. The health benefits of using the medication more widely could be significant if the possible advantages seen are validated in larger research in the future	Zhang et al. (2015)

"functional recovery" and "neuroprotection," which affects comparability and reproducibility. Furthermore, several trials do not adequately justify the choice of dose, frequency, or duration of treatment. Although some studies demonstrate beneficial effects, they often lack detailed investigations into the molecular mechanisms involved, which are essential for scientific validation and regulatory approval.

To overcome these limitations, future research should prioritize the development of standardized formulations with defined phytochemical profiles and optimized dosing protocols. Multicenter, double-blind, placebo-controlled RCTs involving larger and more diverse patient populations are needed to establish clinical efficacy. Mechanistic studies employing systems biology and omics technologies (e.g., genomics, proteomics, metabolomics) can help elucidate how TCIM therapies exert their effects. Comprehensive safety evaluations, including herb-drug interaction studies and long-term toxicity assessments, are crucial. Integrative models that combine conventional and traditional medicine could support a more personalized approach to stroke management. Finally, the use of advanced imaging techniques and validated biomarkers would enable more objective and reliable assessment of therapeutic outcomes.

9 Conclusion

This review underscores the potential of Traditional, Complementary, and Integrative Medicine (TCIM) in addressing the multifactorial pathophysiology of ischemic stroke. The neuroprotective, anti-inflammatory, and antioxidant properties of various herbal and alternative therapies may support improved neurological recovery and reduce the risk of stroke recurrence. Integrating TCIM into conventional stroke care requires a multidisciplinary framework that fosters collaboration among clinicians, researchers, and traditional medicine practitioners. However, to enable safe and effective implementation, further rigorous studies are needed to standardise formulations, establish therapeutic efficacy, and evaluate safety profiles. Such evidence-based integration could ultimately transform the global paradigm of ischemic stroke management.

Author contributions

SS: Writing – original draft, Data curation. RB: Formal Analysis, Resources, Supervision, Validation, Visualization, Writing – review and editing. LD: Data curation, Investigation, Project administration, Supervision, Writing – review and editing. PR: Writing – original draft. NH: Investigation, Project administration, Resources, Supervision, Visualization, Writing – review and editing. ASS: Conceptualization, Investigation, Methodology, Project administration, Resources, Supervision, Writing – review and editing.

Funding

The author(s) declare that no financial support was received for the research and/or publication of this article.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declare that no Generative AI was used in the creation of this manuscript.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

Abdelsalam, S. A., Renu, K., Zahra, H. A., Abdallah, B. M., Ali, E. M., Veeraraghavan, V. P., et al. (2023). Polyphenols mediate neuroprotection in cerebral ischemic stroke—an update. *Nutrients* 15, 1107. doi:10.3390/nu15051107

Ajoolabady, A., Wang, S., Kroemer, G., Penninger, J. M., Uversky, V. N., Pratico, D., et al. (2021). Targeting autophagy in ischemic stroke: from molecular mechanisms to clinical therapeutics. *Pharmacol. Ther.* 225, 107848. doi:10.1016/j.pharmthera.2021.107848

Akashlal, M., Nair, P.P., Nair, D.R., Ahmad, A., Chandrasekhararao, B., and Sudhakar, D. (2025). A systematic review on safety and efficacy of ayurvedic interventions in hemiplegia (pakshaghata). J. Evid. Based Integr. Med. 30, 2515690X241304523. doi:10.1177/2515690X241304523

Ara, I., Maqbool, M., Gani, I., Kashmir, O., and Kashmir, I. (2022). Neuroprotective activity of herbal medicinal products: a review. *Int. J. Cyrrent Reseach Physiology Pharmacol.* 6. Available online at: https://ijcrpp.com/index.php/ijcrpp/article/view/53.

Barclay, R. E., Stevenson, T. J., Poluha, W., Semenko, B., and Schubert, J. (2020). Mental practice for treating upper extremity deficits in individuals with hemiparesis after stroke. *Cochrane Database Syst. Rev.* 2020. doi:10.1002/14651858.cd005950.pub5

Briones-Valdivieso, C., Briones, F., Orellana-Urzúa, S., Chichiarelli, S., Saso, L., and Rodrigo, R. (2024). Novel multi-antioxidant approach for ischemic stroke therapy targeting the role of oxidative stress. *Biomedicines* 12, 501. doi:10.3390/biomedicines12030501

Cebova, M., and Pechanova, O. (2020). Protective effects of polyphenols against ischemia/reperfusion injury. *Molecules* 25, 3469. doi:10.3390/molecules25153469

Chang, C. C., Lee, Y. C., Lin, C. C., Chang, C. H., Chiu, C. D., Chou, L. W., et al. (2016). Characteristics of traditional Chinese medicine usage in patients with stroke in Taiwan: a nationwide population-based study. *J. Ethnopharmacol.* 186, 311–321. doi:10.1016/j.jep.2016.04.018

Chen, B., and Jin, W. (2023). A comprehensive review of stroke-related signaling pathways and treatment in western medicine and traditional Chinese medicine. *Front. Neurosci.* 17, 1200061. doi:10.3389/fnins.2023.1200061

Chen, H., Chen, X., Luo, Y., and Shen, J. (2018). Potential molecular targets of peroxynitrite in mediating blood–brain barrier damage and haemorrhagic transformation in acute ischaemic stroke with delayed tissue plasminogen activator treatment. *Free Radic. Res.* 52 (11–12), 1220–1239. doi:10.1080/10715762.2018.1521519

Chen, Z., Mo, J., Xu, J., Qin, H., Zheng, H., Pan, Y., et al. (2019). Risk profile of ischemic stroke caused by small-artery occlusion vs. Deep intracerebral hemorrhage. *Front. Neurol.* 10, 10. doi:10.3389/fneur.2019.01213

Cheng, F., Wang, X., Lu, Y., Zhong, X., Zhao, Y., and Wang, Q. (2012). Chinese medicine injection qingkailing for treatment of acute ischemia stroke: a systematic review of randomized controlled trials. *Evidence-Based Complementary Altern. Med.* 2012, 213172–213177. doi:10.1155/2012/213172

Ciancarelli, I., Morone, G., Iosa, M., Cerasa, A., Calabrò, R. S., and Tozzi Ciancarelli, M. G. (2024). Neuronutrition and its impact on post-stroke neurorehabilitation: modulating plasticity through diet. *Nutrients* 16 (21), 3705. doi:10.3390/nu16213705

Contrada, M., Cerasa, A., Tonin, P., Bagetta, G., and Scuteri, D. (2021). Aromatherapy in stroke patients: is it time to begin? *Front. Behav. Neurosci.* 15, 749353. doi:10.3389/fnbeh.2021.749353

Cui, P., Hou, H., Song, B., Xia, Z., and Xu, Y. (2024). Vitamin D and ischemic stroke - association, mechanisms, and therapeutics. *Ageing Res. Rev.* 96, 102244. doi:10.1016/j.arr.2024.102244

Deijle, I. A., Jonkers, I. M., Hooghiemstra, A. M., Engels, G., Twisk, J. W. R., Weinstein, H. C., et al. (2024). Effects of a 1 year aerobic and strength training on cognitive functioning after transient ischemic attack or minor stroke: a randomized controlled trial. *J. Stroke Cerebrovasc. Dis.* 33 (1), 107441. doi:10.1016/j.jstrokecerebrovasdis.2023.107441

Dong, W., Gong, F., Zhao, Y., Bai, H., and Yang, R. (2023). Ferroptosis and mitochondrial dysfunction in acute central nervous system injury. *Front. Cell. Neurosci.* 17, 1228968. doi:10.3389/fncel.2023.1228968

Dong, Y., Jiang, K., Li, Z., Zhou, Y., Ju, B., Min, L., et al. (2024). Tongxinluo and functional outcomes among patients with acute ischemic stroke: a randomized clinical trial. *JAMA Netw. Open* 7 (9), e2433463. doi:10.1001/jamanetworkopen.2024.33463

Du, X., Amin, N., Xu, L., Botchway, B. O. A., Zhang, B., and Fang, M. (2023). *Pharmacological intervention of curcumin via the NLRP3 inflammasome in ischemic stroke*, 14. Frontiers Media SA. Frontiers in Pharmacology. doi:10.3389/fphar.2023.1249644

Dubey, S. (2024). A review on yoga practice and its effects. Available online at: https://www.researchgate.net/publication/359157970.

Fan, C., Li, Y., Lan, T., Wang, W., Mao, X., and Yu, S. Y. (2021). Prophylactic treatment of curcumin in a rat model of depression by attenuating hippocampal synaptic loss. *Food Funct.* 12 (22), 11202–11213. doi:10.1039/d1fo02676c

fan, Li Y., fan, Z. Y., Huang, C., and Jiang, J. (2025). Baicalin improves neurological outcomes in mice with ischemic stroke by inhibiting astrocyte activation and neuroinflammation. *Int. Immunopharmacol.* 149, 114186. doi:10.1016/j.intimp.2025.114186

Fodor, K., Tit, D. M., Pasca, B., Bustea, C., Uivarosan, D., Endres, L., et al. (2018). Long-Term resveratrol supplementation as a secondary prophylaxis for stroke. *Oxid. Med. Cell. Longev.* 2018, 4147320. doi:10.1155/2018/4147320

Fu, C., Wu, Y., Liu, S., Luo, C., Lu, Y., Liu, M., et al. (2022). Rehmannioside A improves cognitive impairment and alleviates ferroptosis via activating PI3K/AKT/Nrf2 and SLC7A11/GPX4 signaling pathway after ischemia. *J. Ethnopharmacol.* 289, 115021. doi:10.1016/j.jep.2022.115021

Gokce, E. C., Kahveci, R., Gokce, A., Sargon, M. F., Kisa, U., Aksoy, N., et al. (2016). Curcumin attenuates inflammation, oxidative stress, and ultrastructural damage induced by spinal cord ischemia–reperfusion injury in rats. *J. Stroke Cerebrovasc. Dis.* 25 (5), 1196–1207. doi:10.1016/j.jstrokecerebrovasdis.2016.01.008

Granger, D. N., and Kvietys, P. R. (2015). Reperfusion injury and reactive oxygen species: the evolution of a concept. *Redox Biol.* 6, 524–551. doi:10.1016/j.redox.2015.08.020

Gudarzi, S., Jafari, M., Pirzad Jahromi, G., Eshrati, R., Asadollahi, M., and Nikdokht, P. (2022). Evaluation of modulatory effects of saffron (*Crocus sativus* L.) aqueous extract on oxidative stress in ischemic stroke patients: a randomized clinical trial. *Nutr. Neurosci.* 25 (6), 1137–1146. doi:10.1080/1028415X.2020.1840118

Guo, J., Tuo, Q., and Lei, P. (2023). Iron, ferroptosis, and ischemic stroke. *J. Neurochem.* 165 (4), 487–520. doi:10.1111/jnc.15807

Han, S. Y., Hong, Z. Y., Xie, Y. H., Zhao, Y., and Xu, X. (2017). Therapeutic effect of Chinese herbal medicines for post stroke recovery: a traditional and network meta-analysis. *Med. Madr.* 96, e8830. doi:10.1097/MD.0000000000008830

Haupt, M., Gerner, S. T., Bähr, M., and Doeppner, T. R. (2023). Neuroprotective strategies for ischemic stroke—future perspectives. *Int. J. Mol. Sci.* 24, 4334. doi:10.3390/ijms24054334

Huang, W. H., Dou, Z. L., Jin, H. M., Cui, Y., Li, X., and Zeng, Q. (2021). The effectiveness of music therapy on hand function in patients with stroke: a systematic review of randomized controlled trials. *Front. Neurol.* 12, 641023. doi:10.3389/fneur.2021.641023

Hung, I. L., Hung, Y. C., Wang, L. Y., Hsu, S. F., Chen, H. J., Tseng, Y. J., et al. (2015). Chinese herbal products for ischemic stroke. *Am. J. Chin. Med.* 43 (7), 1365–1379. doi:10.1142/S0192415X15500779

Hung, Y. C., Cheng, Y. C., Muo, C. H., Chiu, H. E., Liu, C. T., and Hu, W. L. (2016). Adjuvant Chinese herbal products for preventing ischemic stroke in patients with atrial fibrillation. *PLoS One.* 11 (7), e0159333. doi:10.1371/journal.pone.0159333

Hurd, M. D., Goel, I., Sakai, Y., and Teramura, Y. (2021). Current status of ischemic stroke treatment: from thrombolysis to potential regenerative medicine. *Regen. Ther.* 18, 408–417. doi:10.1016/j.reth.2021.09.009

Ibáñez, B., Melero, A., Montoro, A., Merino-Torres, J. F., Soriano, J. M., and San Onofre, N. (2023). A narrative review of the herbal preparation of ayurvedic, traditional Chinese, and kampō medicines applied as radioprotectors. *Antioxidants* 12 (7), 1437. doi:10.3390/antiox12071437

Jasti, N., Reddy, A. V., Ramakrishna, K. K., Bhargav, H., and Kulkarni, G. B. (2022). "Role of yoga in stroke management: current evidence and future directions," in *The principles and practice of yoga in cardiovascular medicine* (Springer Nature), 253–265.

Jittiwat, J. (2017). Laser acupuncture at GV20 improves brain damage and oxidative stress in animal model of focal ischemic stroke. *J. Acupunct. Meridian Stud.* 10 (5), 324–330. doi:10.1016/j.jams.2017.08.003

Juan, C. A., Pérez de la Lastra, J. M., Plou, F. J., and Pérez-Lebeña, E. (2021). The chemistry of reactive oxygen species (ROS) revisited: outlining their role in biological macromolecules (DNA, lipids and proteins) and induced pathologies. *Int. J. Mol. Sci.* 22 (9), 4642. doi:10.3390/ijms22094642

Jurcau, A., and Ardelean, A. I. (2022). Therapeutic strategies in huntington's disease: from genetic defect to gene therapy. *Oxidative Stress Ischemia/Reperfusion Inj. Follow. Acute Ischemic Stroke* 10, 1895. doi:10.3390/biomedicines10081895

Jurcau, A., and Ardelean, I. A. (2021). Molecular pathophysiological mechanisms of ischemia/reperfusion injuries after recanalization therapy for acute ischemic stroke. *NeuroSignals* 20, 727–744. doi:10.31083/j.jin2003078

Kim, K., and Park, K. (2019). A review of antiplatelet activity of traditional medicinal herbs on integrative medicine studies. *Evid. Based. Complement. Altern. Med.* 2019, 7125162. Il. doi:10.1155/2019/7125162

Koutsaliaris, I. K., Moschonas, I. C., Pechlivani, L. M., Tsouka, A. N., and Tselepis, A. D. (2021). Inflammation, oxidative stress, vascular aging and atherosclerotic ischemic stroke. *Curr. Med. Chem.* 29 (34), 5496–5509. doi:10.2174/0929867328666210921161711

Kuang, H., Zhu, X., Chen, H., Tang, H., and Zhao, H. (2024). Corrigendum: the immunomodulatory mechanism of acupuncture treatment for ischemic stroke: research progress, prospects, and future direction. *Front. Immunol.* 15, 1468179. doi:10.3389/fimmu.2024.1468179

Kuriakose, D., and Xiao, Z. (2020). Pathophysiology and treatment of stroke: present status and future perspectives. *Int. J. Mol. Sci.* 21, 7609–7624. doi:10.3390/ijms21207609

Lai, Y. T., Huang, H. L., Hsieh, C. C., Lin, C. H., Yang, J. C., Tsou, H. H., et al. (2023). The effects of yoga exercise on blood pressure and hand grip strength in chronic stroke patients: a pilot controlled study. *Int. J. Environ. Res. Public Health* 20 (2), 1108. doi:10.3390/ijerph20021108

Lee, B. K., Hyun, S. W., and Jung, Y. S. (2020a). Yuzu and hesperidin ameliorate blood-brain barrier disruption during hypoxia via antioxidant activity. *Antioxidants* 9 (9), 843. doi:10.3390/antiox9090843

Lee, J. J., Hsu, W. H., Yen, T. L., Chang, N. C., Luo, Y. J., Hsiao, G., et al. (2011). Traditional Chinese medicine, Xue-Fu-Zhu-Yu decoction, potentiates tissue plasminogen activator against thromboembolic stroke in rats. *J. Ethnopharmacol.* 134 (3), 824–830. doi:10.1016/j.jep.2011.01.033

Lee, S. S., Kim, C. J., Shin, M. S., and Lim, B. V. (2020b). Treadmill exercise ameliorates memory impairment through ERK-Akt-CREB-BDNF signaling pathway in cerebral ischemia gerbils. *J. Exerc Rehabil.* 16 (1), 49–57. doi:10.12965/jer.2040014.007

Lenoir dit, C. R., Verdun, S., Triquenot-Bagan, A., Tourny, C., and Coquart, J. (2024). Yoga in the rehabilitation of post-stroke sequelae: a non-inferiority randomized controlled trial. *J. Integr. Complementary Med.* 30 (6), 543–553. doi:10.1089/jicm.2023.0315

Li, L., Zhu, W., Lin, G., Chen, C., Tang, D., Lin, S., et al. (2022). Effects of acupuncture in ischemic stroke rehabilitation: a randomized controlled trial. *Front. Neurol.* 13, 13. doi:10.3389/fneur.2022.897078

Li, M. Z., Zhang, Y., Zou, H. Y., Ouyang, J. Y., Zhan, Y., Yang, L., et al. (2018). Investigation of Ginkgo biloba extract (EGb 761) promotes neurovascular restoration and axonal remodeling after embolic stroke in rat using magnetic resonance imaging and histopathological analysis. *Biomed. & Pharmacother.* 103, 989–1001. doi:10.1016/j.biopha.2018.04.125

Li, Y., Wu, J., Du, F., Tang, T., Lim, J. C. W., Karuppiah, T., et al. (2024). Neuroprotective potential of glycyrrhizic acid in ischemic stroke: mechanisms and therapeutic prospects. *Pharmaceuticals* 17 (11), 1493. doi:10.3390/ph17111493

Li, Y., Zhong, W., Jiang, Z., and Tang, X. (2019). New progress in the approaches for blood–brain barrier protection in acute ischemic stroke. *Brain Res. Bull.* 144, 46–57. doi:10.1016/j.brainresbull.2018.11.006

Lieber, A. C., Hong, E., Putrino, D., Nistal, D. A., Pan, J. S., and Kellner, C. P. (2018). Nutrition, energy expenditure, dysphagia, and self-efficacy in stroke rehabilitation: a review of the literature. *Brain Sci.* 8 (12), 218. doi:10.3390/brainsci8120218

Lim, A. R., and Kim, H. K. (2024). The effects of aromatherapy on stroke symptoms in stroke patients: a systematic review and meta-analysis. *Korean J. Adult Nurs.* 36 (2), 85. doi:10.7475/kjan.2024.36.2.85

Liu, J., Guo, Z. N., Yan, X. L., Huang, S., Ren, J. X., Luo, Y., et al. (2020). Crosstalk between autophagy and ferroptosis and its putative role in ischemic stroke. *Front. Cell. Neurosci.* 14, 577403. doi:10.3389/fncel.2020.577403

Liu, L., Anderson, G. A., Fernandez, T. G., and Doré, S. (2019). Efficacy and mechanism of Panax ginseng in experimental stroke. *Front. Neurosci.* 13, 13. doi:10.3389/fnins.2019.00294

Liu, S., Lin, F., Wang, J., Pan, X., Sun, L., and Wu, W. (2022a). Polyphenols for the treatment of ischemic stroke: new applications and insights. *Molecules* 27, 4181. doi:10.3390/molecules27134181

Liu, W., Zhou, X., Zeng, K., Nie, C., Huang, J., Zhu, L., et al. (2023). Study on the action mechanism of Buyang Huanwu Decoction against ischemic stroke based on S1P/S1PR1/PI3K/Akt signaling pathway. *J. Ethnopharmacol.* 312, 116471. doi:10.1016/j.jep.2023.116471

Liu, Z., Zhou, Z., Ai, P., Zhang, C., Chen, J., and Wang, Y. (2022b). Astragaloside IV attenuates ferroptosis after subarachnoid hemorrhage via Nrf2/HO-1 signaling pathway. *Front. Pharmacol.* 13, 13. doi:10.3389/fphar.2022.924826

Lopez, M. S., Dempsey, R. J., and Vemuganti, R. (2015). Resveratrol neuroprotection in stroke and traumatic CNS injury. *Neurochem. Int.* 89, 75–82. doi:10.1016/j.neuint.2015.08.009

Lu, W., Chen, Z., and Wen, J. (2024). Flavonoids and ischemic stroke-induced neuroinflammation: focus on the glial cells. *Biomed. Pharmacother.* 170. doi:10.1016/j.biopha.2023.115847

Mao, X. Y., Yin, X. X., Guan, Q. W., Xia, Q. X., Yang, N., Zhou, H. H., et al. (2021). Dietary nutrition for neurological disease therapy: current status and future directions. *Pharmacol. Ther.* 226, 107861. doi:10.1016/j.pharmthera.2021.107861

Maqbool, M., and Zehravi, M. (2021). Neuroprotective role of polyphenols in treatment of neurological disorders: a review. *Interventional Pain Med. Neuromodulation* 1 (1). doi:10.5812/ipmn.117170

McFarlane, S. I., Sica, D. A., and Sowers, J. R. (2005). Stroke in patients with diabetes and hypertension. *J. Clin. Hypertens.* 7 (5), 286–292. doi:10.1111/j.1524-6175.2005.04379.x

Mirończuk, A., Kapica-Topczewska, K., Socha, K., Soroczyńska, J., Jamiołkowski, J., Kułakowska, A., et al. (2021). Selenium, copper, zinc concentrations and Cu/Zn, Cu/Se molar ratios in the serum of patients with acute ischemic stroke in northeastern Poland—a new insight into stroke pathophysiology. *Nutrients* 13 (7), 2139. doi:10.3390/nu13072139

Mo, Y., Sun, Y. Y., and Liu, K. Y. (2020). Autophagy and inflammation in ischemic stroke. *Neural Regen. Res.* 15, 1388–1396. doi:10.4103/1673-5374.274331

Mu, J. D., Ma, L. X., Zhang, Z., Qian, X., Zhang, Q. Y., Ma, L. H., et al. (2023). The factors affecting neurogenesis after stroke and the role of acupuncture. *Front. Neurol.* 14, 1082625. doi:10.3389/fneur.2023.1082625

Ni, X., Lin, H., Li, H., Liao, W., Luo, X., Wu, D., et al. (2020). Evidence-based practice guideline on integrative medicine for stroke 2019. *J. Evid. Based Med.* 13 (2), 137–152. doi:10.1111/jebm.12386

Owjfard, M., Rahimian, Z., Karimi, F., Borhani-Haghighi, A., and Mallahzadeh, A. (2024). A comprehensive review on the neuroprotective potential of resveratrol in ischemic stroke. *Heliyon* 10, e34121. doi:10.1016/j.heliyon.2024.e34121

Pacifici, F., Rovella, V., Pastore, D., Bellia, A., Abete, P., Donadel, G., et al. (2021). Polyphenols and ischemic stroke: insight into one of the best strategies for prevention and treatment. *Nutrients* 13, 1967. doi:10.3390/nu13061967

Pal, R., Adhikari, D., Heyat, M. B., Ullah, I., and You, Z. (2023). Yoga meets intelligent internet of things: recent challenges and future directions. *Bioengineering* 10 (4), 459. doi:10.3390/bioengineering10040459

Pawluk, H., Woźniak, A., Grześk, G., Kołodziejska, R., Kozakiewicz, M., Kopkowska, E., et al. (2020). The role of selected pro-inflammatory cytokines in pathogenesis of ischemic stroke. *Clin. Interventions Aging* 15, 469–484. doi:10.2147/CIA.S233909

Pogrebnoy, D., and Dennett, A. (2020). Exercise programs delivered according to guidelines improve mobility in people with stroke: a systematic review and meta-analysis. *Arch. Phys. Med. Rehabil.* 101 (1), 154–165. doi:10.1016/j.apmr.2019.06.015

Poljsak, B. (2011). Strategies for reducing or preventing the generation of oxidative stress. *Oxid. Med. Cell. Longev.* 2011, 1–15. doi:10.1155/2011/194586

Qin, C., Yang, S., Chu, Y. H., Zhang, H., Pang, X. W., Chen, L., et al. (2022a). Signaling pathways involved in ischemic stroke: molecular mechanisms and therapeutic interventions. *Signal Transduct. Target Ther.* 7 (1), 215. doi:10.1038/s41392-022-01064-1

Qin, S., Zhang, Z., Zhao, Y., Liu, J., Qiu, J., Gong, Y., et al. (2022b). The impact of acupuncture on neuroplasticity after ischemic stroke: a literature review and perspectives. *Front. Cell. Neurosci.* 16, 817732. doi:10.3389/fncel.2022.817732

Qu, Y., Wang, L., and Mao, Y. (2022). Gallic acid attenuates cerebral ischemia/reperfusion-induced blood–brain barrier injury by modifying polarization of microglia. *J. Immunotoxicol.* 19 (1), 17–26. doi:10.1080/1547691X.2022.2043494

Rajahthurai, S. D., Farrukh, M. J., Makmor-Bakry, M., Tan, H. J., Fatokun, O., Mohd, S. S., et al. (2022). Use of complementary and alternative medicine and adherence to medication therapy among stroke patients: a meta-analysis and systematic review. *Front. Pharmacol.* 13, 870641. doi:10.3389/fphar.2022.870641

Ranjan, K. N. (2023). Herbal drugs for neuroprotection in ischemic stroke. *Certif. J. | Kar World J. Pharm. Res* 12. doi:10.20959/wjpr202314-29334

Reis, D., and Jones, T. (2017). Aromatherapy: using essential oils as a supportive therapy. *Clin. J. Oncol. Nurs.* 21 (1), 16–19. doi:10.1188/17.CJON.16-19

Reverse Electron Transport at Mitochondrial (2024). Reverse electron transport at mitochondrial complex I in ischemic stroke. *Aging Age-Related Dis.*

Saceleanu, V. M., Toader, C., Ples, H., Covache-Busuioc, R. A., Costin, H. P., Bratu, B. G., et al. (2023). Integrative approaches in acute ischemic stroke: from symptom recognition to future innovations. *Biomedicines* 11 (10), 2617. doi:10.3390/biomedicines11102617

Sakakima, H. (2019). Endogenous neuroprotective potential due to preconditioning exercise in stroke. *Phys. Ther. Res.* 22 (2), 45–52. doi:10.1298/ptr.R0006

Salehi, S. S., Lorigooini, Z., Jivad, N., and Ghadimi, K. (2019). Research paper: effect of aromatherapy with lavender 10% essential oil on motor function, speech and delirium in patients with acute thrombotic cerebral ischemia. *Casp. J. Neurol. Sci.* 5 (2), 49–55. doi:10.29252/CJNS.5.17.49

Salvagno, M., Sterchele, E. D., Zaccarelli, M., Mrakic-Sposta, S., Welsby, I. J., Balestra, C., et al. (2024). Oxidative stress and cerebral vascular tone: the role of reactive oxygen and nitrogen species. *Int. J. Mol. Sci.* 25 (5), 3007. doi:10.3390/ijms25053007

Sankaran, R., Kamath, R., Nambiar, V., and Kumar, A. (2019). A prospective study on the effects of Ayurvedic massage in post-stroke patients. *J. Ayurveda Integr. Med.* 10 (2), 126–130. doi:10.1016/j.jaim.2018.02.137

Schmid, A. A., Miller, K. K., Van Puymbroeck, M., and DeBaun-Sprague, E. (2014). Yoga leads to multiple physical improvements after stroke, a pilot study. *Complement. Ther. Med.* 22 (6), 994–1000. doi:10.1016/j.ctim.2014.09.005

Shah, Z. A., Nada, S. E., and Doré, S. (2011). Heme oxygenase 1, beneficial role in permanent ischemic stroke and in Gingko biloba (EGb 761) neuroprotection. *Neuroscience* 180, 248–255. doi:10.1016/j.neuroscience.2011.02.031

Shang, Y., Zhang, Z., Tian, J., and Li, X. (2022). Anti-inflammatory effects of natural products on cerebral ischemia. *Front. Pharmacol.* 13, 13. doi:10.3389/fphar.2022.914630

She, R., Liu, D., Liao, J., Wang, G., Ge, J., and Mei, Z. (2023). Mitochondrial dysfunctions induce PANoptosis and ferroptosis in cerebral ischemia/reperfusion injury: from pathology to therapeutic potential. *Front. Cell. Neurosci.* 17, 1191629. doi:10.3389/fncel.2023.1191629

Shu, J., Yang, L., Wei, W., and Zhang, L. (2022). Identification of programmed cell death-related gene signature and associated regulatory axis in cerebral ischemia/reperfusion injury. *Front. Genet.* 13, 13. doi:10.3389/fgene.2022.934154

Siotto, M., Germanotta, M., Santoro, M., Canali, R., Pascali, S., Insalaco, S., et al. (2022). Oxidative stress status in post stroke patients: sex differences. *Healthcare* 10 (5), 869. doi:10.3390/healthcare10050869

Spence, J. D. (2019). Nutrition and risk of stroke. *Nutrients* 11 (3), 647. doi:10.3390/nu11030647

Spoelstra-de Man, A. M. E., Elbers, P. W. G., and Oudemans-van Straaten, H. M. (2018). Making sense of early high-dose intravenous vitamin C in ischemia/reperfusion injury. *Crit. Care* 22 (1), 70. doi:10.1186/s13054-018-1996-y

Sun, Y., and Zhu, C. (2023). Potential role of PANoptosis in neuronal cell death: commentary on "PANoptosis-like cell death in ischemia/reperfusion injury of retinal neurons.". *Neural Regen. Res.* 18 (2), 339–340. doi:10.4103/1673-5374.346483

Sun, Y. Y., Zhu, H. J., Zhao, R. Y., Zhou, S. Y., Wang, M. Q., Yang, Y., et al. (2023). Remote ischemic conditioning attenuates oxidative stress and inflammation via the Nrf2/HO-1 pathway in MCAO mice. *Redox Biol.* 66, 102852. doi:10.1016/j.redox.2023.102852

Tang, X., Liu, H., Xiao, Y., Wu, L., and Shu, P. (2022). Vitamin C intake and ischemic stroke. *Front. Nutr.* 9, 935991. doi:10.3389/fnut.2022.935991

Thayabaranathan, T., Immink, M. A., Stevens, P., Hillier, S., Thrift, A. G., Brodtmann, A., et al. (2018). Understanding the potential for yoga and tai chi interventions to moderate risk factors for stroke – a scoping review. *Future Neurol.* 13 (4), 239–252. doi:10.2217/fnl-2018-0005

Tian, H. Y., Lei, Y. X., Zhou, J. T., Liu, L. J., Yang, T., Zhou, Y., et al. (2025). Insight into interplay between PANoptosis and autophagy: novel therapeutics in ischemic stroke. *Front. Mol. Neurosci.* 17, 17. doi:10.3389/fnmol.2024.1482015

Tong, X., Wang, Y., Fiehler, J., Bauer, C. T., Jia, B., Zhang, X., et al. (2021). Thrombectomy versus combined thrombolysis and thrombectomy in patients with acute stroke: a matched-control study. *Stroke* 52 (5), 1589–1600. doi:10.1161/STROKEAHA.120.031599

Tong, Y., Forreider, B., Sun, X., Geng, X., Zhang, W., Du, H., et al. (2015). Music-supported therapy (MST) in improving post-stroke patients' upper-limb motor function: a randomised controlled pilot study. *Neurol. Res.* 37 (5), 434–440. doi:10.1179/1743132815Y.0000000034

Ueno, Y., Miyamoto, N., Yamashiro, K., Tanaka, R., and Hattori, N. (2019). Omega-3 polyunsaturated fatty acids and stroke burden. *Int. J. Mol. Sci.* 20 (22), 5549. doi:10.3390/ijms20225549

Wang, H., Liu, C., Zhao, Y., and Gao, G. (2020). Mitochondria regulation in ferroptosis. *Eur. J. Cell. Biol.* 99 (1), 151058. doi:10.1016/j.ejcb.2019.151058

Wang, J., Hu, J., Chen, X., Lei, X., Feng, H., Wan, F., et al. (2021). Traditional Chinese medicine monomers: novel strategy for endogenous neural stem cells activation after stroke. *Front. Cell. Neurosci.* 15, 628115. doi:10.3389/fncel.2021.628115

Wang, J., and Xiong, X. (2012). Current situation and perspectives of clinical study in integrative medicine in China. *Evidence-Based Complementary Altern. Med.* 2012, 268542–268611. doi:10.1155/2012/268542

Wang, T., Duan, S., Wang, H., Sun, S., Han, B., and Fu, F. (2016). Neurological function following cerebral ischemia/reperfusion is improved by the Ruyi Zhenbao pill in a rats. *Biomed. Rep.* 4 (2), 161–166. doi:10.3892/br.2016.568

Wessels, I., Maywald, M., and Rink, L. (2017). Zinc as a gatekeeper of immune function. *Nutrients* 9 (12), 1286. doi:10.3390/nu9121286

Wierońska, J. M., Cieślik, P., and Kalinowski, L. (2021). Nitric oxide-dependent pathways as critical factors in the consequences and recovery after brain ischemic hypoxia. *Biomolecules* 11 (8), 1097. doi:10.3390/biom11081097

Wu, L., Song, H., Zhang, C., Wang, A., Zhang, B., Xiong, C., et al. (2023). Efficacy and safety of *Panax notoginseng* saponins in the treatment of adults with ischemic stroke in China. *JAMA Netw. Open* 6 (6), e2317574. doi:10.1001/jamanetworkopen.2023.17574

Xie, C., Gu, A., Cai, J., Wu, Y., and Chen, R. (2018). Curcumin protects neural cells against ischemic injury in N2a cells and mouse brain with ischemic stroke. *Brain Behav.* 8 (2), e00921. doi:10.1002/brb3.921

Xing, Y., Yang, S. D., Dong, F., Wang, M. M., Feng, Y. S., and Zhang, F. (2018). The beneficial role of early exercise training following stroke and possible mechanisms. *Life Sci.* 198, 32–37. doi:10.1016/j.lfs.2018.02.018

Xu, C., He, Z., Shen, Z., and Huang, F. (2022). Potential Benefits of Music Therapy on Stroke Rehabilitation. *Oxid Med Cell Longev.* 9386095. doi:10.1155/2022/9386095

Xu, J. H., Huang, Y. M., Ling, W., Li, Y., Wang, M., Chen, X. Y., et al. (2015). Wen Dan Decoction for hemorrhagic stroke and ischemic stroke. *Complement. Ther. Med.* 23 (2), 298–308. doi:10.1016/j.ctim.2015.01.001

Xu, M., Wu, R. X., Li, X. L., Zeng, Y. S., Liang, J. Y., Fu, K., et al. (2022). Traditional medicine in China for ischemic stroke: bioactive components, pharmacology, and mechanisms. *J. Integr. Neurosci.* 21 (1), 26. doi:10.31083/j.jin2101026

Xu, S., Lu, J., Shao, A., Zhang, J. H., and Zhang, J. (2020). Glial cells: role of the immune response in ischemic stroke. *Front. Immunol.* 11, 294. doi:10.3389/fimmu.2020.00294

Xu, Y., Li, K., Zhao, Y., Zhou, L., Liu, Y., and Zhao, J. (2023). Role of ferroptosis in stroke. *Cell. Mol. Neurobiol.* 43 (1), 205–222. doi:10.1007/s10571-022-01196-6

Yan-shu, P., Yao, W., Bing, W., and Peng-tao, L. (2008). Effects of qingkailing injection on transforming growth factor-β1 expression in cerebral ischemia-reperfusion injury in rats. *World Sci. Technol.* 10 (5), 29–32. doi:10.1016/s1876-3553(09)60023-5

Younis, N., and Mohamed, M. (2020). Sandalwood oil neuroprotective effects on middle cerebral artery occlusion model of ischemic brain stroke. *Pharmacogn. Mag.* 16 (68), 117. doi:10.4103/pm.pm_398_19

Yu, C. C., Liu, L., Chen, S. Y., Wang, X. F., Wang, L., and Du, Y. J. (2022). Ancient Chinese herbal recipe huanglian jie du decoction for ischemic stroke: an overview of current evidence. *Aging Dis.* 13 (6), 1733–1744. doi:10.14336/AD.2022.0311

Yu, M., Sun, Z. J., Li, L. T., Ge, H. Y., Song, C. Q., and Wang, A. J. (2015). The beneficial effects of the herbal medicine Di-huang-yin-zi (DHYZ) on patients with ischemic stroke: a Randomized, Placebo controlled clinical study. *Complement. Ther. Med.* 23 (4), 591–597. doi:10.1016/j.ctim.2015.06.003

Zahiruddin, S., Basist, P., Parveen, A., Parveen, R., Khan, W., et al. (2020). Ashwagandha in brain disorders: a review of recent developments. *J. Ethnopharmacol.* 257, 112876. doi:10.1016/j.jep.2020.112876

Zhang, H., Jin, B., You, X., Yi, P., Guo, H., Niu, L., et al. (2023). Pharmacodynamic advantages and characteristics of traditional Chinese medicine in prevention and treatment of ischemic stroke. *Chin. Herb. Med.* 15 (4), 496–508. doi:10.1016/j.chmed.2023.09.003

Zhang, H., Lee, J. Y., Borlongan, C., and Tajiri, N. (2019). A brief physical activity protects against ischemic stroke. *Brain Circ.* 5 (3), 112–118. doi:10.4103/bc.bc_32_19

Zhang, L., Ma, J., Yang, F., Li, S., Ma, W., Chang, X., et al. (2022). Neuroprotective effects of quercetin on ischemic stroke: a literature review. *Front. Pharmacol.* 13, 854249. doi:10.3389/fphar.2022.854249

Zhang, S., Wu, B., Liu, M., Li, N., Zeng, X., Liu, H., et al. (2015). Acupuncture efficacy on ischemic stroke recovery: multicenter randomized controlled trial in China. *Stroke* 46 (5), 1301–1306. doi:10.1161/STROKEAHA.114.007659

Zhang, W. W., Xu, F., Wang, D., Ye, J., and Cai, S. Q. (2018). Buyang Huanwu Decoction ameliorates ischemic stroke by modulating multiple targets with multiple components: *in vitro* evidences. *Chin. J. Nat. Med.* 16 (3), 194–202. doi:10.1016/S1875-5364(18)30047-5

Zhao, H., Li, Y., Zhang, Y., He, W. Y., and Jin, W. N. (2022). Role of immune and inflammatory mechanisms in stroke: a review of current advances. *Neuroimmunomodulation* 29 (4), 255–268. doi:10.1159/000524951

Zhao, L., Li, H., Gao, Q., Xu, J., Zhu, Y., Zhai, M., et al. (2021b). Berberine attenuates cerebral ischemia-reperfusion injury induced neuronal apoptosis by down-regulating the CNPY2 signaling pathway. *Front. Pharmacol.* 12, 12. doi:10.3389/fphar.2021.609693

Zhao, S., Zheng, H., Du, Y., Zhang, R., Chen, P., Ren, R., et al. (2021a). The clinical efficacy of Ginkgo biloba leaf preparation on ischemic stroke: a systematic review and meta-analysis. *Evid. Based. Complement. Altern. Med.* 2021, 4265219. doi:10.1155/2021/4265219

Zheng, L., Meng, L., Liang, H., and Yang, J. (2023). Sanhua decoction: current understanding of a traditional herbal recipe for stroke. *Front. Neurosci.* 17, 1149833. doi:10.3389/fnins.2023.1149833

Zhong, D. Y., Cheng, H., Luo, H. S., Li, Q., Li, L., Li, H. J., et al. (2023). Study on mechanisms and molecular verification of Buyang Huanwu decoction in treating ischemic stroke from the perspective of cuproptosis. *TMR Integr. Med.* 7 (0), e23013. doi:10.53388/tmrim202307013

Zhou, J., Sun, F., Zhang, W., Feng, Z., Yang, Y., and Mei, Z. (2024). Novel insight into the therapeutical potential of flavonoids from traditional Chinese medicine against cerebral ischemia/reperfusion injury. *Front. Pharmacol.* 15, 1352760. Frontiers in Pharmacology. Frontiers Media SA. doi:10.3389/fphar.2024.1352760

Zhou, Y., Zhang, S., and Fan, X. (2021). Role of polyphenols as antioxidant supplementation in ischemic stroke. *Oxid. Med. Cell. Longev.* 2021, 5471347. doi:10.1155/2021/5471347

Zhu, B., Cao, H., Sun, L., Li, B., Guo, L., Duan, J., et al. (2018). Metabolomics-based mechanisms exploration of Huang-Lian Jie-Du decoction on cerebral ischemia via UPLC-Q-TOF/MS analysis on rat serum. *J. Ethnopharmacol.* 216, 147–156. doi:10.1016/j.jep.2018.01.015

Zhu, H., Hu, S., Li, Y., Sun, Y., Xiong, X., Hu, X., et al. (2022). Interleukins and ischemic stroke. *Front. Immunol.* 13. doi:10.3389/fimmu.2022.828447

Zhu, T., Wang, L., Feng, Y., Sun, G., and Sun, X. (2021). Classical active ingredients and extracts of Chinese herbal medicines: pharmacokinetics, pharmacodynamics, and molecular mechanisms for ischemic stroke. *Oxid. Med. Cell. Longev.* 2021, 8868941. doi:10.1155/2021/8868941

Zhu, W., Jia, Q., Ferreira, A. C., Jiang, H., Zhang, J., Li, B., et al. (2024). Acupuncture for ischemic stroke: where are we now? *Acupunct. Herb. Med.* 4, 36–55. doi:10.1097/hm9.0000000000000094

Zuo, W., Yan, F., Zhang, B., Hu, X., and Mei, D. (2018). Salidroside improves brain ischemic injury by activating PI3K/Akt pathway and reduces complications induced by delayed tPA treatment. *Eur. J. Pharmacol.* 830, 128–138. doi:10.1016/j.ejphar.2018.04.001

frontiers | Frontiers in Pharmacology

TYPE Clinical Trial
PUBLISHED 12 June 2025
DOI 10.3389/fphar.2025.1608719

Check for updates

OPEN ACCESS

EDITED BY
Bin Yu,
Nanjing University of Chinese Medicine, China

REVIEWED BY
Hong-He Xiao,
Liaoning University of Traditional Chinese Medicine, China
Ming Ruan,
Nanjing Xiaozhuang University, China

*CORRESPONDENCE
Haitong Wan,
whtong@126.com

RECEIVED 09 April 2025
ACCEPTED 27 May 2025
PUBLISHED 12 June 2025

CITATION
Pan D, Wan H, He Y, Yang J, Guo Y, Yu L, Zhang F, Zheng G, Xu B, Song Y, Zhao M, Liu X, Liu J, Sun G and Lin Y (2025) Efficacy of danhong injection adjuvant therapy in patients with acute ischemic stroke: a real-world, multicenter, retrospective study.
Front. Pharmacol. 16:1608719.
doi: 10.3389/fphar.2025.1608719

COPYRIGHT
© 2025 Pan, Wan, He, Yang, Guo, Yu, Zhang, Zheng, Xu, Song, Zhao, Liu, Liu, Sun and Lin. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

Efficacy of danhong injection adjuvant therapy in patients with acute ischemic stroke: a real-world, multicenter, retrospective study

Danping Pan[1], Haitong Wan[1,2]*, Yu He[3], Jiehong Yang[1], Yilei Guo[1], Li Yu[1], Feihu Zhang[1], Guoqing Zheng[4], Bin Xu[5], Yaohong Song[6], Mingjun Zhao[7], Xiangzhe Liu[8], Jianhe Liu[9], Gang Sun[10] and Yaming Lin[11]

[1]School of Basic Mecicine Sciences, Zhejiang Chinese Medical University, Hangzhou, China, [2]Academy of Chinese Medical Sciences, Henan University of Chinese Medicine, Zhengzhou, China, [3]School of Pharmaceutical Sciences, Zhejiang Chinese Medical University, Hangzhou, China, [4]Department of Neurology, The First Affiliated Hospital of Zhejiang Chinese Medical University (Zhejiang Provincial Hospital of Chinese Medicine), Hangzhou, China, [5]Department of Neurology, The Second Affiliated Hospital of Zhejiang Chinese Medical University, Hangzhou, China, [6]Department of Cardiology, Nanjing Hospital of Chinese Medicine Affiliated to Nanjing University of Chinese Medicine, Nanjing, China, [7]Department of Cardiology, Affliated Hospital of Shaanxi University of Chinese Medicine, Xianyang, China, [8]Department of Encephalology, The First Affiliated Hospital of Henan University of Chinese Medicine, Zhengzhou, China, [9]Department of Cardiology, The First Hospital of Hunan University of Chinese Medicine, Changsha, China, [10]Department of Cardiology, The First Affiliated Hospital of Guizhou University of Traditional Chinese Medicine, Guiyang, China, [11]Department of Neurology, Yunnan Provincial Hospital of Traditional Chinese Medicine, Kunming, China

Background: Previous clinical and experimental studies indicate that Danhong injection (DHI) confers protective effects against acute ischemic stroke (AIS). However, due to limited sample sizes, large-scale clinical studies are still needed to confirm its efficacy.

Methods: This real-world, multicenter retrospective study used inpatient data from eight centers across Mainland China. AIS patients were divided into a DHI group or a Non-DHI group depending on whether they received DHI (7–14 consecutive days). Propensity score matching (PSM) was applied to balance baseline differences, and multiple analytical methods (crude analysis, multivariate regression, stabilized inverse probability of treatment weighting [sIPTW], and PSM combined with multivariate regression) were conducted. The primary outcome was the NIHSS score at discharge. Secondary outcomes included the proportions of patients with post-treatment NIHSS scores ≤4 or ≤1, the mRS score, the proportion of patients achieving mRS ≤1, and the incidence of in-hospital complications (IHC).

Results: A total of 3,560 patients were enrolled, including 1,425 in the DHI group, and 2,135 in the Non-DHI group, with 1,415 matched pairs identified via PSM. After treatment, the NIHSS score in the DHI group was 2.01 ± 3.10, compared with 2.50 ± 3.26 in the Non-DHI group, indicating significantly lower scores in the DHI group (adjusted RR = 0.81, 95% CI: 0.74–0.88, $P < 0.001$). These findings were consistent across multiple analytical methods (RR = 0.79–0.82). After treatment, the proportion of patients with NIHSS ≤4 and ≤1 was higher in the DHI group (adjusted RR = 1.02, 95% CI: 1.01–1.03, $P = 0.005$; adjusted RR = 1.07, 95% CI:

1.05–1.10, $P < 0.001$). The DHI group also had a lower mRS score ($P < 0.001$) and a higher proportion of patients achieving mRS ≤1 (adjusted RR = 1.12, 95% CI: 1.10–1.15, $P < 0.001$). No noteworthy difference was found between the two groups in the incidence of IHC (adjusted RR = 1.01, 95% CI: 0.99–1.03, $P = 0.320$).

Conclusion: DHI adjunctive therapy may improve neurological outcomes in patients with AIS. However, additional randomized controlled trials (RCTs) are needed to confirm its effectiveness in routine biomedicine-based clinical practice.

Clinical Trial Registration: https://www.chictr.org.cn/bin/project/edit?pid=211769, identifier ChiCTR2400079391.

KEYWORDS

danhong injection, acute ischemic stroke, clinical efficacy, real-world multicenter retrospective study, traditional Chinese medicine

Introduction

Globally, stroke is the third most common cause of mortality (10.7% of total deaths) and stands fourth in disability-adjusted life years (DALYs) lost (5.6% of total DALYs) (GBD 2021 Diseases and Injuries Collaborators, 2024; GBD 2021 Stroke Risk Factor Collaborators et al., 2024). In China, stroke imposes a notably high disease burden. According to the latest Global Burden of Disease estimates, 3.94 million new stroke cases were recorded nationwide in 2019, including 2.87 million new Ischemic Stroke (IS) events, and 1.03 million patients died from IS (Wang et al., 2022). Despite guideline-based optimal treatment and secondary prevention strategies, the 1-year stroke recurrence rate remains 9.6%–17.7% (Chinese Society of Neurology, and Chinese Stroke Society, 2022; Pan et al., 2021). In clinical practice across China, traditional Chinese medicine (TCM) is commonly used as an adjunctive therapy for acute ischemic stroke (AIS), largely due to its potential multi-target and multi-pathway effects.

Danhong injection (DHI) is composed of *Salvia miltiorrhiza* (Danshen) and *Carthamus tinctorius* (Honghua), promoting blood circulation, resolving blood stasis, and relaxing meridians. As one of the most commonly used Chinese botanical drug injections in China, DHI is approved for the treatment of ischemic cardiovascular and cerebrovascular diseases (Fu et al., 2018). DHI exhibits multiple pharmacological effects, including inhibiting oxidative stress and inflammation, anticoagulation, lowering lipids, preventing apoptosis, inducing vasodilation, and promoting angiogenesis (Feng et al., 2019). In animal experiments, DHI alleviated neuronal injury in the ischemic penumbra of rats after cerebral ischemia/reperfusion (CI/R) (Zeng et al., 2021). In IS model rats, DHI reduced infarct volume and enhanced neurological recovery by promoting neurogenesis (Li et al., 2023). Moreover, a meta-analysis involving 67 randomized controlled trials (RCTs) indicated that combining DHI with biomedicine significantly enhanced neurological outcomes, self-care capacity, and blood lipid profiles in IS patients (Ma et al., 2022).

Nevertheless, previous clinical research was constrained by relatively small sample sizes and tended to focus on specific endpoints, resulting in insufficient evidence regarding the overall efficacy of DHI as an adjunct therapy in AIS. To our knowledge, no large-scale real-world study has comprehensively evaluated the clinical effectiveness of adjunctive DHI therapy in routine biomedicine-based AIS management. Hence, this real-world, multicenter study set out to evaluate the impact of combining DHI with standard treatment on neurological function and other clinical outcomes in AIS patients, ultimately supplying stronger clinical evidence for DHI's use as an adjunct therapy.

Abbreviations: DALYs, disability-adjusted life years; IS, Ischemic Stroke; TCM, traditional Chinese medicine; AIS, acute ischemic stroke; DHI, Danhong injection; CI/R, cerebral ischemia/reperfusion; RCTs, randomized controlled trials; MACCE, major adverse cardiovascular and cerebrovascular events; NIHSS, National Institutes of Health Stroke Scale; mRS, modified Rankin Scale; IHC, in-hospital complications; LAA, large-artery atherosclerosis; SVO, small-vessel occlusion; LI, lacunar infarction; FLI, focal or large-area infarction; PSM, Propensity score matching; SD, standardized difference; SMD, standardized mean difference; RR, risk ratio; CI, confidence interval; sIPTW, stabilized inverse probability of treatment weighting; MD, mean difference; HSYA, hydroxysafflor yellow A; AEs, adverse events.

Methods

Study design

This multicenter retrospective cohort study drew on hospital medical records from eight medical centers in Mainland China from June 2018 to May 2023. A list of all participating centers is provided in Supplementary Table S1. The study protocol was granted by the Ethics Committee of the Second Affiliated Hospital of Zhejiang Chinese Medical University (No.2023-072-01) and registered at the Chinese Clinical Trial Registry (ChiCTR2400079391). Reporting adhered to recommended guidelines for observational studies using routinely collected health data (Von Elm et al., 2007; Langan et al., 2018) (Supplementary Table S2). As the dataset uses anonymous identifiers to protect patient privacy, informed consent was not required.

Study participants

Participants qualified for inclusion if they met the following conditions: (1) Patients discharged with a primary biomedicine-based diagnosis of AIS (Chinese Society of Neurology, and Chinese Stroke Society, 2024), with a disease course of ≤2 weeks; (2) Patients

TABLE 1 Baseline characteristics of patients receiving or not receiving DHI before and after PSM.

Characteristics a	Unmatched patients			Propensity-score-matched patients		
	DHI group (n = 1,425)	Non-DHI group (n = 2,135)	SMD b	DHI group (n = 1,415)	Non-DHI group (n = 1,415)	SMD
Sex, n(%)						
Male	883 (62.0)	1,334 (62.5)	0.011	875 (61.8)	883 (62.4)	0.012
Female	542 (38.0)	801 (37.5)		540 (38.2)	532 (37.6)	
Age, mean (SD)	68.37 (11.84)	67.61 (11.55)	0.065	68.29 (11.82)	68.23 (11.51)	0.005
District, n(%) c						
East	479 (33.6)	594 (27.8)	0.178	469 (33.1)	475 (33.6)	0.011
Middle	476 (33.4)	660 (30.9)		476 (33.6)	477 (33.7)	
West	470 (33.0)	881 (41.3)		470 (33.2)	463 (32.7)	
Smoking status, n(%)						
Current smoker	392 (27.5)	615 (28.8)	0.046	390 (27.6)	392 (27.7)	0.004
Former smoker	110 (7.7)	182 (8.5)		109 (7.7)	108 (7.6)	
Never smoker	923 (64.8)	1,338 (62.7)		916 (64.7)	915 (64.7)	
Drinking, n (%)	345 (24.2)	562 (26.3)	0.049	344 (24.3)	338 (23.9)	0.010
Medical history, n(%) d						
Stroke	525 (36.8)	671 (31.4)	0.114	519 (36.7)	504 (35.6)	0.022
Ischemic stroke	504 (35.4)	620 (29.0)	0.136	498 (35.2)	488 (34.5)	0.015
Heart disease	332 (23.3)	451 (21.1)	0.052	328 (23.2)	337 (23.8)	0.015
Hypertension	1,103 (77.4)	1704 (79.8)	0.059	1,097 (77.5)	1,108 (78.3)	0.019
Type 2 diabetes	508 (35.6)	766 (35.9)	0.005	503 (35.5)	509 (36.0)	0.009
Hyperlipidemia	341 (23.9)	605 (28.3)	0.100	341 (24.1)	343 (24.2)	0.003
Disease course, n(%)						
≤1d	714 (50.1)	1,121 (52.5)	0.048	710 (50.2)	704 (49.8)	0.008
>1d	711 (49.9)	1,014 (47.5)		705 (49.8)	711 (50.2)	
TOAST classification, n(%)						
LAA	357 (25.1)	576 (27.0)	0.068	356 (25.2)	363 (25.7)	0.032
SVO	395 (27.7)	622 (29.1)		393 (27.8)	408 (28.8)	
Other types e	673 (47.2)	937 (43.9)		666 (47.1)	644 (45.5)	
Infarction size, n(%)						
LI	1,079 (75.7)	1,562 (73.2)	0.059	1,071 (75.7)	1,072 (75.8)	0.002
FLI	346 (24.3)	573 (26.8)		344 (24.3)	343 (24.2)	
Baseline NIHSS score, n(%)						
≤4	1,008 (70.7)	1,528 (71.6)	0.019	1,005 (71.0)	1,006 (71.1)	0.002
5–20	402 (28.2)	586 (27.4)		395 (27.9)	394 (27.8)	
≥21	15 (1.1)	21 (1.0)		15 (1.1)	15 (1.1)	

[a]Values are presented as n (%) or mean (SD).
[b]The SMD, was used to compare characteristics between the DHI, and Non-DHI, groups, with an SMD <0.1 indicating balanced and comparable covariates.
[c]"District" refers to Eastern (Zhejiang, Nanjing), Central (Shaanxi, Henan, Hunan), or Western (Guizhou, Yunnan) regions.
[d]"Stroke" includes ischemic stroke and hemorrhagic stroke; "Heart disease" includes coronary artery disease, myocardial infarction, atrial fibrillation, and heart failure.
[e]"Other types" refers to all AIS, patients other than LAA and SVO.
Abbreviations: DHI, danhong injection; SMD, standardized mean difference; SD, standard deviation; LAA, large-artery atherosclerosis; SVO, small-vessel occlusion; LI, lacunar infarction; FLI, focal or large-area infarction; NIHSS, national institutes of health stroke scale.

aged ≥40 years; (3) A hospital stay of 7–21 days; (4) Patients who received DHI therapy for 7–14 consecutive days during hospitalization, or did not receive DHI treatment.

Individuals were excluded under any of these conditions: (1) Patients with insufficiently detailed medical records, preventing the extraction of key information; (2) Patients who met the criteria for brain death upon admission or who were discharged against medical advice, died in hospital, or experienced major adverse cardiovascular and cerebrovascular events (MACCE) during hospitalization; (3) Patients who did not receive DHI therapy in accordance with the

currently recommended guideline dosage (20 mL once daily, administered intravenously) (Fu et al., 2018).

Procedures

The exposure factor in this cohort study was whether patients received adjunctive DHI therapy. Those in the DHI group were administered DHI at the guideline-recommended dose (20 mL once daily, given intravenously) for at least seven consecutive days (Fu et al., 2018). Most patients initiated DHI treatment within 48 h of admission. The treatment duration ranged from 7 to 14 days, as determined by the attending physicians. Due to the retrospective nature of the study and variability in clinical practice, no subgroup analysis was conducted based on the timing of initiation or treatment duration.

Both groups received standard biomedicine-based therapy in accordance with existing guidelines (Chinese Society of Neurology, and Chinese Stroke Society, 2024). Baseline characteristics at admission were collected for each patient, including clinical data (Table 1), laboratory tests, and the baseline National Institutes of Health Stroke Scale (NIHSS) score. At discharge, the NIHSS and modified Rankin Scale (mRS) scores were recorded, and the occurrence of in-hospital complications (IHC) was evaluated. The manually extracted data then underwent secondary review and verification to ensure consistency.

Description of DHI

DHI is a standardized Chinese herbal formulation composed of *Salvia miltiorrhiza* Bunge [Lamiaceae; *Salviae miltiorrhizae radix et rhizoma*] and *Carthamus tinctorius* L. [Asteraceae; *Carthami flos*], both of which are listed in the Chinese Pharmacopoeia (2020 edition). The preparation used in this study was a finished commercial product approved by the China Food and Drug Administration (CFDA, Approval No. Z20026866). The HPLC profile of DHI has been reported previously (Li et al., 2023) and provides quantitative information on its representative metabolites.

Outcomes

The primary outcome was the NIHSS score at discharge (approximately 14 ± 7 days post-treatment). Secondary outcomes included the proportions of patients with NIHSS scores ≤4 and ≤1 at discharge, the mRS score, the proportion of patients with an mRS score ≤1, and the incidence of IHC. The NIHSS measures the severity of neurological deficits in AIS, with ≤4 indicating mild stroke, 4–21 indicating moderate stroke, and ≥21 indicating severe stroke. The mRS evaluates neurological recovery following AIS, ranging from 0 (no residual stroke symptoms) to 6 (death). IHC encompass pneumonia, urinary tract infection, cerebral edema or intracranial hypertension, and deep vein thrombosis or embolism.

Covariates

To examine the association between DHI adjunct therapy and outcomes, we collected multiple covariates: baseline sex, age, smoking status, drinking, past medical history (IS, stroke, heart disease, hypertension, diabetes, hyperlipidemia), disease course, TOAST classification (large-artery atherosclerosis [LAA], small-vessel occlusion [SVO], other types), laboratory parameters, infarction size (lacunar infarction [LI], focal or large-area infarction [FLI]), and the baseline NIHSS score. Given potential differences in healthcare levels across regions, the study center was included as a confounding factor. Detailed information on all 27 covariates is provided in Supplementary Table S3.

Statistical analyses

Propensity score matching (PSM) was employed to balance baseline variables between the DHI and Non-DHI groups. A multivariable logistic regression model was used to estimate propensity scores, incorporating previously identified covariates (Kainz et al., 2017). Patients were matched on a 1:1 basis via nearest neighbor matching with a caliper width equal to 0.2 times the standardized difference (SD) of the logit-transformed propensity score (caliper = 0.2 × SD [logit (PS)]) (Makhnevich et al., 2024). A fast matching algorithm was applied without replacement. For both pre- and post-matching datasets, continuous variables were reported as mean ± standard deviation, and categorical variables were presented as frequency (percentage). The standardized mean difference (SMD) was used to compare intergroup differences in baseline characteristics before and after matching, with an SMD <0.1 indicating adequate balance and comparability.

After PSM, outcomes were analyzed according to their data type. For discrete outcomes (e.g., NIHSS score), Poisson regression was first used to calculate the risk ratio (RR) and 95% confidence interval (CI). If overdispersion was detected, negative binomial regression was applied instead (Schober and Vetter, 2021). For binary outcomes (e.g., NIHSS score ≤4), the RR and 95% CI were derived using modified Poisson regression (Zou, 2004). A two-sided P-value <0.05 was deemed statistically significant. As this study was exploratory, no adjustments were made for multiple comparisons. Aside from laboratory parameters, which were <20% missing and recategorized as dichotomous variables, all variables in this study were complete. Detailed information regarding missing data is presented in Supplementary Table S4, and multiple imputation was performed to address these missing values.

To further clarify the relationship between non-random DHI therapy and NIHSS scores, we applied several analytical methods: crude analysis, multivariable regression, and propensity score–based approaches (PSM, stabilized inverse probability of treatment weighting [sIPTW], and PSM combined with multivariable regression) (Geleris et al., 2020). Except for the crude analysis, all other methods incorporated the covariates from Model two for adjustment. We also generated a forest plot to visualize the incidence of post-treatment "NIHSS score >4" across key clinical subgroups, including demographic factors such as age and sex. Other sensitivity analyses included: (1) Fitting multiple PSM models. Model one included sex, age, district, lifestyle factors, and past medical history. Model two added disease course, TOAST classification, infarction size, and baseline NIHSS score. Model three further incorporated

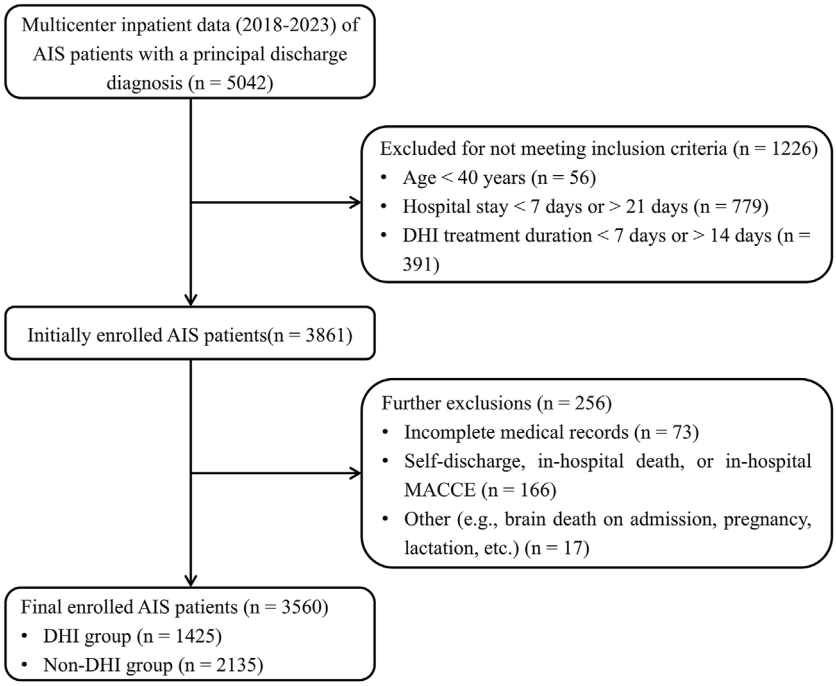


FIGURE 1
Flow diagram of the study selection process. MACCE includes ischemic stroke, hemorrhagic stroke, myocardial infarction, and vascular-related mortality. Abbreviations: AIS, acute ischemic stroke; DHI, Danhong Injection; MACCE, major adverse cardiovascular and cerebrovascular events.

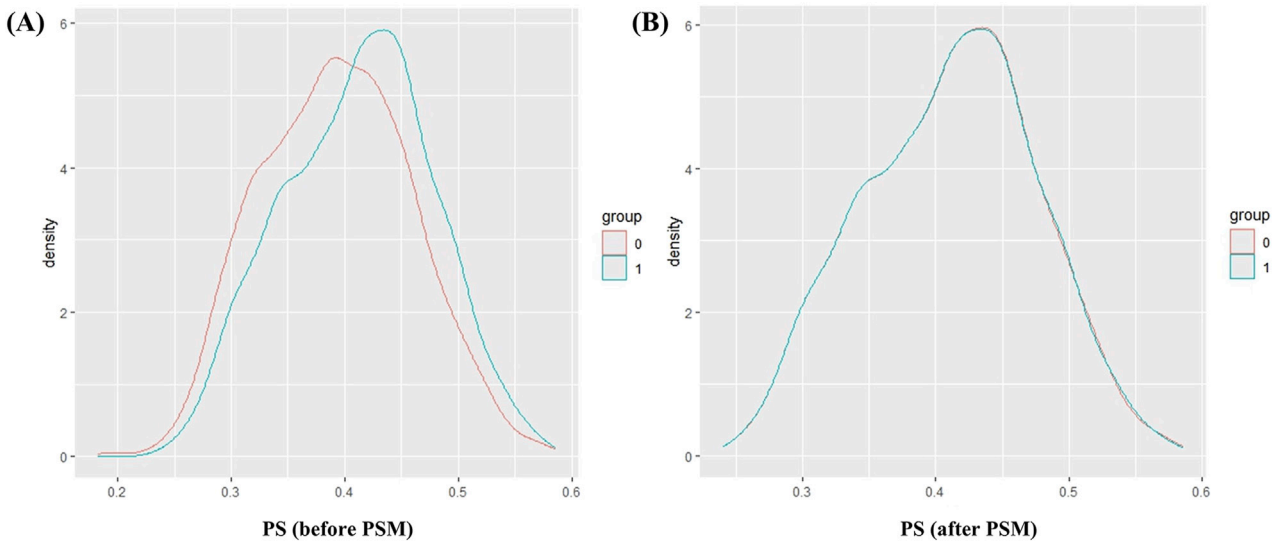


FIGURE 2
Density plots of propensity scores before and after PSM. **(A)** PS density curve before PSM; **(B)** PS density curve after PSM. Abbreviations: PS, propensity score; PSM, propensity score matching.

laboratory parameters. Model two served as the primary analysis model. (2) Excluding patients who underwent intravenous thrombolysis or endovascular intervention. (3) Excluding patients with severe stroke. (4) Performing a *post hoc* E-value analysis to gauge how large an unmeasured confounder must be to eliminate the observed association (Haneuse et al., 2019). All statistical analyses were performed using R software (version 4.2.1).

Results

Patients and baseline characteristics

A total of 5,042 patients discharged with a primary diagnosis of AIS from eight medical centers in Mainland China between June 2018 and May 2023 were screened. Of these, 3,816 met the inclusion

TABLE 2 Association between DHI use and the primary outcomes.

Analysis	DHI group a	Non-DHI group a	RR (95% CI)	p-value	MD b
PSM	2.01 (3.10)	2.50 (3.26)	0.81 (0.74–0.88)	<0.001	0.61 (2.81)
Crude analysis	2.02 (3.10)	2.45 (3.13)	0.82 (0.76–0.89)	<0.001	0.62 (2.66)

[a]Shown is the post-treatment NIHSS, score, presented as mean (SD).
[b]MD is calculated as the difference in NIHSS (pre-to post-treatment) for the DHI, group minus the difference in NIHSS (pre-to post-treatment) for the Non-DHI, group, and is also presented as mean (SD).
Abbreviations: DHI, danhong injection; RR, risk ratio; CI, confidence interval; MD, mean difference; PSM, propensity score matching.

criteria. After applying the exclusion criteria, 3,560 patients were ultimately included in the analysis, comprising 1,425 in the DHI group and 2,135 in the Non-DHI group (Figure 1).

The baseline characteristics of patients in the DHI and Non-DHI groups are presented in Table 1 (a full listing of the 27 characteristics is available in Supplementary Table S3). Before matching, all SMD values were below 0.1 except for district, stroke, ischemic stroke, and hyperlipidemia. After matching, all baseline characteristics (1,415 patients per group) were balanced between the DHI and Non-DHI cohorts (SMD <0.1). As a result of PSM, the predicted likelihood distribution (represented by propensity scores) was similar in both groups (Figure 2).

Primary outcome

In the primary analysis conducted after PSM, the NIHSS score in the DHI group was 2.01 ± 3.10, compared with 2.50 ± 3.26 in the Non-DHI group. Negative binomial regression revealed that the NIHSS score in the DHI group was significantly lower (adjusted RR = 0.81, 95% CI: 0.74–0.88, $P < 0.001$), corresponding to a mean difference (MD) of 0.61 ± 2.81 between the two groups (Table 2).

In the unadjusted (crude) analysis, the post-treatment NIHSS score was 2.02 ± 3.10 in the DHI group and 2.45 ± 3.13 in the Non-DHI group. Univariate negative binomial regression again showed the DHI group's NIHSS score was significantly lower (adjusted RR = 0.82, 95% CI: 0.76–0.89, $P < 0.001$), with an MD of 0.62 ± 2.66 (Table 2). Further multivariable and propensity score analyses showed consistent findings (RR = 0.79–0.82) (Table 3).

TABLE 3 Associations between DHI use and the primary outcome in crude, multivariable, and propensity-score analyses.

Analysis	RR (95% CI)
Crude analysis	0.82 (0.76–0.89)
Multivariable analysis a	0.79 (0.75–0.84)
Propensity-score analyses	
With matching b	0.81 (0.74–0.88)
With sIPTW c	0.81 (0.74–0.90)
With matching and multivariable analysis d	0.79 (0.74–0.83)

[a]The RR, is derived from a multivariable negative binomial regression model adjusted for 15 covariates (Table 1). Analysis includes all 3,560 patients.
[b]This primary analysis uses a univariable negative binomial regression model where 15 covariates are matched according to the propensity score. It includes 2,830 patients (1,415 receiving DHI, 1,415 not).
[c]The RR, is obtained from a univariable negative binomial regression model applying sIPTW, to the same 15 covariates, covering all patients.
[d]The RR, is generated from a multivariable negative binomial regression model using the same 15 covariates matched by the propensity score, analyzing 2,830 patients.
Abbreviations: RR, risk ratio; CI, confidence interval; sIPTW, stable inverse probability of treatment weighting.

Secondary outcome

Among the 2,830 patients after PSM (1,415 in the DHI group and 1,415 in the Non-DHI group), the proportion of patients with NIHSS scores ≤4 and ≤1 were significantly higher in the DHI group (adjusted RR = 1.02, 95% CI: 1.01–1.03, $P = 0.005$; adjusted RR = 1.07, 95% CI: 1.05–1.10, $P < 0.001$). The DHI group also had significantly lower mRS score than the Non-DHI group ($P < 0.001$) (Figure 3), and a greater proportion of patients achieved mRS ≤1 (adjusted RR = 1.12, 95% CI: 1.10–1.15, $P < 0.001$). No significant differences were observed in the incidence of IHC or pneumonia between the two groups (adjusted RR = 1.01, 95% CI: 0.99–1.03, $P = 0.320$; adjusted RR = 1.01, 95% CI: 0.99–1.02, $P = 0.478$) (Table 4).

Subgroup and sensitivity analyses

Patients with NIHSS score >4 are generally considered to have moderate-to-severe stroke, indicating substantial neurological impairment that may affect prognosis. A *post hoc* subgroup analysis evaluated the incidence of NIHSS score >4 in different AIS subgroups after treatment. The findings revealed that the DHI group had an overall lower relative risk, demonstrating consistent effectiveness across multiple subgroups (Figure 4). Apart from infarction size ($P = 0.048$), the interaction *P*-values for all other subgroups were nonsignificant ($P > 0.05$), indicating that infarct size significantly modulates the risk of having an NIHSS score >4. Notably, compared with the FLI subgroup, the LI subgroup had a markedly reduced relative risk.

Multiple *post hoc* sensitivity analyses—including fitting various PSM models, excluding patients who underwent intravenous thrombolysis or endovascular intervention, and excluding those with severe stroke—produced similar results (Supplementary Table S5). The E-value for the RR was 1.77, and the upper confidence bound for the primary outcome was 1.53, indicating that a strong unmeasured confounder would be required to invalidate the observed association or its 95% CI (Supplementary Figure S1).

Discussion

To our knowledge, this is the first real-world, multicenter, retrospective cohort study to evaluate the efficacy of adjunctive DHI therapy in patients with AIS. Of the 3,560 patients with complete data on both primary and secondary outcomes, 2,830

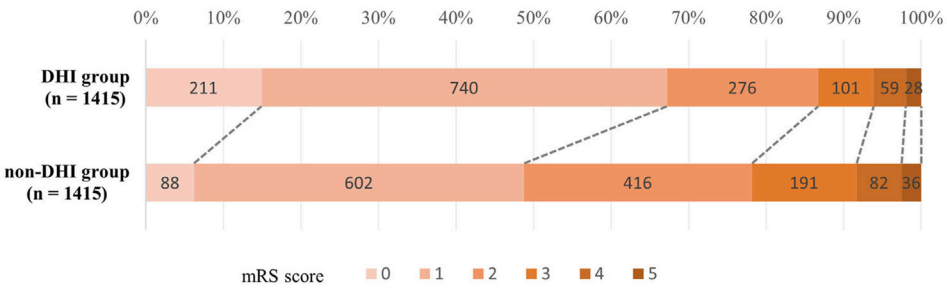


FIGURE 3
Distribution of mRS scores in AIS patients.

TABLE 4 Association between DHI use and the secondary outcomes.

Secondary outcomes	DHI group (n = 1,415)	Non-DHI group (n = 1,415)	Adjusted RR (95% CI)	p-value
NIHSS score ≤4, n (%)	1,271 (89.82)	1,223 (86.43)	1.02 (1.01–1.03)	0.005
NIHSS score ≤1, n (%)	813 (57.46)	659 (46.57)	1.07 (1.05–1.10)	<0.001
mRS score ≤1, n (%)	951 (67.21)	690 (48.76)	1.12 (1.10–1.15)	<0.001
IHC, n (%)	174 (12.30)	157 (11.10)	1.01 (0.99–1.03)	0.320
Pneumonia, n (%)	138 (9.75)	127 (8.98)	1.01 (0.99–1.02)	0.478

PSM, was used to balance baseline differences between the DHI, and Non-DHI, groups. The *p*-value indicates the comparison between these two groups. Abbreviations: DHI, danhong injection; RR, risk ratio; CI, confidence interval; IHC, in-hospital complications.

(1,415 in each group) were successfully matched by PSM. These findings suggest that adjunctive DHI therapy not only lowers post-treatment NIHSS scores and increases the proportion of patients achieving NIHSS ≤4 or ≤1, but also reduces mRS scores and raises the proportion of patients with mRS ≤1. Hence, it appears to enhance both neurological function and quality of life. Notably, no effect of DHI on IHC was observed in AIS patients.

DHI is prepared from *Salvia miltiorrhiza* (Danshen) and *Carthamus tinctorius* (Honghua), and is commonly used in Chinese clinical practice to treat ischemic cardiovascular and cerebrovascular diseases due to its blood-activating and stasis-resolving properties. According to the literature, DHI contains multiple active metabolites, including danshensu, salvianolic acids, and hydroxysafflor yellow A (HSYA), which act on diverse molecular targets to improve hemorheology, protect vascular endothelium, and attenuate inflammation and oxidative stress (Feng et al., 2019; Ma et al., 2022). Experimental studies have shown that DHI reduces infarct volume, promotes neurovascular remodeling, and facilitates neurological recovery in IS models (Feng et al., 2019). These effects are thought to be mediated by suppression of the NF-κB and NLRP3 inflammasome pathways (Du et al., 2021; Wang J. et al., 2024), enhancement of antioxidant capacity via the Nrf2/ARE signaling axis (Guo et al., 2014), and regulation of neuronal apoptosis and autophagy through the PI3K/Akt/mTOR and BDNF/CREB pathways (Zhang et al., 2023; Li et al., 2023). Among its bioactive metabolites, danshensu exhibits anti-inflammatory and autophagy-regulating effects (Hu et al., 2022); salvianolic acids inhibit oxidative stress and platelet aggregation (Zhao et al., 2024; Liu et al., 2023); and HSYA exerts neuroprotective actions, potentially by modulating SIRT1-related pathways (Fangma et al., 2021). Collectively, these multi-target mechanisms support the therapeutic potential of DHI in the treatment of AIS.

Building on these advantages, these studies have explored the efficacy of DHI in patients with AIS and angina. They found that DHI improved neurological function and self-care ability in AIS patients in a dose-dependent manner (Du et al., 2018; Ma et al., 2022). In angina patients, add-on DHI therapy reduced the frequency of anginal episodes, alleviated myocardial ischemic symptoms, and improved TCM syndromes (Liu et al., 2021; Chen et al., 2022). These reports further indicated that adjunct DHI therapy did not introduce additional safety risks. However, their conclusions relied mainly on relatively small sample sizes, limiting the ability to fully characterize DHI's effectiveness in real-world practice.

The results of our study align with outcomes reported in other adjunctive therapy trials. For example, the TISS trial indicated that adding Tongxinluo markedly increased the proportion of AIS patients attaining a 90-day NIHSS score of 0–1 or a reduction of ≥4 points (Dong et al., 2024). In the TASTE trial, Edaravone Dexborneol lowered the NIHSS score by an additional 0.4 points compared with Edaravone alone in the acute phase (Xu et al., 2021). The BAST trial showed that at 90 days, butylphthalide reduced the NIHSS score by about one point more than the control group (Wang et al., 2023). Using a large-scale, multicenter, real-world cohort, our study similarly confirmed the beneficial effects of DHI on NIHSS and mRS scores, supporting its role in adjunctive AIS treatment. However, whether these short-term improvements in neurological function and disability reduction translate into better long-term outcomes remains unclear and will require extended follow-up and large-scale RCTs.

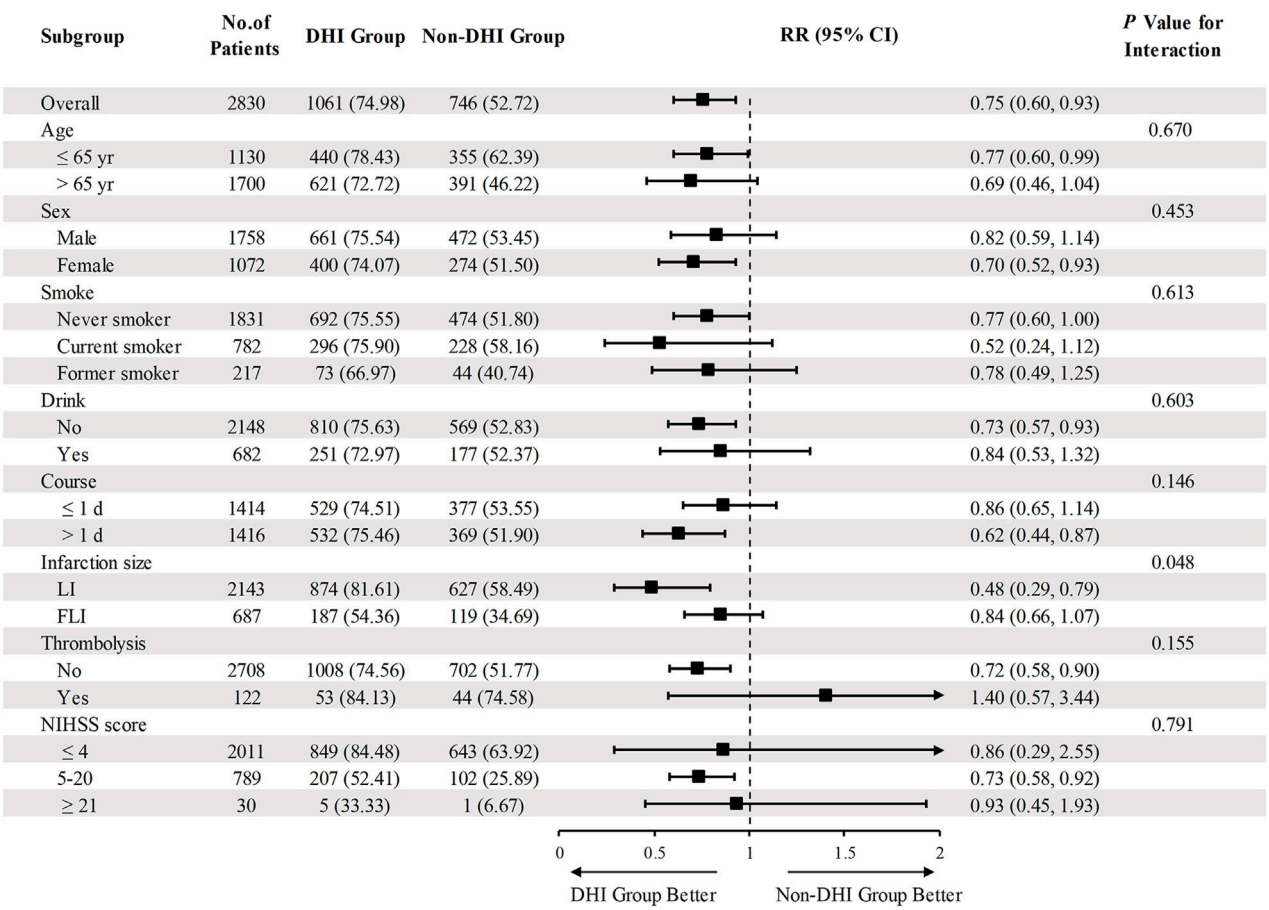


FIGURE 4
Subgroup analysis of the relationship between DHI use and "NIHSS score >4" in AIS patients. Abbreviations: DHI, Danhong Injection; RR, risk ratio; CI, confidence interval; LI, lacunar infarction; FLI, focal or large-area infarction.

This study used a multicenter, retrospective, real-world cohort design, providing data closer to clinical practice, including treatment pathways, concomitant medications, and disease heterogeneity, than conventional RCTs. It thereby offers greater external validity for applying botanical drug–based therapy in AIS (Tan et al., 2022). In contrast to prior RCTs or preclinical reports, this multicenter analysis provides pragmatic and clinically relevant evidence on DHI use in real-world AIS patients across diverse hospital settings. Nonetheless, real-world observational studies may still be influenced by unaccounted confounding factors (Psaty et al., 1999). To mitigate potential confounding, we conducted crude analysis, PSM, sIPTW, and multivariable regression. Subgroup and sensitivity analyses were then performed to verify the robustness and consistency of our findings. No effect of adjunct DHI therapy on IHC was identified, possibly because patients who develop such complications often present with advanced age, severe stroke, or multi-organ dysfunction.

As this was a retrospective study, active monitoring of medication-related adverse events (AEs) was not performed. Nonetheless, large-scale pharmacovigilance studies in China have reported a low incidence of DHI-related adverse drug reactions (ADRs), approximately 3.5‰, primarily type A reactions (e.g., sweating, dizziness, headache, flushing), which typically resolved after drug withdrawal (Liu et al., 2021; Chen et al., 2022). Notably, no cases of anaphylactic shock or allergic respiratory distress were reported. Moreover, RCTs have reported no significant difference in AEs incidence between DHI-treated and control groups (Li et al., 2015; Ge et al., 2022). DHI has also shown good compatibility and safety when administered with common intravenous solvents (Wang A. et al., 2024). These findings collectively support the favorable safety profile of DHI in both clinical trials and routine practice.

This large-scale, multicenter study closely reflects real-world TCM clinical settings, offering a model for examining adjunctive AIS therapy. Although high-quality evidence supporting TCM interventions in AIS remains limited, TCM's multi-component and multi-target properties suggest broader clinical utility. By evaluating DHI's impact on neurological function and disability in AIS patients, our results confirm that adjunct DHI therapy can improve clinical outcomes.

Nonetheless, our study has certain limitations. First, although we used multiple approaches to address confounding, unmeasured factors (e.g., prior medication use) may still bias the results, and E-value analysis only provides supplementary insights. Second, excluding AIS patients hospitalized for longer than 21 days reduced the representation of severely ill patients requiring

extended hospitalization. Third, our findings were limited to short-term clinical outcomes (discharge NIHSS and mRS scores), as follow-up imaging, biomarker, and other relevant data were not consistently available across centers, making it infeasible to assess radiographic or mechanistic responses. Fourth, all eight participating centers were affiliated with TCM institutions, potentially limiting generalizability. Fifth, as a retrospective observational study without randomization or blinding, there may be a risk of residual bias, though we applied multiple statistical strategies to enhance internal validity and transparency. In light of these limitations, our results should be interpreted with caution. Looking ahead, well-designed multicenter RCTs are needed to validate the efficacy of adjunctive DHI therapy and to provide stronger evidence for its clinical application in AIS.

Conclusion

In conclusion, adjunctive DHI therapy can lower NIHSS and mRS scores in AIS patients, thereby enhancing clinical outcomes. These findings support the use of DHI as an adjunctive treatment for AIS. However, larger-scale, multicenter RCTs are still needed to further validate its efficacy and safety in routine biomedicine-based practice.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving humans were approved by the Ethics Committee of the Second Affiliated Hospital of Zhejiang Chinese Medical University. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements.

Author contributions

DP: Data curation, Visualization, Writing – original draft, Formal Analysis. HW: Funding acquisition, Project administration, Supervision, Writing – review and editing. YH: Writing – original draft, Methodology. JY: Writing – review and editing, Conceptualization. YG: Writing – original draft, Validation. LY: Writing – original draft, Formal Analysis, Visualization. FZ: Writing – original draft, Validation. GZ: Data curation, Writing – original draft. BX: Data curation, Writing – original draft. YS: Data curation, Writing – original draft. MZ: Data curation, Writing – original draft. XL: Data curation, Writing – original draft. JL: Writing – original draft, Data curation. GS: Writing – original draft, Data curation. YL: Data curation, Writing – original draft.

Funding

The author(s) declare that financial support was received for the research and/or publication of this article. This work was supported by the National Science and Technology Major Project (grant no.2024ZD0528100,2024ZD0528104).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The handling editor BY declared a shared parent affiliation with the author YS at the time of review.

Generative AI statement

The authors declare that no Generative AI was used in the creation of this manuscript.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2025.1608719/full#supplementary-material

References

Chen, L., Fu, G., Hua, Q., Zhu, H., Deng, Y., Wu, W., et al. (2022). Efficacy of add-on Danhong injection in patients with unstable angina pectoris: a double-blind, randomized, placebo-controlled, multicenter clinical trial. *J. Ethnopharmacol.* 284, 114794. doi:10.1016/j.jep.2021.114794

Chinese Society of Neurology, and Chinese Stroke Society (2022). Chinese guideline for the secondary prevention of ischemic stroke and transient ischemic attack 2022. *Chin. J. Neurol.* 55 (10), 1071–1110. doi:10.3760/cma.j.cn113694-20220714-00548

Chinese Society of Neurology, and Chinese Stroke Society (2024). Chinese guidelines for diagnosis and treatment of acute ischemic stroke 2023. *Chin. J. Neurol.* 57 (6), 523–559. doi:10.3760/cma.j.cn113694-20240410-00221

Dong, Y., Jiang, K., Li, Z., Zhou, Y., Ju, B., Min, L., et al. (2024). Tongxinluo and functional outcomes among patients with acute ischemic stroke: a randomized clinical trial. *JAMA Netw. Open* 7 (9), e2433463. doi:10.1001/jamanetworkopen.2024.33463

Du, H., He, Y., Pan, Y., Zhao, M., Li, Z., Wang, Y., et al. (2021). Danhong injection attenuates cerebral ischemia-reperfusion injury in rats through the suppression of the neuroinflammation. *Front. Pharmacol.* 12, 561237. doi:10.3389/fphar.2021.561237

Du, Y., Zhong, L., Wu, S., and Wang, X. (2018). Comparative study of the effects of Danhong injection with different doses on ischemic stroke: a substudy of hospital-based Danhong injection registry. *J. Tradit. Chin. Med.* 38 (6), 917–925. doi:10.1016/s0254-6272(18)30992-0

Fangma, Y., Zhou, H., Shao, C., Yu, L., Yang, J., Wan, H., et al. (2021). Hydroxysafflor yellow A and anhydrosafflor yellow B protect against cerebral ischemia/reperfusion injury by attenuating oxidative stress and apoptosis via the silent information regulator 1 signaling pathway. *Front. Pharmacol.* 12, 739864. doi:10.3389/fphar.2021.739864

Feng, X., Li, Y., Wang, Y., Li, L., Little, P. J., Xu, S., et al. (2019). Danhong injection in cardiovascular and cerebrovascular diseases: pharmacological actions, molecular mechanisms, and therapeutic potential. *Pharmacol. Res.* 139, 62–75. doi:10.1016/j.phrs.2018.11.006

Fu, C., Liu, L., Wang, Y., and Li, C. (2018). Chinese experts consensus on application of Danhong injection. *Chin. J. Integr. Tradit. West. Med.* 38 (4), 389–397. doi:10.7661/j.cjim.20180312.064

GBD 2021 Diseases and Injuries Collaborators (2024). Global incidence, prevalence, years lived with disability (YLDs), disability-adjusted life-years (DALYs), and healthy life expectancy (HALE) for 371 diseases and injuries in 204 countries and territories and 811 subnational locations, 1990-2021: a systematic analysis for the Global Burden of Disease Study 2021. *Lancet* 403 (10440), 2133–2161. doi:10.1016/S0140-6736(24)00757-8

GBD 2021 Stroke Risk Factor CollaboratorsAbate, M. D., Abate, Y. H., Abd ElHafeez, S., Abd-Allah, F., Abdelalim, A., et al. (2024). Global, regional, and national burden of stroke and its risk factors, 1990-2021: a systematic analysis for the Global Burden of Disease Study 2021. *Lancet Neurol.* 23 (10), 973–1003. doi:10.1016/S1474-4422(24)00369-7

Ge, L., Shen, Y., Liu, X., Xue, L., He, X., Yuan, X., et al. (2022). Safety reevaluation of Danhong injections based on hospital pharmacovigilance system and nested case control study. *Pharm. Clin. Res.* 30 (4), 368–370. doi:10.13664/j.cnki.pcr.2022.04.016

Geleris, J., Sun, Y., Platt, J., Zucker, J., Baldwin, M., Hripcsak, G., et al. (2020). Observational study of hydroxychloroquine in hospitalized patients with covid-19. *N. Engl. J. Med.* 382 (25), 2411–2418. doi:10.1056/NEJMoa2012410

Guo, H., Li, M., Liu, Q., Guo, L., Ma, M., Wang, S., et al. (2014). Danhong injection attenuates ischemia/reperfusion-induced brain damage which is associating with Nrf2 levels *in vivo* and *in vitro*. *Neurochem. Res.* 39 (9), 1817–1824. doi:10.1007/s11064-014-1384-1

Haneuse, S., Vanderweele, T. J., and Arterburn, D. (2019). Using the E-value to assess the potential effect of unmeasured confounding in observational studies. *JAMA* 321 (6), 602–603. doi:10.1001/jama.2018.21554

Hu, S., Chen, Y., Huang, S., Liu, M., Liu, Y., and Huang, S. (2022). Sodium Danshensu protects against oxygen glucose deprivation/reoxygenation-induced astrocytes injury through regulating NOD-like receptor pyrin domain containing 3 (NLRP3) inflammasome and tuberous sclerosis complex-2 (TSC2)/mammalian target of rapamycin (mTOR) pathways. *Ann. Transl. Med.* 10 (20), 1097. doi:10.21037/atm-22-2143

Kainz, K., Greifer, N., Givens, A., Swietek, K., Lombardi, B. M., Zietz, S., et al. (2017). Improving causal inference: recommendations for covariate selection and balance in propensity score methods. *J. Soc. Soc. Work Res.* 8 (2), 279–303. doi:10.1086/691464

Langan, S. M., Schmidt, S. A., Wing, K., Ehrenstein, V., Nicholls, S. G., Filion, K. B., et al. (2018). The reporting of studies conducted using observational routinely collected health data statement for pharmacoepidemiology (RECORD-PE). *BMJ* 363, k3532. doi:10.1136/bmj.k3532

Li, L., Yang, J., Li, C., Zhou, H., Yu, L., Wu, X., et al. (2023). Danhong injection improves neurological function in rats with ischemic stroke by enhancing neurogenesis and activating BDNF/AKT/CREB signaling pathway. *Biomed. Pharmacother.* 163, 114887. doi:10.1016/j.biopha.2023.114887

Li, X., Tang, J., Li, W., Li, C., Zhao, T., Zhao, B., et al. (2015). Postmarketing safety surveillance and reevaluation of Danhong injection: clinical study of 30888 cases. *Evid. Based Complement. Altern. Med.* 2015, 610846. doi:10.1155/2015/610846

Liu, C., Du, L., Zhang, S., Wang, H., Kong, L., and Du, G. (2023). Network pharmacology and experimental study of phenolic acids in salvia miltiorrhiza bung in preventing ischemic stroke. *Front. Pharmacol.* 14, 1108518. doi:10.3389/fphar.2023.1108518

Liu, J., Li, D., Dong, W., Liu, Y., Wu, Y., Tang, D., et al. (2021). Detection of an anti-angina therapeutic module in the effective population treated by a multi-target drug Danhong injection: a randomized trial. *Signal Transduct. Target. Ther.* 6 (1), 329. doi:10.1038/s41392-021-00741-x

Ma, Y., Deng, K., Liu, J., Ma, B., Mei, F., Hui, W., et al. (2022). The add-on effects of Danhong injection among patients with ischemic stroke receiving Western medicines: a systematic review and meta-analysis. *Front. Pharmacol.* 23, 937369. doi:10.3389/fphar.2022.937369

Makhnevich, A., Perrin, A., Talukder, D., Liu, Y., Izard, S., Chiuzan, C., et al. (2024). Thick liquids and clinical outcomes in hospitalized patients with alzheimer disease and related dementias and dysphagia. *JAMA Intern. Med.* 184 (7), 778–785. doi:10.1001/jamainternmed.2024.0736

Pan, Y., Li, Z., Li, J., Jin, A., Lin, J., Jing, J., et al. (2021). Residual risk and its risk factors for ischemic stroke with adherence to guideline-based secondary stroke prevention. *J. Stroke* 23 (1), 51–60. doi:10.5853/jos.2020.03391

Psaty, B. M., Koepsell, T. D., Lin, D., Weiss, N. S., Siscovick, D. S., Rosendaal, F. R., et al. (1999). Assessment and control for confounding by indication in observational studies. *J. Am. Geriatr. Soc.* 47 (6), 749–754. doi:10.1111/j.1532-5415.1999.tb01603.x

Schober, P., and Vetter, T. R. (2021). Count data in medical research: Poisson regression and negative binomial regression. *Anesth. Analg.* 132 (5), 1378–1379. doi:10.1213/ANE.0000000000005398

Tan, Y. Y., Papez, V., Chang, W. H., Mueller, S. H., Denaxas, S., and Lai, A. G. (2022). Comparing clinical trial population representativeness to real-world populations: an external validity analysis encompassing 43 895 trials and 5 685 738 individuals across 989 unique drugs and 286 conditions in England. *Lancet Healthy Longev.* 3 (10), e674–e689. doi:10.1016/S2666-7568(22)00186-6

Von Elm, E., Altman, D. G., Egger, M., Pocock, S. J., Gøtzsche, P. C., Vandenbroucke, J. P., et al. (2007). The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *Lancet* 370 (9596), 1453–1457. doi:10.1016/S0140-6736(07)61602-X

Wang, A., Jia, B., Zhang, X., Huo, X., Chen, J., Gui, L., et al. (2023). Efficacy and safety of butylphthalide in patients with acute ischemic stroke: a randomized clinical trial. *JAMA Neurol.* 80 (8), 851–859. doi:10.1001/jamaneurol.2023.1871

Wang, A., Zhu, Y., Han, S., Han, Z., Zhu, G., Jia, X., et al. (2024). Stability and safety evaluation of Danhong injection with different clinical solvents based on sequential analysis. *Chin. J. Pharm. Anal.* 44 (3), 493–500. doi:10.16155/j.0254-1793.2024.03.15

Wang J., J., Guo, Y., Zhou, H., Hua, Y., Wan, H., and Yang, J. (2024). Investigating the mechanistic of Danhong injection in brain damage caused by cardiac I/R injury via bioinformatics, computer simulation, and experimental validation. *ACS Omega* 9 (16), 18341–18357. doi:10.1021/acsomega.4c00200

Wang, Y., Li, Z., Gu, H., Zhai, Y., Zhou, Q., Jiang, Y., et al. (2022). China stroke statistics: an update on the 2019 report from the national center for healthcare quality management in neurological diseases, China national clinical research center for neurological diseases, the Chinese stroke association, national center for chronic and non-communicable disease control and prevention, Chinese center for disease control and prevention and institute for global neuroscience and stroke collaborations. *Stroke Vasc. Neurol.* 7 (5), 415–450. doi:10.1136/svn-2021-001374

Xu, J., Wang, A., Meng, X., Yalkun, G., Xu, A., Gao, Z., et al. (2021). Edaravone dexborneol versus edaravone alone for the treatment of acute ischemic stroke: a phase III, randomized, double-blind, comparative trial. *Stroke* 52 (3), 772–780. doi:10.1161/STROKEAHA.120.031197

Zeng, M., Zhou, H., He, Y., Wang, Z., Shao, C., Yin, J., et al. (2021). Danhong injection alleviates cerebral ischemia/reperfusion injury by improving intracellular energy metabolism coupling in the ischemic penumbra. *Biomed. Pharmacother.* 140, 111771. doi:10.1016/j.biopha.2021.111771

Zhang, H., Wang, X., Chen, W., Yang, Y., Wang, Y., Wan, H., et al. (2023). Danhong injection alleviates cerebral ischemia-reperfusion injury by inhibiting autophagy through miRNA-132-3p/ATG12 signal axis. *J. Ethnopharmacol.* 300, 115724. doi:10.1016/j.jep.2022.115724

Zhao, C., Bai, X., Wen, A., Wang, J., and Ding, Y. (2024). The therapeutic effects of salvianolic acids on ischemic stroke: from molecular mechanisms to clinical applications. *Pharmacol. Res.* 210, 107527. doi:10.1016/j.phrs.2024.107527

Zou, G. (2004). A modified Poisson regression approach to prospective studies with binary data. *Am. J. Epidemiol.* 159 (7), 702–706. doi:10.1093/aje/kwh090

frontiers | Frontiers in Pharmacology

TYPE Review
PUBLISHED 30 June 2025
DOI 10.3389/fphar.2025.1561702

Check for updates

OPEN ACCESS

EDITED BY
Chun Guang Li,
Western Sydney University, Australia

REVIEWED BY
Majid Sharifi-Rad,
Zabol University, Iran
Hee Geun Jo,
Gachon University, Republic of Korea

*CORRESPONDENCE
S. Ankul Singh,
ankulsingh57@gmail.com
Rukaiah Fatma Begum,
rukaiah786@gmail.com

RECEIVED 16 January 2025
ACCEPTED 04 June 2025
PUBLISHED 30 June 2025

CITATION
Sneha Sri R, Pavithra T, Vinciya T, Santhosh Kumar V, Harikrishnan N, Begum RF and Ankul Singh S (2025) Integrative approaches in Alzheimer's disease: evaluating the potential of traditional, complementary, and integrative medicine (TCIM).
Front. Pharmacol. 16:1561702.
doi: 10.3389/fphar.2025.1561702

COPYRIGHT
© 2025 Sneha Sri, Pavithra, Vinciya, Santhosh Kumar, Harikrishnan, Begum and Ankul Singh. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

Integrative approaches in Alzheimer's disease: evaluating the potential of traditional, complementary, and integrative medicine (TCIM)

R. Sneha Sri[1], T. Pavithra[1], T. Vinciya[1], V. Santhosh Kumar[1], N. Harikrishnan[2], Rukaiah Fatma Begum[3]* and S. Ankul Singh[1]*

[1]Department of Pharmacology, Dr. M.G.R. Educational and Research Institute, Chennai, Tamil Nadu, India, [2]Department of Pharmaceutical Analysis, Dr. M.G.R. Educational and Research Institute, Chennai, Tamil Nadu, India, [3]Institute of Pharmaceutical Research, GLA University, Mathura, Uttar Pradesh, India

This review explores the potential of Traditional, Complementary, and Integrative Medicine (TCIM) as an adjunct to conventional therapies for Alzheimer's Disease (AD). Unlike pharmaceutical treatments that primarily offer symptomatic relief, TCIM encompasses holistic approaches that target multiple pathophysiological pathways involved in AD, including tau pathology, oxidative stress, mitochondrial dysfunction, and neuroinflammation. Herbal therapies such as *Withania somnifera*, *Ginkgo biloba*, and *Curcuma longa* have shown promising neuroprotective effects in preclinical and limited clinical studies. Mind-body practices like Kirtan Kriya meditation have also demonstrated stress-reduction benefits, addressing modifiable risk factors for AD. While current evidence highlights the potential of TCIM interventions to complement standard care, rigorous validation through high-quality randomized controlled trials remains essential. This review underscores the need for integrative, personalized approaches that synergize traditional and modern medical systems to enhance therapeutic outcomes in AD.

KEYWORDS

Alzheimer's disease (AD), traditional medicine, complementary and integrative medicine (TCIM), herbal therapies, neuroinflammation, cognitive decline

1 Introduction

Globally, Alzheimer's disease (AD) is the primary cause of dementia and cognitive loss in those over 65. Its frequency in this age group varies from 1.9 to 5.8 instances per 100 individuals, and rates rise significantly with age, especially in females (Atri, 2019) (Rocca et al., 1986). Alzheimer's disease is caused by tau tangles, Aβ plaques, and neuroinflammation, all contributing to the disease (Kabra, 2022; Webers et al., 2020). Aβ aggregation influences tau hyperphosphorylation and microglial inflammation, linking these clinical hallmarks (Kabra, 2022). Pathophysiological changes that characterize this progressive neurodegenerative disease include amyloid-beta plaque buildup, hyperphosphorylated tau protein neurofibrillary tangles, and neurodegeneration due to inflammation and microglial activation (Jack et al., 2013; Li et al., 2022).

In its clinical form, AD first appears as cognitive decline and memory loss, which progresses to neuropsychiatric symptoms including mood swings, disorientation, and severe hallucinations (Harada et al., 2013). Traditional, Complementary, and Integrative

Medicine (TCIM) may offer therapeutic alternatives for AD. Mind-body therapy and lifestyle treatments are examples of TCIM techniques that report the multidimensional aspect of AD and complement the limited pharmacological choices available (Nguyen et al., 2024). However, despite the increased interest in TCIM for AD, the number of existing studies and the quality of research are still quite limited. Most research is discredited due to inadequate procedures, such as the lack of strong statistical analysis or RCTs. For this reason, very intensive assessment and cross-disciplinary research must be conducted for TCIM methodologies to be validated and integrated with conventional treatments (Kong et al., 2023). Glutamate antagonists and cholinesterase inhibitors are two examples of current treatments for Alzheimer's disease that target symptoms rather than the fundamental causes of the illness. This restriction emphasizes how urgently therapies that focus on the intricate pathophysiological pathways of AD are needed (Miculas et al., 2022). TCIM is emphasized in treating Alzheimer's disease because it considers the human person as a totality-the environmental, behavioral, emotional, and physical factors all contribute to total wellness. More compelling arguments include the less-than-desirable effects of present-day pharmaceutical therapy, the harmful effects they often incur, and further factors. With the complicated nature of AD, this all-embracing approach makes TCIM a useful additional care and preventive approach (Nguyen et al., 2024). The incidence of AD is predicted to triple by 2050, impacting one in every 85 individuals, making it a rapidly expanding worldwide health concern (Brookmeyer et al., 2007). With direct medical expenses varying by country and disease severity, Alzheimer's disease has a significant financial impact (Takizawa et al., 2014).

Although studies frequently have methodological issues, such as small sample sizes, a lack of controls, and inconsistent results, TCIM shows promise for treating AD. Another issue is publication bias, which favors favorable outcomes while keeping negative discoveries unreported. Extensive, multi-center RCTs are necessary to validate the safety and effectiveness of TCIM. While numerous reviews have explored the potential of Traditional, Complementary, and Integrative Medicine (TCIM) in Alzheimer's disease (AD), the present manuscript addresses a critical gap by focusing on the multi-targeted and synergistic therapeutic mechanisms of TCIM interventions. Unlike monotherapeutic strategies that often target a single pathological hallmark of AD, TCIM modalities offer a unique advantage through their ability to modulate multiple pathways simultaneously, including oxidative stress, neuroinflammation, mitochondrial dysfunction, and cholinergic deficits. This review consolidates current evidence on the pleiotropic effects of TCIM interventions, offering a systems-level perspective that has not been comprehensively synthesized before. In doing so, we aim to provide a nuanced understanding of how TCIM-based multi-target strategies could complement conventional treatments and improve therapeutic outcomes.

2 Methodology

2.1 Search strategy

A comprehensive literature search was conducted across PubMed, Scopus, and Web of Science databases from inception to December 2024. The search strategy included combinations of keywords and MeSH terms such as "Alzheimer's Disease," "Traditional Medicine," "Complementary and Integrative Medicine (TCIM)," "Herbal Therapies," "Neuroinflammation," "Cognitive Decline," and "Botanical Drugs." The Boolean operators "AND" and "OR" were used to refine and expand the search results.

2.2 Inclusion and exclusion criteria

To preserve the review's quality and applicability, stringent inclusion and exclusion criteria were used. Clinical or preclinical results about neuroprotection, anti-inflammatory qualities, and cognitive function improvement were specifically highlighted in studies that examined the therapeutic potential of TCIM in Alzheimer's disease. Additionally, articles that addressed how TCIM addresses fundamental pathogenic pathways, including amyloid-beta pathology and oxidative stress, were given priority.

On the other hand, research that was unrelated to Alzheimer's disease or published in a language other than English was disqualified. Furthermore, papers that were only abstracts, editorials, or comments, or those that were not available in full text, were not included. This guaranteed that only comprehensive, peer-reviewed research publications were included in the study.

3 Pathogenic pathways in Alzheimer's disease

Alzheimer's disease involves a complex interplay of multiple pathological mechanisms including amyloid-beta (Aβ) accumulation, tau protein hyperphosphorylation, oxidative stress, mitochondrial dysfunction, neuroinflammation, and cholinergic deficits. These interconnected pathways contribute to progressive synaptic failure and cognitive decline. A schematic overview of these multifactorial pathogenic mechanisms is presented in Figure 1.

4 TCIM approaches for AD

4.1 Foundations and therapeutic principles of TCIM

TCIM, which refers to traditional, complementary, and integrative medicine, is are health practice not very commonly applied by mainstream medicine, but rather serves to supplement the conventional ways of doing things. WHO describes the collective knowledge, skills, and practices acquired from culture and used for the discovery, treatment, and prevention of illness and health-related problems as traditional medicine (Ng et al., 2024). According to the WHO, complementary medicine refers to medical procedures that are utilized in addition to traditional medicine but are not entirely included in the prevailing healthcare system (Ventola, 2010). The term "integrative health," which refers to the systematic blending of complementary and conventional treatments for patient-centered care, is further expanded by the National Center for Complementary and

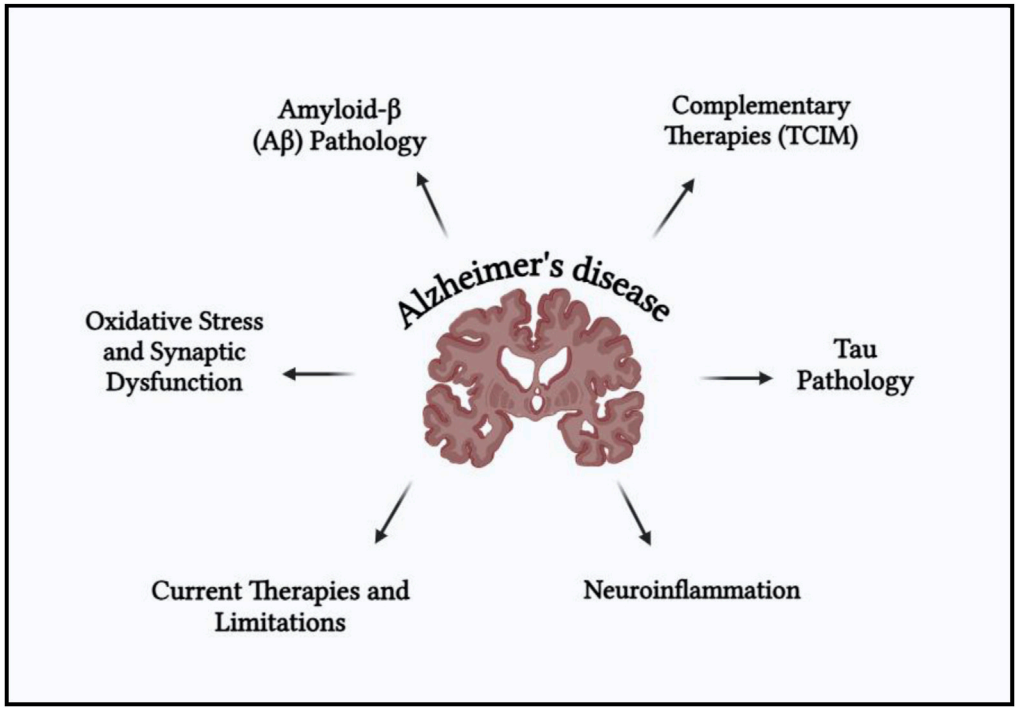


FIGURE 1
Schematic representation of the multifactorial pathogenesis of Alzheimer's disease (AD), highlighting key neurobiological mechanisms.

Integrative Health (NCCIH) (Adib-Hajbaghery et al., 2021). By combining conventional medical procedures with complementary therapies including acupuncture, botanical drugs (e.g., phytochemicals with known antioxidant or anti-inflammatory effects), and to a lesser extent, practices like yoga or meditation that may influence neuroendocrine or oxidative stress pathways. Activities like Kirtan Kriya, a meditation technique that incorporates hand gestures, chanting, and concentrated attention, have demonstrated the potential to lower stress, which is an acknowledged risk factor for AD (Ashford et al., 2015). New studies further reveal that TCIM can be used for the treatment of biological factors involved in AD, such as inflammation and insulin resistance. TCIM thus provides a hopeful scope for management and prevention, yet its validity, safety, and assimilation into conventional practice have to be proved by deeper exploration (Phutrakool and Pongpirul, 2022).

4.2 Botanical drugs and phytochemicals

4.2.1 Botanical drugs overview

The three oldest and most vigorous active medical practices are traditional Chinese medicine, traditional Indian medicine, and Ayurveda. The two traditions have sound empirical, experimental, and philosophical evidence. Alternative and complementary medicine have regained popularity for many reasons, such as growing side effects, microbial resistance, emergent diseases, new medications' high costs, and no efficient treatments for chronic diseases for which no cure is known (Schaffner, 2001). Over 1,500 botanical drugs sold as supplements were among the alternative medicines that two-thirds of Americans were predicted to utilize by 2010 (Legal Status of Traditional Medicine, 2001). With projects in China, Singapore, and Europe, pharmaceutical companies worldwide concentrate on natural product medication development, effectively globalizing Traditional Chinese Medicine (TCM). India's Ayurveda is also becoming more popular worldwide thanks to government initiatives. According to the Western scientific community, despite their increasing popularity, traditional medicines need scientific confirmation and high-quality research to gain worldwide recognition (Patwardhan and Vaidya, 2014; Patwardhan et al., 2005). *In vitro* models, which lack the intricacy of genuine AD pathophysiology, are used in many TCIM investigations. Although botanical drugs may exhibit anti-inflammatory properties in computational models or cell cultures, their practical effectiveness is still unknown in the absence of *in vivo* validation or clinical studies. Human trials with appropriate controls and well-planned animal experiments should be given top priority in future research.

4.2.2 Specific botanical drugs

Although they are becoming ever more popular, most herbal treatments still do not have concrete scientific evidence. Traditional use oftentimes relies upon empirical use or centuries of experience based on anecdotes, while Western biologic systems require evidence from systematic clinical experience and RCTs. As an example:

Although it is used extensively to enhance memory, in AD research, ginkgo biloba had contradictory effects (Xie et al., 2022). There has been little or uncontrolled investigation of the potential

cognitive benefit of Bacopa monnieri, a leading Ayurvedic herb [Bacopa_monnieri_UPDATE_(supplements)]. While curcumin, the active ingredient of turmeric, possesses anti-inflammatory and antioxidant activity, its clinical bioavailability remains challenging (Lopresti, 2018). The disconnect between ancient claims and modern pharmaceutical standards is illustrated by these scenarios, where safety, dose control, repeatability, and bioavailability must be taken into careful consideration (Barnes, 2003).

4.2.3 Mechanisms of action

Several herbal compounds demonstrate interesting biological activity in early-stage investigations, yet much of the data is from *in vitro* studies that fail to convey the sophistication of AD pathology. Many of the studies of TCIM are based on cell models that greatly simplify processes such as amyloid deposition, oxidative stress, and neuroinflammation (shortt et al., 2017).

For instance, it is known to be observed that Icariin inhibits tau phosphorylation and Aβ deposition by modulating the PI3K/Akt/GSK-3β pathway (Wu et al., 2024; Yan et al., 2023).

Ashwagandha and *Centella asiatica* are said to possess anti-inflammatory activity through modulation of cytokines like TNF-α and IL-6, according to studies (Weerasinghe, 2024).

Acetylcholinesterase-inhibiting and able to cross the blood-brain barrier, huperzine A, which is extracted from Chinese club moss, has, in some experiments, been found to bring about symptomatic relief equivalent to that of existing medication, though with fewer side effects (Zhang, 2012).

It is important to validate these pathways using human trials and properly designed *in vitro* animal models. The transition from bench to clinical use has to be bridged in future research to allow for the safe and effective incorporation of candidate herbal therapies into AD therapy.

4.3 The role of traditional medicine in Alzheimer's disease

Although AD is becoming increasingly prevalent across the world and much research is conducted to find a cure, a long-term remedy has yet to be seen. Concerning the devastating loss of cognitive function that patients afflicted with AD experience, powerful therapeutic and preventive treatments are desperately needed to neutralize it. This is very timely as traditional medicine has brought out several promising remedies and drugs for neurodegenerative disorders (Cooper and Ma, 2017). TCM has been practiced for over 2,000 years. TCM or modern pharmacological theories have recently served as the foundation for Chinese botanical drugs used to treat AD; this method has discovered an impact on the causes and mechanisms of AD, TCM treatment, and natural extracts that effectively treat AD. Additionally, there is proof that Chinese botanical drugs may offer some additional cognitive advantages for single-target antagonists (Liu et al., 2014). *Tetradium ruticarpum* (A.Juss.) T.G. Hartley—Family: Rutaceae Bentham is one neuroprotective plant that is frequently used in Traditional Chinese Medicine (TCM) as a herbal remedy. Evodiamine, an extract of *E. rutaecarpa* Bentham, has a wide range of advantageous qualities, including anti-inflammatory and anti-AD roles, as well as antiobesity, antinociceptive, anticancer, antinomic, and antimetastatic effects, all of which are extremely beneficial for treating neurodegenerative diseases (Liu et al., 2013). Additionally, TCM has provided us with substances that need to be improved or substituted for the current AD. Targetin, an agonist of the retinoid X receptor (RXR), effectively treats AD in mouse models. Strong contenders for RXR agonists include the TCM substances sulfonic acid and β-lipoic acid. These TCM chemicals show promise for becoming anti-dementia medications by forming strong interactions via the RXR protein receptors (Chen et al., 2014). The citrus flavonoid nobiletin, which is used in Kampo, or Traditional Japanese Medicine, may be a natural anti-dementia treatment, according to research. Despite these results, the majority of the data supporting Kampo and TCM's cognitive advantages comes from preclinical or small-scale research. These claims are limited in their robustness by methodological errors, a dearth of placebo-controlled studies, and variations in herbal composition. It has been demonstrated to improve memory and learning, reduce oxidative stress, combat neurodegeneration, encourage synaptic plasticity, and stop plaque development. In a similar vein, ninjin'yoeito, another Kampo drug, has been investigated for enhancing mood and cognitive abilities in Alzheimer's patients. Depression was decreased, and cognitive function was enhanced when both therapies were combined (Cooper and Ma, 2017). *Centella asiatica* (L.) Urb. — Family: Apiaceae, a plant used as a brain tonic, has been shown to have neuroprotective benefits such as decreasing oxidative stress, inhibiting enzymes, and stopping amyloid plaque formation in AD. Ayurveda, a traditional Indian medicine, has been discovered to include valuable substances for treating problems with the nervous system, such as memory-related conditions like dementia (Nishteswar et al., 2014). Key pathogenic processes in Alzheimer's disease, such as Aβ aggregation and enzymes involved in the generation of Aβ peptide, can be inhibited by bioactive metabolites found in Traditional Chinese Medicine (TCM). This realization emphasizes the necessity of further research into TCM-derived plant metabolites to identify new mechanisms and synergistic pathways that conventional treatments may influence. Certain TCM elements can reduce inflammation and neuronal cytotoxicity brought on by Aβ. The review raises the idea of using TCM to treat AD and the opportunity for further TCM ingredient screening for therapeutic uses (Cheung et al., 2015). AD may benefit from the antioxidant and anti-apoptotic properties of several herbal medications and phytochemicals. Herbal medications and substances have been shown in preclinical investigations in animal models of AD to have antioxidant properties, enhance cognition, and offer neuroprotection. However, drug-herb interactions, dosage consistency, and long-term effectiveness must all be carefully considered when combining conventional therapy with contemporary pharmaceutical treatments. Guidelines based on evidence would aid in bridging the gap between conventional medicine. According to traditional wisdom, herbal treatments may be able to address the pathophysiology of AD at several molecular and cellular levels (Wadhawan et al., 2024). Medicinal plants, such as *Tinospora cordifolia* (Willd.) Miers—Family: Menispermaceae, *Hericium erinaceus* (Bull.) Pers. — Family:

Hericiaceae, *Commiphora wightii* (Arn.) Bhandari—Family: Burseraceae, *Hypericum perforatum* L. — Family: Hypericaceae, *Convolvulus pluricaulis* Choisy—Family: Convolvulaceae, *Rhodiola rosea* L. — Family: Crassulaceae, *Moringa oleifera Lam.* — Family: Moringaceae, and *Camellia sinensis* (L) Kuntze—Family: Theaceae are a few examples of medicinal plants that have both preventative and therapeutic effects on AD. Information from observational research is scarce (Perry et al., 1999; Sutalangka et al., 2013; Zieneldien et al., 2022). Although Randomized controlled trials and well-designed longitudinal investigations are required to determine the effectiveness, safety, and therapeutic range of traditional herbal treatments, to expand the body of evidence.

- *Ginkgo biloba* L. — Family: Ginkgoaceae, a well-studied herbal extract in the context of neurodegenerative diseases, has shown promising effects in various experimental models. *In vitro* studies reveal that it exerts antioxidant and neuroprotective effects by protecting neurons from oxidative stress and β-amyloid-induced cytotoxicity. *In vivo* findings in animal models of Alzheimer's disease demonstrate improved cognitive performance, enhanced cerebral blood flow, and restored mitochondrial function. However, while some *clinical trials* have reported improved memory and cognitive scores, particularly with long-term administration, the overall quality of evidence remains mixed. Meta-analyses indicate heterogeneity in outcomes, underscoring the need for more robust, large-scale randomized controlled trials (RCTs) (Yang et al., 2015).
- Huperzine A, an alkaloid derived from *Huperzia serrata* (Thunb.) Trevis. — Family: Lycopodiaceae, has been identified as a potent reversible inhibitor of acetylcholinesterase (AChE). *In vitro*, it shows neuroprotective activity against glutamate-induced excitotoxicity and oxidative injury. *In vivo* studies report enhancements in memory retention, learning behavior, and synaptic density in rodent models of Alzheimer's disease. In *clinical settings*, small-scale RCTs have demonstrated cognitive improvements in individuals with Alzheimer's and vascular dementia, with favorable tolerability. Despite encouraging findings, caution is warranted when interpreting these results due to limitations in trial design and sample size (Rafii et al., 2011).
- *Centella asiatica* (L.) Urb. — Family: Apiaceae has long been used in traditional medicine for cognitive support. Its *in vitro* profile includes promotion of neurite outgrowth, enhanced collagen synthesis, and modulation of antioxidant enzymes. In *in vivo* models, it has been shown to improve spatial learning and memory, increase hippocampal dendritic arborization, and support synaptic plasticity. Preliminary *clinical evidence* supports its potential, particularly in elderly populations where improvements in mood and memory have been observed. Nonetheless, these results require validation through well-powered clinical studies (Wright et al., 2022).
- *Curcuma longa* L. — Family: Zingiberaceae, the principal curcuminoid of *C. longa*, exhibits a diverse range of bioactivities. *In vitro*, it demonstrates antioxidant, anti-inflammatory, and anti-amyloidogenic effects by modulating signaling pathways like NF-κB, Nrf2, and β-secretase activity. *In vivo* animal models show that *C. longa* L. — Family: Zingiberaceae can reduce amyloid plaque burden, promote neurogenesis, and enhance memory performance. However, *clinical trials* have yielded inconsistent outcomes, largely due to curcumin's poor oral bioavailability. While some trials suggest modest cognitive benefits, further studies employing bioavailability-enhanced formulations are necessary (Kuszewski et al., 2018).
- *Withania somnifera* (L.) Dunal—Family: Solanaceae has demonstrated promising neuroprotective effects across multiple experimental levels. *In vitro*, it stimulates neurite outgrowth, suppresses oxidative stress, and downregulates inflammatory mediators. *In vivo* studies reveal its ability to reverse cognitive deficits in stress-induced and transgenic mouse models of Alzheimer's disease, with noted improvements in learning and memory. Clinical investigations have also shown favorable results in improving attention, executive function, and information processing speed in both cognitively impaired and healthy adults. Despite its traditional use and growing scientific support, large-scale, blinded RCTs are still needed to substantiate its clinical efficacy in dementia care (Xing et al., 2022)

4.4 Complementary and integrative medicine

The National Center for Complementary and Integrative Health (NCCIH) classifies TCIM approaches into three categories (a) mind-body practices (e.g., yoga, meditation, acupuncture): that may exert effects through modulation of stress hormones like cortisol or neurotrophic factors such as BDNF; (b) traditional medical systems such as Ayurveda and Traditional Chinese Medicine (TCM), which offer pharmacologically active herbal agents; and (c) natural products, including botanical medicines, vitamins, and probiotics with potential neuroprotective or anti-inflammatory properties (Nguyen et al., 2022).

It has been shown that using these adjunct methods along with drug treatments results in synergistic outcomes. For example, in AD patients, donepezil and yoga or mindfulness-based stress reduction have been associated with improved mood, reduced anxiety, and enhanced cognitive functioning. Cholinesterase inhibitors also seem to facilitate neuroprotection and delay cognitive decline when used along with nutritional therapies like the Mediterranean diet. These combined strategies illustrate how an integrated, multimodal approach could enhance overall outcomes of Alzheimer's disease treatment (García-Casares et al., 2021).

Although preclinical and clinical research have shown encouraging benefits, there is still conflicting evidence in favor of complementary and integrative therapies for AD. Thorough clinical studies with specified objectives are required to confirm their effectiveness and clarify the exact processes behind their possible advantages.

4.5 Modulation of the microbiota-gut-brain axis in Alzheimer's disease through TCIM approaches

The microbiota-gut-brain axis includes bidirectional communication via neuroimmune, neuroendocrine, and direct

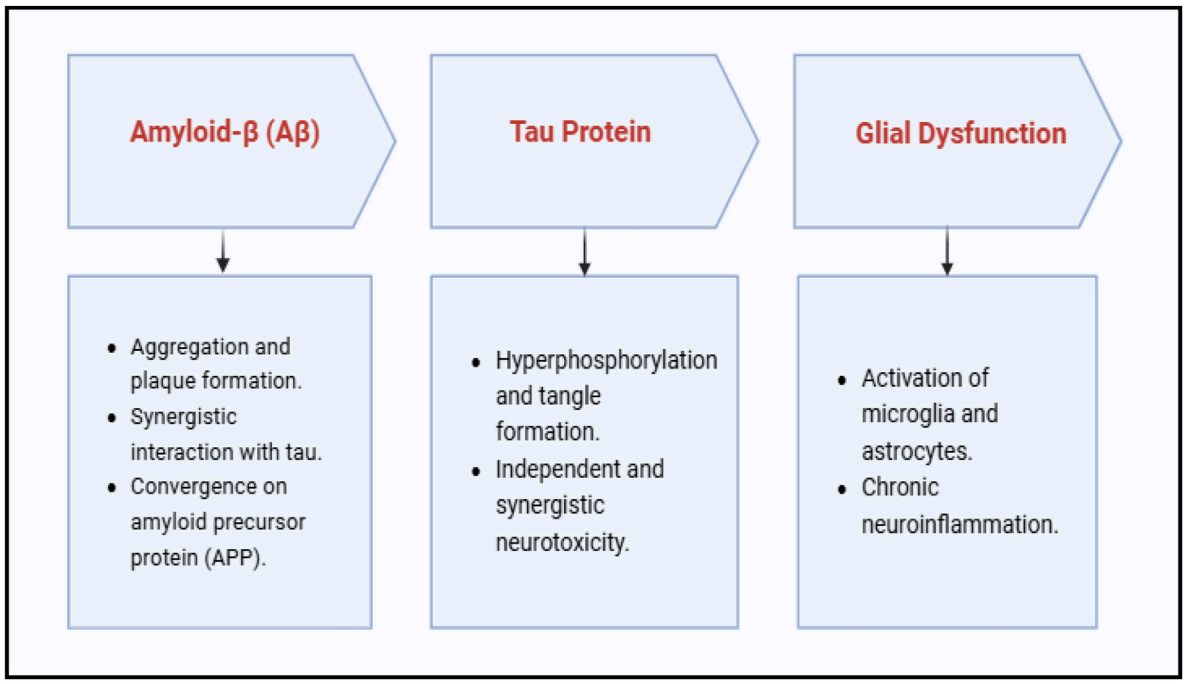


FIGURE 2
Overview of molecular and cellular mechanisms modulated by selected TCIM interventions.

neural routes, such as the vagus nerve (Peterson, 2020). The condition contributes to AD pathology by impairing host immunological responses and increasing inflammation, potentially acting as a trigger for the start and development of AD. TCM is a viable resource for treating Alzheimer's disease because of its chemical diversity and multi-target properties. It modulates the gut microbiota, which is a key target for its ability to cure Alzheimer's disease by modifying the microbiota-gut-brain axis (Ma et al., 2023).

Traditional Chinese medicine can prevent and treat Alzheimer's disease by regulating the gut microbiota (Long et al., 2024).

Mechanism: Changes in the gut plants may contribute to the buildup of amyloid beta. The relationship between the gut and the brain, and metabolites produced by the gastrointestinal microbiome, is involved in Alzheimer's disease pathogenesis.

Clinical trial evidence: Recent preclinical and clinical research suggests that gut microbiota changes may play a role in Alzheimer's disease. Antibiotics, prebiotics, probiotics, fecal microbiota transplantation, and dietary changes are all possible treatments.

Emphasizes the efficacy of antibiotics, prebiotics, probiotics, feces microbe transplantation, and dietary changes as complementary therapy for Alzheimer's disease, demonstrating their incorporation in mainstream pharmacological practice (Guo et al., 2020).

5 Mechanisms of action in TCIM

Amyloid β, tau, and glial dysfunction are the main contributors to the growth of AD (Han et al., 2020). Key Contributors and Therapeutic Pathways in AD are illustrated in Figure 2.

Curcuma longa L. — Family: Zingiberaceae has been explored as a treatment that influences pathways such as PI3K/Akt signaling and neurotrophin signaling (Bashir et al., 2022). TMS has been shown to promote synaptic plasticity, change gene expression, and boost cognitive function in AD patients (Zhao et al., 2019). In AD mouse models, trans-cinnamaldehyde (TCA) has also been demonstrated to have neuroprotective effects via improving NMDA receptor activity and decreasing neuroinflammation. TCA lowers pro-inflammatory mediator levels and microglial activation by blocking the NF-κB pathway (Prasad et al., 2002). AD is a neurodegenerative condition complicated by oxidative stress, neuroinflammation, and loss of cognitive functions (Papagiouvannis et al., 2021) (Murphy and Park, 2017). Studies indicate that treatment of AD is possibly more effective using the multi-target approach, such as including antioxidants, anti-inflammatory drugs, and neurotrophic factors, rather than single-modal therapy (Papagiouvannis et al., 2021) (Onaolapo et al., 2021).

Activating the Nrf2-mediated antioxidant defense system and stimulating the neurotrophic signaling pathway can potentially change AD pathology (Onaolapo et al., 2021). Additionally, nutritional treatment and lifestyle modifications may be crucial in lowering the oxidative stress and neuroinflammation associated with Alzheimer's. Though preclinical studies have shown that anti-inflammatory medicines are beneficial, this finding has yet to be confirmed in clinical investigations, necessitating additional studies into more viable treatment strategies (Ghosh et al., 2013). Tau immunotherapy can regulate both tau and upstream amyloid pathology, while persistent interleukin-1β overexpression worsens tau pathology despite reduced amyloid burden (Castillo-Carranza et al., 2015) (Haut et al., 2023). The "Amyloid Cascade Hypothesis," which holds

that tau and Aβ separately produce neurotoxicity, is called into question by these findings (Busche and Hyman, 2020) Research shows that tau and Aβ work in concert, convergently acting on the amyloid precursor protein (APP) as a common downstream effector (Busche and Hyman, 2020) (Wang C. et al., 2014). This emphasizes the necessity of concurrently focusing efforts on both (Wang C. et al., 2014). It has been shown in traditional medicine that *C. longa*, or *C. longa* L. — Family: Zingiberaceae, activates the PI3K/Akt pathway, increasing neuronal survival and lowering damage brought on by Aβ (Limpeanchob et al., 2008). *Bacopa monnieri* (L.) Wettst. — Family: Plantaginaceae also improves Nrf2 pathway activity, which reduces oxidative stress and increases cellular resilience (Dhanasekaran et al., 2009). According to recent research, *Centella asiatica* (L.) Urb. — Family: Apiaceae extracts considerably lower oxidative stress indicators and hippocampus Aβ levels in AD mouse models, confirming its function in protective effects on neurons and cognitive improvement (Jayaprakasam et al., 2010). Clinical investigations verifying these cognitive advantages are still few, despite preclinical research suggesting *C. asiatica* (L.) Urb. — Family: Apiaceae may have a neuroprotective function. Furthermore, *in vivo* and clinical studies have demonstrated that *Withania somnifera* reduces tau aggregation and enhances antioxidant defense (Kuboyama et al., 2002; Li et al., 2008). TCIM drugs like Fuzhisan that increase acetylcholine content with additional anti-inflammatory and antioxidant activities share the same aim of achieving and maintaining adequate acetylcholine levels that conventional therapy, including cholinesterase inhibitors, aims to achieve. Because TCIM mechanisms can target various pathways implicated in AD pathogenesis, they can complement traditional therapies, which highlights the promise of integrative therapy methods (King et al., 2020). However, the absence of established procedures, dosage fluctuations, and a dearth of solid clinical data pose serious obstacles to the broader use of TCIM. Evidence-based studies that align TCIM techniques with traditional medical procedures may offer the necessary scientific support for their broad application.

TCIM-based therapies have tremendous promise for the treatment of Alzheimer's disease, as they can target most of the pathogenic pathways, such as oxidative stress, inflammation, tau pathology, and cholinergic deficits. Overcoming critical obstacles such as bioavailability, pharmacokinetics, and herbal metabolite standardization will, however, need to be overcome for preclinical success to translate to clinical application. Integrative techniques that combine TCIM with conventional medicines have the potential to provide synergistic advantages in AD management.

5.1 Pharmacological and clinical validation of herb-derived agents in Alzheimer's disease

5.1.1 Pharmacological mechanisms of action

Herb-derived phytochemicals target multiple pathogenic pathways in AD (Table 1). The pharmacological mechanisms of key herbal agents in AD are summarized in Table 1.

Curcuma longa L. — Family: Zingiberaceae: Inhibits Aβ aggregation, activates PI3K/Akt pathway, and downregulates NF-κB-mediated neuroinflammation. [IC50 for AChE inhibition: ~67.69 μM] (Sehgal et al., 2012).

Withania somnifera (L.) Dunal—Family: Solanaceae: Inhibits acetylcholinesterase, reduces TNF-α and IL-6 levels, and promotes neurogenesis via LRP1 receptor upregulation (Huang et al., 2008).

Bacopa monnieri (L.) Wettst. — Family: Plantaginaceae: Enhances BDNF expression and reduces oxidative stress through upregulation of antioxidant enzymes (SOD, catalase) (Yang et al., 2015).

Ginkgo biloba L. — Family: Ginkgoaceae: Reduces tau hyperphosphorylation, enhances cerebral blood flow, and exerts antioxidant effects (Yin et al., 2009).

Centella asiatica (L.) Urb. — Family: Apiaceae: Protects neurons via antioxidant activity and modulates MAPK/ERK and PI3K/Akt signaling pathways (Anand et al., 2007).

5.1.2 Pharmacokinetics and pharmacodynamics (PK/PD)

Despite promising effects, botanical drugs face PK/PD challenges: Table 2 outlines the absorption, metabolism, BBB penetration, and half-life of commonly used herbs in AD.

5.1.3 Quality control aspects

Standardization and quality assurance of botanical drugs remain significant concerns in TCIM. Phytochemical content variation can arise due to environmental factors like climate, harvesting season, and post-harvest storage conditions (Saper et al., 2008). This inconsistency affects therapeutic efficacy and reproducibility. Additionally, herbal products may be contaminated with pesticides, heavy metals, or microbial agents, posing potential health risks (WHO, 2024). Regulatory agencies, including the World Health Organization (WHO), advocate for the implementation of Good Agricultural and Collection Practices (GACP) and standardized manufacturing methods such as High-Performance Liquid Chromatography (HPLC) to ensure quality and safety (Kunle, 2012). Identification and quantification of specific marker metabolites, such as withanolides in *Withania somnifera* (L.) Dunal—Family: Solanaceae, bacosides in *Bacopa monnieri* (L.) Wettst. — Family: Plantaginaceae, and the standardized extract EGb761 in *Ginkgo biloba* L. — Family: Ginkgoaceae, are essential for product consistency and efficacy validation (Flory et al., 2021).

5.1.4 Herb-drug interactions

Several herb-drug interactions can affect conventional AD treatments. Common herb-drug interactions that may influence pharmacotherapy in AD are detailed in Table 3.

5.1.5 Clinical evidence and efficacy evaluation

Several human studies have explored herbal efficacy, but limitations remain.

6 Enhanced therapeutic potential of herbal combinations

Multi-metabolite botanical drugs are widely recognized for potential use in treating complex diseases such as AD. These alternatives have several key advantages over more conventional

TABLE 1 Pharmacological mechanisms of action of selected herb-derived agents in Alzheimer's disease.

Herb	Absorption	Metabolism	BBB penetration	Half-life
Curcuma longa L. — Family: Zingiberaceae	Poor (<1%)	Extensive hepatic glucuronidation	Low	~1–2 h (Gupta et al., 2022)
Withania somnifera (L.) Dunal — Family: Solanaceae	Moderate	Hepatic (CYP3A4)	Moderate	~6 h (Aguiar and Borowski, 2013)
Bacopa	Variable	Hepatic	Moderate	~4–6 h (Zullino et al., 2003)
Ginkgo	Good	CYP2C19, 2D6 interaction	Yes	~4–6 h (Diniz et al., 2023)
Centella	Low	Liver via phase I/II	Moderate	~3–5 h (Patwardhan et al., 2005)

TABLE 2 Pharmacokinetic properties of key herbal drugs used in Alzheimer's disease.

Herb	Affected drug	Mechanism	Clinical concern
Ginkgo	Donepezil	CYP2D6/3A4 modulation	Altered plasma levels
Curcuma longa L. — Family: Zingiberaceae	Rivastigmine	Inhibits P-gp	Potential toxicity (Shoukat et al., 2023)
Bacopa	Galantamine	Additive AChE inhibition	Cholinergic excess risk (Candelario et al., 2015)
Withania somnifera (L.) Dunal — Family: Solanaceae	Benzodiazepines	GABAergic potentiation	Excess sedation (Small et al., 2018)

TABLE 3 Herb-drug interactions relevant to conventional Alzheimer's disease therapies.

Agent	Study design	Sample size	Outcome	Limitations
Curcuma longa L. — Family: Zingiberaceae	RCT	n = 132	Reduced inflammatory markers; no cognitive improvement	Poor bioavailability (Choudhary et al., 2017)
Withania somnifera (L.) Dunal — Family: Solanaceae	RCT	n = 50	Memory improvement (MMSE)	Small sample, short duration (Yang et al., 2015)
Ginkgo	Meta-analysis	21 trials, n > 2600	Mild cognitive benefit (MMSE, ADL)	Heterogeneous studies (Hutchison and Rodgers, 2001)
Bacopa	RCT	n = 98	Improved memory retention	Variability in extracts (Pullar et al., 2018)
Centella	reclinical	n/a	Reduced Aβ burden in mice	Limited human data (Article-034.).

treatments, among which are improved safety profiles, relative cost-effectiveness, and potential multi-target efficacy. Traditional Oriental herbal formulations have been shown to address multiple aspects of AD by diverse mechanisms (Jeon et al., 2019). Illustrations of Synergistic Properties of Multi-metabolite Herbal Therapies in Figure 3.

The multi-metabolite nature of herbal preparations and the existence of unknown plant metabolites pose challenges in metabolism studies. Nevertheless, recent improvements in bioanalytical technologies have improved our capacity to assess the pharmacokinetics and metabolic interactions of plant metabolites (Xin et al., 2011).

These treatments have also been able to counteract some of the chemotherapy-induced toxicities, including neurotoxicity and cardiotoxicity (Fu et al., 2018). The neuropathological factors involved, such as tau, α-synuclein, and β-amyloid (Aβ), are synergistic and interact with each other in a complex manner, different from the classical linear progression model (Clinton et al., 2010). Thus, a single protein cannot be targeted, since the anti-Aβ clinical studies were not successful enough (Wang J. et al., 2014). Promising approaches are multi-target therapies, which include polyphenolic metabolites. Comparison between grape seed extract, resveratrol, and purple grape juice extract showed better outcomes than single metabolite treatment on cognition scores and lowering of amyloid loads in AD models of animal systems (Calfio et al., 2020). It forms a significant problem in producing effective treatments against AD. Recent studies focused on using the possibilities of multi-target treatments of natural substances and phytochemicals, which could serve to mitigate Alzheimer's disease (Tuzimski and Petruczynik, 2022) (Calfio et al., 2020). Multi-target therapies, including polyphenolic compounds, have emerged as promising strategies. Research comparing grape seed extract, resveratrol, and purple grape juice extract with single-metabolite treatments found improved cognitive outcomes and reduced amyloid load in animal models of AD (Tuzimski and Petruczynik, 2022) (Iqubal et al., 2021). Natural compounds, however, tend to attack the complexities of Alzheimer's disease from various angles, whereas synthetic drugs tend to target a particular target (Wang J. et al., 2014). Unlike synthetic drugs, which often target a single pathway,

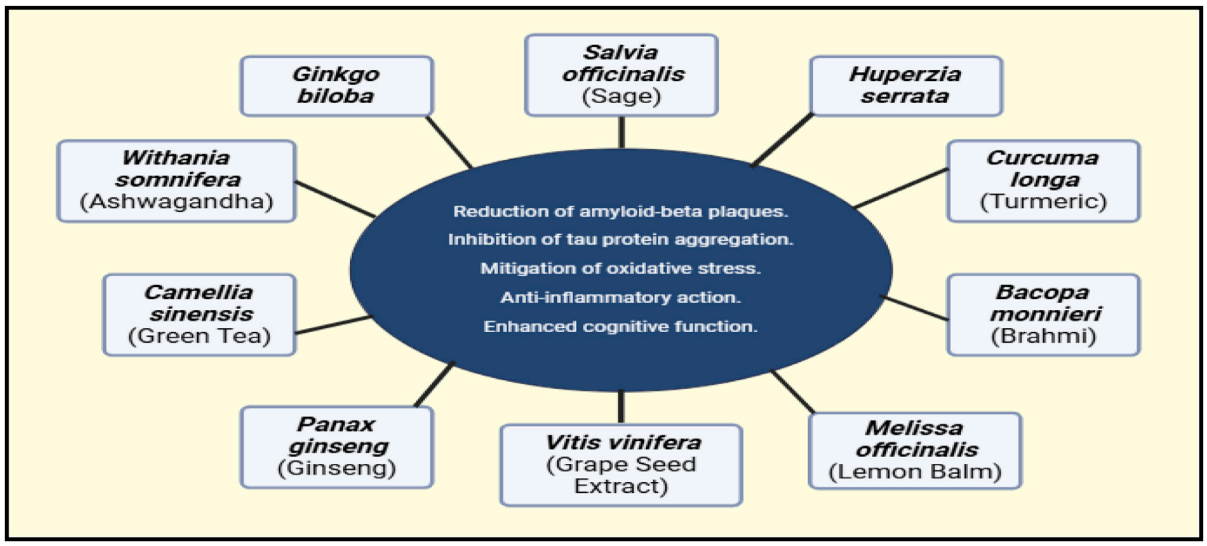


FIGURE 3
Proposed synergistic effects of various TCIM interventions in AD. This figure is based on theoretical predictions derived from existing literature and mechanistic insights, rather than direct experimental evidence.

natural compounds exhibit a broader therapeutic scope. Plant extracts and isolated phytochemicals are gaining attention as potential AD therapeutics, with many showing promise in preclinical studies (Bhat et al., 2022; Oxford et al., 2020).

7 Evaluation of clinical studies and therapeutic efficacy

Recent AD pharmacological trials have focused on beta-amyloid, which has usually failed to enhance cognitive function (Tripathi et al., 2024). The other targets that scientists consider include tau protein, neuroinflammation, and oxidative stress. A summary of clinical investigations evaluating TCIM interventions in Alzheimer's disease is provided in Table 4. Similarly, Table 5 provides an overview of experimental studies elucidating the mechanisms of action of TCIM approaches in Alzheimer's disease. Selected landmark clinical trials investigating both conventional and herbal interventions in Alzheimer's disease are summarized in Table 6. Plant-based traditional remedies like *Ginkgo biloba* L. — Family: Ginkgoaceae, *Huperzia serrata* (Thunb.) Trevis. — Family: Lycopodiaceae, and *Curcuma longa* have been shown to influence these targets through plant metabolites (Lee et al., 2020). Randomized controlled trials have been conducted to look at botanical drug therapies for AD, and Mini-Mental State Checkup scores have been used as a key outcome measure (Gharat et al., 2024). Cholinesterase inhibitors are already in use for the treatment of Alzheimer's disease symptoms, and there is an urgent need for disease-modifying medication (Cocchiara et al., 2020). Management of AD requires initial diagnosis and proper treatment, encompassing both drug and nondrug therapies (Pimperkhede et al., 2023).

Acetylcholinesterase inhibitors and memantine are the two most commonly prescribed pharmacotherapies; however, herbal treatments are also being researched (Pavlik et al., 2022). The CONCORD-AD network consists of seven multinational cohorts and aims to provide greater insight into the natural history of AD, thereby informing the design of clinical trials (Parfenov, 2020). However, management mistakes prevail; these include underdiagnosis, misunderstanding contemporary medicines, and the use of anti-dementia drugs. Non-pharmacological interventions like stimulation, exercise, and antioxidant foods are often overlooked (Andrade et al., 2022). The education and support of the caregivers are essential to provide excellent patient care (Pimperkhede et al., 2023). More knowledge among healthcare providers, improved diagnosis techniques, and access to effective medications would be vital in enhancing the management of AD (Andrade et al., 2022) (Pimperkhede et al., 2023). Meta-analyses and systematic reviews in recent times have shown significant insights into the prevention and treatment of AD. Exercise, specifically aerobic and multimodal exercise, has been proven to decrease AD symptoms, most importantly, cognitive (G et al., 2021).

Some gene variations, including CD33, BIN1, and MTHFR, have been identified as contributing to the risk of AD in several studies (Yu et al., 2020). A comprehensive assessment of modifiable factors disclosed 21 evidence-based notions for preventing AD, with great evidence for education, cognitive activity, and health conditions (Ou et al., 2020). Lastly, infectious agents have also been linked to the risk for Alzheimer's disease, which has been found to increase with many infections, including *Chlamydia* pneumoniae, Human herpesvirus-6, and Herpes simplex virus-1, with significant connections (Kumar et al., 2016). New-generation improvements in diagnostic tools and biomarker-based assessments will even increase trial reliability. Currently, the landscape of AD clinical trials is characterized by narrow target approaches, potential biases, small sample sizes, and variability in interventions. These must be overcome with multi-target therapeutic approaches, long follow-ups, and standardized protocols in drug and non-drug therapy.

TABLE 4 Summary of clinical studies evaluating TCIM in Alzheimer's disease.

Study design	Sample size and characteristics	Intervention	Outcome measures	Effect size/ Results	Statistical significance	Reference
Randomized Controlled Trial (RCT)	72 patients with mild-to-moderate AD	Sailuotong (Ginseng, Ginkgo, Crocus)	MMSE, ADAS-Cog	Improved MMSE scores by 3.2 ± 1.4 points	$p < 0.01$	Steiner-Lim et al. (2023)
Double-blind RCT	60 elderly patients diagnosed with AD	Bushen capsule	ADAS-Cog, memory score	Memory scores improved significantly over placebo	$p < 0.05$	Zhang et al. (2019)
Pilot RCT	34 AD patients (aged 65–85)	Acupuncture (weekly sessions for 12 weeks)	ADAS-Cog, behavior rating	ADAS-Cog improved by 2.7 points on average	Not reported	Lin et al. (2021)
Case-control	40 patients, 40 controls	Tai Chi exercise program (12 weeks)	Cognitive subtests	Delayed decline in executive function	$p < 0.05$	Yassine and Finch (2020)
Open-label clinical trial	50 patients with mild AD	Ginkgo biloba L. — Family: Ginkgoaceae extract (EGb761)	MMSE, Clock Drawing Test	MMSE scores improved by 2.4 ± 0.9	$p < 0.05$	Strandberg (2019)

TABLE 5 Mechanistic studies on TCIM modalities in Alzheimer's disease.

Type of study	Model/ Sample	Intervention	Outcome/ Mechanism	Effect size/ Results	Statistical significance	Reference
In vivo (mice)	APP/PS1 transgenic mice	Ginseng extract	Reduced Aβ deposition, improved memory function	40% reduction in Aβ plaques	$p < 0.01$	Choi et al. (2017)
In vitro	SH-SY5Y neuroblastoma cells	Resveratrol	Inhibited tau phosphorylation and oxidative stress	60% decrease in p-tau levels	$p < 0.001$	Inoue et al. (2016)
In vivo	Rat model of AD	Acupuncture	Increased BDNF and synaptic plasticity	BDNF upregulated 2.5-fold	$p < 0.01$	Nakamura and Sugaya (2014)
In vivo	SAMP8 mice	Curcuma longa L. — Family: Zingiberaceae	Anti-inflammatory and antioxidant effects	IL-6 levels decreased by 30%	$p < 0.05$	Kabak et al. (2021)
In vitro	Astrocyte cell line	Ginkgo biloba L. — Family: Ginkgoaceae extract	Increased mitochondrial viability	ATP production increased by 45%	$p < 0.01$	Qiu et al. (2022)

TABLE 6 Clinical trials related to Alzheimer's disease.

Trial name/Study	Intervention	Key outcome	Limitations
EXPEDITION-3 Trial	Solanezumab (Anti-Aβ monoclonal antibody)	Failed to enhance cognitive function in mild AD patients (Tripathi et al., 2024)	Targeted only Aβ; ignored tau and other mechanisms
Ginkgo Evaluation of Memory Study (GEMS)	*Ginkgo biloba* L. — Family: Ginkgoaceae extract	There was no significant decrease in the incidence of AD compared to the placebo (Lee et al., 2020)	The study population is mostly elderly with high comorbidities
HUPER Study	Huperzine A	Exhibited moderate improvement in cognition and decreased oxidative stress (Lee et al., 2020)	Small sample size; short trial duration
CURCUMIN Study	*Curcuma longa* L. — Family: Zingiberaceae supplements	Marginal effects on cognitive outcomes, but reduced neuroinflammation markers	Poor bioavailability; variability in *Curcuma longa* L. — Family: Zingiberaceae formulations (Lee et al., 2020)
SYNAPSE Study	Acetylcholinesterase inhibitors (donepezil)	Improved cognitive scores in mild to moderate AD patients (Gharat et al., 2024)	No disease-modifying effects; focused only on symptom management
FIT-AD Study	Aerobic exercise	Significant improvement in cognitive and functional abilities (G et al., 2021)	Lack of standardization in exercise regimens; challenging adherence
CONCORD-AD Network	Multinational cohort studies	Provided insights into the natural history of AD and trial design (Parfenov, 2020)	Heterogeneous populations; limited focus on emerging biomarkers

TABLE 7 Emerging therapeutics in Alzheimer's disease management.

Technique	Therapeutic target	Advantages	Challenges
Gene Therapy	Amyloidβ, Tau protein	Potential to modify disease progression	High cost, ethical concerns
Targeted Ultrasound	Amyloid Plaques	Non-invasive, improves drug delivery	Needs advanced equipment and training
Deep Brain Stimulation	Neurotransmission	Improves cognitive functions	Invasive procedure, potential side effects
Stem cell therapy	Neuroregeneration	Promotes neuronal repair	In a few clinical trials, the potential for oncogenesis
Immunotherapy	Amyloid β, Tau Proteins	Decreases pathological aggregates	Limited clinical trials, risk of tumorigenesis
Nanotechnology	Drug Delivery Systems	Increases blood-brain barrier permeability	Difficult manufacturing

8 Novel therapeutic targets: advances and emerging strategies

AD is a neurodegenerative illness caused by tau tangles, β-amyloid plaques, and neuroinflammation. Research efforts are directed toward novel targets and multifunctional compounds to address the multifaceted aspect of Alzheimer's pathogenesis. Computational biology, particularly molecular docking, has emerged as an effective methodology for discovering new therapeutic candidates. Alzheimer's disease is linked to multiple signaling pathways that have been involved in its etiology. Some key processes are abnormal calcium homeostasis, amyloid-beta plaque formation, and tau protein hyperphosphorylation. The PI3K/AKT pathway regulates cell survival and death, which is important for neuroprotection (Long et al., 2021). Autophagy, which interacts with several AD-related processes, is critical for maintaining cellular homeostasis and removing harmful proteins (Ramachandra et al., 2021).

APOE, GSK-3β, Notch, and Wnt signaling have also recently been associated with AD (Long et al., 2021). Some studies are currently on multi-convergence, with studies suggesting that autophagy serves as a potential therapeutic target, with its critical role and multiple interactions (Gadhave et al., 2021). In addition, natural compounds are identified as activators of PI3K/AKT; hence, they may come into use as neuroprotective drugs in the pharmacotherapy of AD. Current treatments focus on early intervention and a holistic approach to the various disease processes. The targets include amyloid beta, tau proteins, neurotransmission, inflammation, metabolism, and oxidative stress (Hroudová and Fišar, 2024). There are only a few medications that are accepted for the treatment of AD, such as cholinesterase inhibitors, NMDA antagonists, and anti-Aβ monoclonal antibodies (Abdallah, 2024) (Athar et al., 2021). The researchers are working on innovative therapeutic methods such as immunotherapy and neuroinflammation (Abdallah, 2024). An overview of promising therapeutic innovations, including gene therapy, nanotechnology, and neuroregenerative strategies, is summarized in Table 7. Other promising alternative techniques for AD treatment include targeted deep brain stimulation, ultrasound, stem cell therapy, and gene therapy, as represented in Table 4 for Innovative Approaches in AD Management.

Yet, there is still the issue of presymptomatic neuronal injury, undesirable effects of medication, and poor clinical trial design (Tatulian, 2022). Future research may be focused on AD pathophysiology, biomarkers, and new diagnostic tools, thus allowing for the development of more effective therapy strategies in the future (Hroudová and Fišar, 2024) (Tatulian, 2022). The limited absorption and quick metabolism of botanical drugs such as *Curcuma longa* L. — Family: Zingiberaceae and *Bacopa monnieri* (L.) Wettst. — Family: Plantaginaceae makes it difficult to translate preclinical findings of TCIM treatments for AD into human trials. Variability in extract quality and irregular dosage further restricts reproducibility. The multi-target mechanisms by which TCIM interventions modulate Alzheimer's pathology are illustrated in Table 8. Animal models often cannot mimic the intricate pathology of Alzheimer's disease observed in humans, which adds to discrepancies between preclinical and clinical outcomes. In addition, short-term follow-up studies with smaller sample sizes limit the capacity of the trials to reveal important disease-modifying benefits. In addition, there are no standard procedures followed, and only a few biomarkers are used, hence, it is impossible to replicate reproducible results. Future studies would be more optimized in drug delivery systems, and further standardization of formulations can be done using advanced biomarkers and lengthier studies to decide the therapeutic efficacy of TCIM.

9 Innovation and future directions

There are numerous limits to current Alzheimer's disease research. While artificial intelligence and language processing show potential for detecting cognitive decline, low standardization and restricted result comparability impede the clinical application (de la Fuente Garcia et al., 2020). Existing pharmacological treatments only provide symptomatic alleviation, while disease-modifying medicines remain unavailable. Central nervous system drug delivery is still challenging. However, nanoliposomes and exosomes have the potential to improve brain function (Passeri et al., 2022). The preclinical animal models are poor at simulating human AD pathology, leading to a poor transformation of hopeful applicants into clinical trials (Akhtar et al., 2022). Neuroimaging techniques have emerged as promising tools for the early diagnosis of Alzheimer's disease and the identification of biomarkers. However, their clinical utility varies in terms of precision and effectiveness (Kim et al., 2022).

TABLE 8 Mechanism of TCIM in Alzheimer's disease.

Therapeutic agent	Pathways targeted	Mechanism of action	Study design	Reference
Curcuma longa L. — Family: Zingiberaceae	PI3K/Akt Signaling	Promotes neuronal survival, reduces Aβ-induced damage	Preclinical models show reduced Aβ plaque levels	Bashir et al. (2022)
Bacopa monnieri (L.) Wettst. — Family: Plantaginaceae	Nrf2 Antioxidant Pathway	Enhances antioxidant defenses, mitigates oxidative stress	Preclinical studies support cognitive improvement	Dhanasekaran et al. (2009)
Centella asiatica (L.) Urb. — Family: Apiaceae	Oxidative Stress and Aβ Pathways	Reduces hippocampal Aβ levels and oxidative stress markers; improves cognitive function	Evidence in AD mouse models	Jayaprakasam et al. (2010)
Withania somnifera	Tau Aggregation and Antioxidant Défense	Reduces tau aggregation, enhances antioxidant defenses	Supported by *in vivo* and clinical studies	Kuboyama et al. (2002), Li et al. (2008)
Fuzhisan	Cholinergic Pathway, Antioxidants, Anti-Inflammatory	Increases acetylcholine levels, provides anti-inflammatory and antioxidant benefits	Clinical trials indicate cognitive enhancement	King et al. (2020)

Future drugs will focus on the fundamental mechanisms of diseases like Aβ plaques and neurofibrillary tangles, besides clinical relief (Yiannopoulou and Papageorgiou, 2020). Biomarkers of Aβ, tau pathology, neurodegeneration, synaptic dysfunction, and inflammation are important for selecting the right people to be studied and assessing the degree of their improvement (Zetterberg and Bendlin, 2021). The growing interest in psychedelic therapy for Alzheimer's disease raises ethical concerns, for example, about the effect on autonomy, consent, and caregiving, not to mention the potential exploitation of desperation in patients (Peterson et al., 2023). As science moves forward toward personalized medicine, various biomarkers combined with cognitive and neuroimaging data might guide treatment regimens (Yiannopoulou and Papageorgiou, 2020). In preparing healthcare systems for disease-modifying medications and addressing the ethical concerns surrounding psychedelic research, much advancement is set for Alzheimer's treatment and research. Among the new treatment options, there are targeting butyrylcholinesterase, tau proteins, and endocannabinoid system involvement. The role of microRNAs in AD research. Advanced diagnostic techniques, including qRTPCR and iPSCs, alter early detection (Sharma et al., 2024). The focus has shifted to studies on prodromal phases and biomarkers for early detection of the disease at preclinical levels (Rayathala et al., 2022). The goal of future drugs is the modulation of pathology via alterations to amyloid beta plaques and neurofibrillary tangles, whereas nowadays therapies are mainly trying to level neurotransmitter abnormalities (Ramachandra et al., 2021). To better, the complex causative basis of Alzheimer's, multiple metabolites like inflammation, the microbiota, hormones, as well as changes in the neurovascular unit, etc., are now being searched for (Giannoni et al., 2020). The discipline is shifting toward personalized medicine, which incorporates different therapies based on individual biomarkers and clinical manifestations (Yiannopoulou and Papageorgiou, 2020). Future Alzheimer's disease research should prioritize the thorough validation of novel medicines via well-designed clinical trials to ensure reproducibility and effectiveness. Regulatory approvals must include rigorous safety and efficacy studies, particularly for new medicines like psychedelics, stem cells, and nanotechnology-based interventions. Integrating evidence-based conventional treatments is critical for developing holistic approaches that address disease pathophysiology and improve cognitive function. There are still several gaps in understanding the multiple causal metabolites of Alzheimer's disease, including neuroinflammation, gut-brain interactions, hormone imbalances, and alterations in neurovascular variables. Further research in new diagnostic tools, such as qRT-PCR and iPSCs, for earlier detection of AD, together with the development of biomarkers to allow for personalized treatment regimens, shall be promising in the future. Standardization of protocols, drug delivery systems, and combination therapies will be the keys to further improvements in AD treatment.

9.1 Limitations and future research priorities

Despite the promising evidence presented for the use of TCIM in managing Alzheimer's disease (AD), several limitations hinder its clinical translation. A major concern is the lack of large-scale, multicenter randomized controlled trials (RCTs) that would provide robust evidence on safety and efficacy. Many existing studies suffer from small sample sizes, inadequate control groups, short follow-up durations, and variability in herbal formulations. The bioavailability and pharmacokinetic profiles of key herbal agents, such as curcumin and bacosides, remain suboptimal, restricting their clinical effectiveness.

Standardization of herbal products is also a critical issue. Variability in active compound concentrations due to differences in cultivation, harvesting, and processing conditions can lead to inconsistent therapeutic outcomes. Moreover, herb-drug interactions are not well-characterized, posing potential safety risks when TCIM is combined with conventional therapies.

Future research should prioritize:

- Rigorous, multicenter RCTs with standardized interventions and outcome measures.
- Development of novel drug delivery systems (e.g., nanocarriers) to improve bioavailability.
- Comprehensive studies on pharmacokinetics and herb-drug interactions.
- Identification and validation of biomarkers to assess treatment response.
- Integration of personalized medicine approaches, leveraging genomics and neuroimaging data.

10 Conclusion

In conclusion, Alzheimer's disease is a very complex and fast-growing global health challenge; hence, an innovative and holistic solution is required. TCIM is a way that might be adopted as a strategy for the nature of the disease; current pharmaceutical treatments offer little symptomatic relief for the condition. Key pharmacologically active agents derived from TCIM, such as *C. longa* L. — Family: Zingiberaceae, *B. monnieri* (L.) Wettst. — Family: Plantaginaceae, *Centella asiatica* (L.) Urb. — Family: Apiaceae, and *Withania somnifera*, exhibit multi-targeted mechanisms, ranging from PI3K/Akt and Nrf2 pathway activation to tau aggregation inhibition and cholinergic enhancement. These compounds demonstrate potential for disease modification in Alzheimer's through antioxidant, anti-inflammatory, and neuroprotective actions. Future integration of such agents into conventional frameworks may open new avenues for holistic yet mechanism-based Alzheimer's therapeutics.

Collaboration between conventional scientists and TCIM practitioners is pivotal to optimizing TCIM's strengths in Alzheimer's treatment. Efficacy, safety, and long-term outcomes will have to be validated by significant, well-planned clinical trials on established protocols. Such an integration will hasten the move of TCIM from adjunct therapies to an established part of mainstream Alzheimer's care.

Emerging therapeutics include multi-targeted approaches, gene therapy, and nanotechnology. New techniques must be established and tested scientifically and in a multidisciplinary collaboration with other traditional healthcare practitioners before they are included in conventional healthcare. Early diagnosis, specific care, and new treatments might reduce the effects of Alzheimer's disease in the future and may even help improve people's lives.

Author contributions

RS: Data curation, Writing – original draft. TP: Data curation, Resources, Writing – original draft. TV: Data curation, Formal Analysis, Resources, Visualization, Writing – review and editing. VS: Data curation, Investigation, Resources, Supervision, Visualization, Writing – review and editing. NH: Formal Analysis, Investigation, Project administration, Resources, Supervision, Writing – review and editing. RB: Investigation, Methodology, Resources, Supervision, Validation, Writing – review and editing. SA: Conceptualization, Investigation, Methodology, Resources, Supervision, Validation, Writing – review and editing.

Funding

The author(s) declare that no financial support was received for the research and/or publication of this article.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declare that no Generative AI was used in the creation of this manuscript.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

Abdallah, A. E. (2024). Review on anti-alzheimer drug development: approaches, challenges and perspectives. *RSC Adv.* 14 (16), 11057–11088. doi:10.1039/d3ra08333k

Adib-Hajbaghery, M., Fattahi Ardakani, M., Sotoudeh, A., and Asadian, A. (2021). Prevalence of complementary and alternative medicine (CAM) among diabetic patients in Eastern Mediterranean country members of the World Health Organization (WHO): a review. *J. Herb. Med.* 29, 100476. doi:10.1016/j.hermed.2021.100476

Aguiar, S., and Borowski, T. (2013). Neuropharmacological review of the nootropic herb *Bacopa monnieri*. *Rejuvenation Res.* 16 (4), 313–326. doi:10.1089/rej.2013.1431

Akhtar, A., Gupta, S. M., Dwivedi, S., Kumar, D., Shaikh, M. F., and Negi, A. (2022). Preclinical models for Alzheimer's disease: past, present, and future approaches. *ACS Omega* 7 (51), 47504–47517. doi:10.1021/acsomega.2c05609

Anand, P., Kunnumakkara, A. B., Newman, R. A., and Aggarwal, B. B. (2007). Bioavailability of curcumin: problems and promises. *Mol. Pharm.* 4 (6), 807–818. doi:10.1021/mp700113r

Andrade, A., Siqueira, T. C., D'Oliveira, A., and Dominski, F. H. (2022). Effects of exercise in the treatment of Alzheimer's disease: an umbrella review of systematic reviews and meta-analyses. *J. Aging Phys. Act.* 30 (3), 535–551. doi:10.1123/japa.2021-0033

Ashford, J. W., Mahoney, L., and Burkett, T. (2015). A role for complementary and integrative medicine in Alzheimer's disease prevention. *J. Alzheimer's Dis.* 48 (1), 13–14. doi:10.3233/JAD-150505

Athar, T., Al Balushi, K., and Khan, S. A. (2021). Recent advances on drug development and emerging therapeutic agents for Alzheimer's disease. *Mol. Biol. Rep.* 48 (7), 5629–5645. doi:10.1007/s11033-021-06512-9

Atri, A. (2019). The Alzheimer's disease clinical spectrum: diagnosis and management. *Med. Clin. N. Am.* 103 (2), 263–293. doi:10.1016/j.mcna.2018.10.009

Barnes, J. (2003). Quality, efficacy and safety of complementary medicines: fashions, facts and the future. Part I. Regulation and quality. *Br. J. Clin. Pharmacol.* 55 (3), 226–233. doi:10.1046/j.1365-2125.2003.01810.x

Bashir, S., Uzair, M., Abualait, T., Arshad, M., Khallaf, R. A., Niaz, A., et al. (2022). Effects of transcranial magnetic stimulation on neurobiological changes in Alzheimer's disease (Review). *Mol. Med. Rep.* 25 (4), 109. doi:10.3892/mmr.2022.12625

Bhat, B. A., Almilaibary, A., Mir, R. A., Aljarallah, B. M., Mir, W. R., Ahmad, F., et al. (2022). Natural therapeutics in aid of treating Alzheimer's disease: a green gateway toward ending quest for treating neurological disorders. *Front. Neurosci.* 16, 884345. doi:10.3389/fnins.2022.884345

Brookmeyer, R., Johnson, E., Ziegler-Graham, K., and Arrighi, H. M. (2007). Forecasting the global burden of Alzheimer's disease. *Alzheimer's and Dementia* 3 (3), 186–191. doi:10.1016/j.jalz.2007.04.381

Busche, M. A., and Hyman, B. T. (2020). Synergy between amyloid-β and tau in Alzheimer's disease. *Nat. Neurosci.* 23 (10), 1183–1193. doi:10.1038/s41593-020-0687-6

Calfio, C., Gonzalez, A., Singh, S. K., Rojo, L. E., and Maccioni, R. B. (2020). The emerging role of nutraceuticals and phytochemicals in the prevention and treatment of Alzheimer's disease. *J. Alzheimer's Dis.* 77 (1), 33–51. doi:10.3233/JAD-200443

Candelario, M., Cuellar, E., Reyes-Ruiz, J. M., Darabedian, N., Feimeng, Z., Miledi, R., et al. (2015). Direct evidence for GABAergic activity of Withania somnifera on mammalian ionotropic GABAA and GABAρ receptors. *J. Ethnopharmacol.* 171, 264–272. doi:10.1016/j.jep.2015.05.058

Castillo-Carranza, D. L., Guerrero-Muñoz, M. J., Sengupta, U., Hernandez, C., Barrett, A. D. T., Dineley, K., et al. (2015). Tau immunotherapy modulates both pathological tau and upstream amyloid pathology in an Alzheimer's disease mouse model. *J. Neurosci.* 35 (12), 4857–4868. doi:10.1523/jneurosci.4989-14.2015

Chen, K. C., Liu, Y. C., Lee, C. C., and Chen, C. Y. C. (2014). Potential retinoid X receptor agonists for treating Alzheimer's disease from traditional Chinese medicine. *Evidence-Based Complementary Altern. Med.* 2014 (1), 278493. doi:10.1155/2014/278493

Cheung, T. S., Song, T. H., Ng, T. B., Wu, F. H., Lao, L. X., Tang, S. C. W., et al. (2015). Therapeutic effects of herbal chemicals in traditional Chinese medicine on Alzheimer's disease. *Curr. Med. Chem.* 22 (19), 2392–2403. doi:10.2174/0929867322666150520095509

Choi, M. R., Kwak, S. M., Bang, S. H., Jeong, J. E., and Kim, D. J. (2017). Chronic saponin treatment attenuates damage to the pancreas in chronic alcohol-treated diabetic rats. *J. Ginseng Res.* 41 (4), 503–512. doi:10.1016/j.jgr.2016.09.002

Choudhary, D., Bhattacharyya, S., and Bose, S. (2017). Efficacy and safety of ashwagandha (*Withania somnifera* (L.) *dunal*) root extract in improving memory and cognitive functions. *J. Diet. Suppl.* 14 (6), 599–612. doi:10.1080/19390211.2017.1284970

Clinton, L. K., Blurton-Jones, M., Myczek, K., Trojanowski, J. Q., and LaFerla, F. M. (2010). Synergistic Interactions between Abeta, tau, and alpha-synuclein: acceleration of neuropathology and cognitive decline. *J. Neurosci.* 30 (21), 7281–7289. doi:10.1523/JNEUROSCI.0490-10.2010

Cocchiara, R. A., de Lucia, F., Koci, L., Lisanti, E., Petruccini, G., and la Torre, G. (2020). Management of the early stage of Alzheimer's disease: a systematic review of literature over the past 10 years. *Clin. Ter. Soc. Ed. Universo* 171, E357–E368. doi:10.7417/CT.2020.2239

Cooper, E. L., and Ma, M. J. (2017). Alzheimer Disease: clues from traditional and complementary medicine. *J. Tradit. Complement. Med.* 7 (4), 380–385. doi:10.1016/j.jtcme.2016.12.003

de la Fuente Garcia, S., Ritchie, C. W., and Luz, S. (2020). Artificial intelligence, speech, and language processing approaches to monitoring Alzheimer's disease: a systematic review. *J. Alzheimer's Dis.* 78 (4), 1547–1574. doi:10.3233/JAD-200888

Dhanasekaran, M., Holcomb, L. A., Hitt, A. R., Tharakan, B., Porter, J. W., Young, K. A., et al. (2009). *Centella asiatica* extract selectively decreases amyloid β levels in hippocampus of Alzheimer's disease animal model. *Phytotherapy Res.* 23 (1), 14–19. doi:10.1002/ptr.2405

Diniz, L. R. L., Calado, L. L., Duarte, A. B. S., and de Sousa, D. P. (2023). *Centella asiatica* and its metabolite asiatic acid: wound healing effects and therapeutic potential. *Metabolites* 13 (2), 276. doi:10.3390/metabo13020276

Flory, S., Männle, R., and Frank, J. (2021). The inhibitory activity of curcumin on P-glycoprotein and its uptake by and efflux from LS180 cells is not affected by its galenic formulation. *Antioxidants (Basel)* 10 (11), 1826. doi:10.3390/antiox10111826

Fu, B., Wang, N., Tan, H. Y., Li, S., Cheung, F., and Feng, Y. (2018). Multi-component herbal products in the prevention and treatment of chemotherapy-associated toxicity and side effects: a review on experimental and clinical evidences. *Front. Pharmacol.* 9, 1394. doi:10.3389/fphar.2018.01394

G, N. S. H. S., Marise, V. L. P., Satish, K. S., Yergolkar, A. V., Krishnamurthy, M., Ganesan, R. S., et al. (2021). Untangling huge literature to disinter genetic underpinnings of Alzheimer's Disease: a systematic review and meta-analysis. *Ageing Res. Rev.* 71, 101421. doi:10.1016/j.arr.2021.101421

Gadhave, K., Kumar, D., Uversky, V. N., and Giri, R. (2021). A multitude of signaling pathways associated with Alzheimer's disease and their roles in AD pathogenesis and therapy. *Med. Res. Rev.* 41 (5), 2689–2745. doi:10.1002/med.21719

García-Casares, N., Gallego Fuentes, P., Barbancho, M. Á., López-Gigosos, R., García-Rodríguez, A., and Gutiérrez-Bedmar, M. (2021). Alzheimer's disease, mild cognitive impairment and mediterranean diet. A systematic review and dose-response meta-analysis. *J. Clin. Med.* 10 (20), 4642. doi:10.3390/jcm10204642

Gharat, R., Dixit, G., Khambete, M., and Prabhu, A. (2024). Targets, trials and tribulations in Alzheimer therapeutics. *Eur. J. Pharmacol.* 962, 176230. doi:10.1016/j.ejphar.2023.176230

Ghosh, S., Wu, M. D., Shaftel, S. S., Kyrkanides, S., LaFerla, F. M., Olschowka, J. A., et al. (2013). Sustained interleukin-1β overexpression exacerbates tau pathology despite reduced amyloid burden in an Alzheimer's mouse model. *J. Neurosci.* 33 (11), 5053–5064. doi:10.1523/JNEUROSCI.4361-12.2013

Giannoni, P., Fossati, S., Marcello, E., and Claeysen, S. (2020). Editorial: identification of multiple targets in the fight against Alzheimer's disease. *Front. Aging Neurosci.* 12, 169. doi:10.3389/fnagi.2020.00169

Guo, T., Zhang, D., Zeng, Y., Huang, T. Y., Xu, H., and Zhao, Y. (2020). Molecular and cellular mechanisms underlying the pathogenesis of Alzheimer's disease. *Mol. Neurodegener.* 15 (1), 40. doi:10.1186/s13024-020-00391-7

Gupta, S. K., Jadhav, S., Gohil, D., Panigrahi, G. C., Kaushal, R. K., Gandhi, K., et al. (2022). Safety, toxicity and pharmacokinetic assessment of oral Withaferin-A in mice. *Toxicol. Rep.* 9, 1204–1212. doi:10.1016/j.toxrep.2022.05.012

Hannan, M. A., Dash, R., Haque, M. N., Sohag, A. A. M., and Moon, I. S. (2020). Mechanistic insights into the curcumin-mediated neuroprotection in alzheimer's disease: an integrated system. *Pharmacol. Mol. Simul. Study*. doi:10.3233/JAD-200581

Harada, C. N., Natelson Love, M. C., and Triebel, K. L. (2013). Normal cognitive aging. *Clin. Geriatr. Med.* 29 (4), 737–752. doi:10.1016/j.cger.2013.07.002

Haut, F., Argyrousi, E. K., and Arancio, O. (2023). Re-arranging the puzzle between the amyloid-beta and tau pathology: an APP-centric approach. *Int. J. Mol. Sci.* 25 (1), 259. doi:10.3390/ijms25010259

Hroudová, J., and Fišar, Z. (2024). Alzheimer's disease approaches - focusing on pathology, biomarkers and clinical trial candidates. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 134, 111069. doi:10.1016/j.pnpbp.2024.111069

Huang, L., Yagura, T., and Chen, S. (2008). Sedative activity of hexane extract of Keampferia galanga L. and its active compounds. *J. Ethnopharmacol.* 120 (1), 123–125. doi:10.1016/j.jep.2008.07.045

Hutchison, C. W., and Rodgers, T. (2001). The chronic effects of an extract of Bacopa monniera (Brahmi) on cognitive function in healthy human subjects. *Psychopharmacol. Berl.* 156 (4), 481–484. doi:10.1007/s002130100815

Inoue, Y., Hayashi, Y., Kangawa, K., Suzuki, Y., Murakami, N., and Nakahara, K. (2016). Des-acyl ghrelin prevents heatstroke-like symptoms in rats exposed to high temperature and high humidity. *Neurosci. Lett.* 615, 28–32. doi:10.1016/j.neulet.2016.01.003

Iqubal, A., Rahman, S. O., Ahmed, M., Bansal, P., Haider, M. R., Iqubal, M. K., et al. (2021). Current quest in natural bioactive compounds for Alzheimer's disease: multi-targeted-designed-ligand based approach with preclinical and clinical based evidence. *Curr. Drug Targets* 22 (6), 685–720. doi:10.2174/1389450121999201209201004

Jack, C. R., Knopman, D. S., Jagust, W. J., Petersen, R. C., Weiner, M. W., Aisen, P. S., et al. (2013). Tracking pathophysiological processes in Alzheimer's disease: an updated hypothetical model of dynamic biomarkers. *Lancet Neurol.* 12 (2), 207–216. doi:10.1016/S1474-4422(12)70291-0

Jayaprakasam, B., Padmanabhan, K., and Nair, M. G. (2010). Withanamides in *Withania somnifera* fruit protect PC-12 cells from β-amyloid responsible for Alzheimer's disease. *Phytotherapy Res.* 24 (6), 859–863. doi:10.1002/ptr.3033

Jeon, S. G., Song, E. J., Lee, D., Park, J., Nam, Y., Kim, J., et al. (2019). Traditional oriental medicines and Alzheimer's disease. *Aging Dis.* 10 (2), 307–328. doi:10.14336/AD.2018.0328

Kabak, M., Çil, B., and Hocanlı, I. (2021). Relationship between leukocyte, neutrophil, lymphocyte, platelet counts, and neutrophil to lymphocyte ratio and polymerase chain reaction positivity. *Int. Immunopharmacol.* 93, 107390. doi:10.1016/j.intimp.2021.107390

Kabra, A. (2022). A systematic review of amyloid-β and tau's contribution to neuroinflammation in Alzheimer's disease progression. *Int. J. High Sch. Res.* 4 (6), 55–61. doi:10.36838/v4i6.10

Kim, J., Jeong, M., Stiles, W. R., and Choi, H. S. (2022). Neuroimaging modalities in Alzheimer's disease: diagnosis and clinical features. *Int. J. Mol. Sci.* 23 (11), 6079. doi:10.3390/ijms23116079

King, M., Tavoulari, S., Mavridou, V., King, A., Mifsud, J., and Kunji, E. (2020). A single cysteine residue in the translocation pathway of the mitosomal ADP/ATP carrier from cryptosporidium parvum confers a broad nucleotide specificity. *Int. J. Mol. Sci.* 21 (23), 8971. doi:10.3390/ijms21238971

Kong, X., Ma, Z., Tang, R., Wang, X., Wei, K., Yang, G., et al. (2023). Efficacy of acupuncture in patients with mild Alzheimer's disease and its impact on gut microbiota: study protocol for a randomized sham-controlled trial. *Front. Med. (Lausanne)* 10, 1014113. doi:10.3389/fmed.2023.1014113

Kuboyama, T., Tohda, C., Zhao, J., Nakamura, N., Hattori, M., and Komatsu, K. (2002). Axon- or dendrite-predominant outgrowth induced by constituents from Ashwagandha. *Neuroreport* 13 (14), 1715–1720. doi:10.1097/00001756-200210070-00005

Kumar, A., Nisha, C. M., Silakari, C., Sharma, I., Anusha, K., Gupta, N., et al. (2016). Current and novel therapeutic molecules and targets in Alzheimer's disease. *J. Formos. Med. Assoc.* 115 (1), 3–10. doi:10.1016/j.jfma.2015.04.001

Kunle, O. F. (2012). Standardization of herbal medicines - a review. *Int. J. Biodivers. Conserv.* 4 (3). doi:10.5897/ijbc11.163

Kuszewski, J. C., Wong, R. H. X., and Howe, P. R. C. (2018). Can curcumin counteract cognitive decline? Clinical trial evidence and rationale for combining ω-3 fatty acids with curcumin. *Adv. Nutr.* 9 (2), 105–113. doi:10.1093/advances/nmx013

Lee, J., Jin, C., Cho, S. Y., Park, S. U., Jung, W. S., Moon, S. K., et al. (2020). Herbal medicine treatment for Alzheimer disease: a protocol for a systematic review and meta-analysis. *Medicine* 99 (33), e21745. doi:10.1097/MD.0000000000021745

Legal Status of traditional medicine (2001). Legal Status of traditional medicine and complementary/alternative medicine: a worldwide review.

Li, Q., Wu, Y., Chen, J., Xuan, A., and Wang, X. (2022). Microglia and immunotherapy in Alzheimer's disease. *Acta Neurol. Scand.* 145 (3), 273–278. doi:10.1111/ane.13551

Li, X. L., Wang, D. S., Zhao, B. Q., Li, Q., Qu, H. Y., Zhang, T., et al. (2008). Effects of Chinese herbal medicine fuzhisan on aged rats. *Exp. Gerontol.* 43 (9), 853–858. doi:10.1016/j.exger.2008.05.018

Limpeanchob, N., Jaipan, S., Rattanakaruna, S., Phrompittayarat, W., and Ingkaninan, K. (2008). Neuroprotective effect of Bacopa monnieri on beta-amyloid-induced cell death in primary cortical culture. *J. Ethnopharmacol.* 120 (1), 112–117. doi:10.1016/j.jep.2008.07.039

Lin, J. C. W., Srivastava, G., and Tseng, V. S. (2021). *International journal of interactive multimedia and artificial intelligence*. London, United Kingdom: Universidad Internacional de la Rioja. Vol. 6, 4–5.

Liu, A. J., Wang, S. H., Hou, S. Y., Lin, C. J., Chiu, W. T., Hsiao, S. H., et al. (2013). Evodiamine induces transient receptor potential vanilloid-1-mediated protective autophagy in U87-MG astrocytes. *Evidence-Based Complementary Altern. Med.* 2013, 354840–354849. doi:10.1155/2013/354840

Liu, P., Kong, M., Yuan, S., Liu, J., and Wang, P. (2014). History and experience: a survey of traditional Chinese medicine treatment for Alzheimer's disease. *Evidence-Based Complementary Altern. Med.* 2014 (1), 642128. doi:10.1155/2014/642128

Long, H. Z., Cheng, Y., Zhou, Z. W., Luo, H. Y., Wen, D. D., and Gao, L. C. (2021). PI3K/AKT signal pathway: a target of natural products in the prevention and treatment of Alzheimer's disease and Parkinson's disease. *Front. Pharmacol.* 12, 12. doi:10.3389/fphar.2021.648636

Long, J., Zhang, J., Zeng, X., Wang, M., and Wang, N. (2024). Prevention and treatment of Alzheimer's disease via the regulation of the gut microbiota with traditional Chinese medicine. *CNS Neurosci. Ther.* 30 (11), e70101. doi:10.1111/cns.70101

Lopresti, A. L. (2018). The problem of curcumin and its bioavailability: could its gastrointestinal influence contribute to its overall health-enhancing effects? *Adv. Nutr.* 9 (1), 41–50. doi:10.1093/advances/nmx011

Ma, L., Jiang, X., Huang, Q., Chen, W., Zhang, H., Pei, H., et al. (2023). Traditional Chinese medicine for the treatment of Alzheimer's disease: a focus on the microbiota–gut–brain axis. *Biomed. and Pharmacother.* 165, 115244. doi:10.1016/j.biopha.2023.115244

Miculas, D. C., Negru, P. A., Bungau, S. G., Behl, T., Hassan, S. S., and Tit, D. M. (2022). Pharmacotherapy evolution in Alzheimer's disease: current framework and relevant directions. *Cells* 12 (1), 131. doi:10.3390/cells12010131

Murphy, K., and Park, J. (2017). Can Co-activation of Nrf2 and neurotrophic signaling pathway slow Alzheimer's disease? *Int. J. Mol. Sci.* 18 (6), 1168. doi:10.3390/ijms18061168

Nakamura, K., and Sugaya, K. (2014). Neuromelanin-sensitive magnetic resonance imaging: a promising technique for depicting tissue characteristics containing neuromelanin. *Neural Regen. Res.* 9 (7), 759–760. doi:10.4103/1673-5374.131583

Ng, J. Y., Cramer, H., Lee, M. S., and Moher, D. (2024). Traditional, complementary, and integrative medicine and artificial intelligence: novel opportunities in healthcare. *Integr. Med. Res.* 13 (1), 101024. doi:10.1016/j.imr.2024.101024

Nguyen, S. A., Ajam Oughli, H., and Lavretsky, H. (2024). Use of complementary and integrative medicine for Alzheimer's disease and cognitive decline. *J. Alzheimers Dis.* 97, p. 523–540. doi:10.3233/JAD-230710

Nguyen, S. A., Oughli, H. A., and Lavretsky, H. (2022). Complementary and integrative medicine for neurocognitive disorders and caregiver health. *Curr. Psychiatry Rep.* 24 (9), 469–480. doi:10.1007/s11920-022-01355-y

Nishteswar, K., Joshi, H., and Karra, R. (2014). Role of indigenous herbs in the management of Alzheimer′s disease. *Anc. Sci. Life* 34 (1), 3–7. doi:10.4103/0257-7941.150763

Onaolapo, O. J., Olofinnade, A. T., Ojo, F. O., and Onaolapo, A. Y. (2021). Neuroinflammation and oxidative stress in Alzheimer's disease; can nutraceuticals and functional foods come to the rescue? *Antiinflamm. Antiallergy Agents Med. Chem.* 21 (2), 75–89. doi:10.2174/1871523021666220815151559

Ou, Y. N., Zhu, J. X., Hou, X. H., Shen, X. N., Xu, W., Dong, Q., et al. (2020). Associations of infectious agents with Alzheimer's disease: a systematic review and meta-analysis. *J. Alzheimer's Dis.* 75 (1), 299–309. doi:10.3233/JAD-191337

Oxford, A. E., Stewart, E. S., and Rohn, T. T. (2020). Clinical trials in Alzheimer's disease: a hurdle in the path of remedy. *Int. J. Alzheimers Dis.* 2020, 5380346. doi:10.1155/2020/5380346

Papagiouvannis, G., Theodosis-Nobelos, P., Kourounakis, P. N., and Rekka, E. A. (2021). Multi-target directed compounds with antioxidant and/or anti- inflammatory properties as potent agents for Alzheimer's disease. *Med. Chem. (Los Angeles)* 17 (10), 1086–1103. doi:10.2174/1573406416666201013161303

Parfenov, V. A. (2020). Alzheimer's disease: clinical management errors. *Meditsinskiy Sov. = Med. Counc.* (19), 23–28. doi:10.21518/2079-701x-2020-19-23-28

Passeri, E., Elkhoury, K., Morsink, M., Broersen, K., Linder, M., Tamayol, A., et al. (2022). Alzheimer's disease: treatment strategies and their limitations. *Int. J. Mol. Sci.* 23 (22), 13954. doi:10.3390/ijms232213954

Patwardhan, B., Warude, D., Pushpangadan, P., and Bhatt, N. (2005). Ayurveda and traditional Chinese medicine: a comparative Overview. *Evidence-Based Complementary Altern. Med.* 2 (4), 465–473. doi:10.1093/ecam/neh140

Patwardhan, B. K., and Vaidya, A. D. B. (2014). Herbal remedies and the bias against Ayurveda arvind chopra centre for rheumatic diseases pune SEE PROFILE. Available online at: https://www.researchgate.net/publication/230607149.

Pavlik, V. N., Burnham, S. C., Kass, J. S., Helmer, C., Palmqvist, S., Vassilaki, M., et al. (2022). Connecting cohorts to diminish Alzheimer's disease (CONCORD-AD): a report of an international research collaboration network. *J. Alzheimer's Dis.* 85 (1), 31–45. doi:10.3233/JAD-210525

Perry, E. K., Pickering, A. T., Wang, W. W., Houghton, P. J., and Perry, N. S. L. (1999). Medicinal plants and Alzheimer's disease: from ethnobotany to phytotherapy. *J. Pharm. Pharmacol.* 51 (5), 527–534. doi:10.1211/0022357991772808

Peterson, A., Largent, E. A., Lynch, H. F., Karlawish, J., and Sisti, D. (2023). Journeying to ixtlan: ethics of psychedelic medicine and research for Alzheimer's disease and related dementias. *AJOB Neurosci.* 14 (2), 107–123. doi:10.1080/21507740.2022.2148771

Peterson, C. T. (2020). Dysfunction of the microbiota-gut-brain Axis in neurodegenerative disease: the promise of therapeutic modulation with prebiotics, medicinal herbs, probiotics, and synbiotics. *J. Evid. Based Integr. Med.* 25, 2515690X20957225. doi:10.1177/2515690X20957225

Phutrakool, P., and Pongpirul, K. (2022). Acceptance and use of complementary and alternative medicine among medical specialists: a 15-year systematic review and data synthesis. *Syst. Rev.* 11 (1), 10. doi:10.1186/s13643-021-01882-4

Pimperkhede, M., Vaibhav, M., and Kute, G. (2023). A systemic review on management of Alzheimer's disease. *Int. J. Res. Publ. Rev.* 4 (4), 4637–4645. doi:10.55248/gengpi.4.423.38127

Prasad, K. N., Cole, W. C., and Prasad, K. C. (2002). Risk factors for Alzheimer's disease: role of multiple antioxidants, non-steroidal anti-inflammatory and cholinergic agents alone or in combination in prevention and treatment. *J. Am. Coll. Nutr.* 21 (6), 506–522. doi:10.1080/07315724.2002.10719249

Pullar, J., Carr, A., Bozonet, S., and Vissers, M. (2018). High vitamin C Status is associated with elevated mood in male tertiary students. *Antioxidants* 7 (7), 91. doi:10.3390/antiox7070091

Qiu, Z. D., Wei, X. Y., Chen, Z. Y., Guo, J., Huang, L. Q., and Lai, C. J. S. (2022). Discovery of the directionally detoxification effect and chemical mechanism of Ginseng-Fuzi co-decoction based on real-time online filtration electrospray ionization mass spectrometry. *Phytomedicine* 100, 154059. doi:10.1016/j.phymed.2022.154059

Rafii, M. S., Walsh, S., Little, J. T., Behan, K., Reynolds, B., Ward, C., et al. (2011). A phase II trial of huperzine A in mild to moderate Alzheimer disease. *Neurology* 76 (16), 1389–1394. doi:10.1212/WNL.0b013e318216eb7b

Ramachandran, A. K., Das, S., Joseph, A., Shenoy, G. G., Alex, A. T., and Mudgal, J. (2021). Neurodegenerative pathways in Alzheimer's disease: a review. *Curr. Neuropharmacol.* 19 (5), 679–692. doi:10.2174/1570159X18666200807130637

Rayathala, J., C, K. K., and P, V. (2022). Review on Alzheimer's disease: past, present and future. *J. Innovations Appl. Pharm. Sci. (JIAPS)*, 28–31. doi:10.37022/jiaps.v7i1.274

Rocca, W. A., Amaducci, L. A., and Schoenberg, B. S. (1986). Epidemiology of clinically diagnosed Alzheimer's disease. *Ann. Neurol.* 19 (5), 415–424. doi:10.1002/ana.410190502

Saper, R. B., Phillips, R. S., Sehgal, A., Khouri, N., Davis, R. B., Paquin, J., et al. (2008). Lead, mercury, and arsenic in US- and Indian-manufactured ayurvedic medicines sold via the internet. *JAMA* 300 (8), 915–923. doi:10.1001/jama.300.8.915

Schaffner, T. E. (2001). The role of complementary and alternative medicine: accommodating pluralism. Available online at: https://books.google.co.in/books?id=ryEnQlgMCT0C.

Sehgal, N., Gupta, A., Valli, R. K., Joshi, S. D., Mills, J. T., Hamel, E., et al. (2012). *Withania somnifera* reverses Alzheimer's disease pathology by enhancing low-density lipoprotein receptor-related protein in liver. *Proc. Natl. Acad. Sci.* 109 (9), 3510–3515. doi:10.1073/pnas.1112209109

Sharma, M., Pal, P., and Gupta, S. K. (2024). Advances in Alzheimer's disease: a multifaceted review of potential therapies and diagnostic techniques for early detection. *Neurochem. Int.* 177, 105761. doi:10.1016/j.neuint.2024.105761

shortt, J., Ott, C. J., Johnstone, R. W., and Bradner, J. E. (2017). Erratum: a chemical probe toolbox for dissecting the cancer epigenome. *Nat. Rev. Cancer* 17 (4), 268. doi:10.1038/nrc.2017.26

Shoukat, S., Zia, M. A., Uzair, M., Attia, K. A., Abushady, A. M., Fiaz, S., et al. (2023). Bacopa monnieri: a promising herbal approach for neurodegenerative disease treatment supported by *in silico* and *in vitro* research. *Heliyon* 9 (11), e21161. doi:10.1016/j.heliyon.2023.e21161

Small, G. W., Siddarth, P., Li, Z., Miller, K. J., Ercoli, L., Emerson, N. D., et al. (2018). Memory and brain amyloid and tau effects of a bioavailable form of curcumin in non-demented adults: a double-blind, placebo-controlled 18-month trial. *Am. J. Geriatric Psychiatry* 26 (3), 266–277. doi:10.1016/j.jagp.2017.10.010

Steiner-Lim, G. Z., Bensoussan, A., Andrews-Marney, E. R., Al-Dabbas, M. A., Cave, A. E., Chiu, C. L., et al. (2023). A randomized, double-blind, placebo-controlled, parallel-group 12-week pilot phase II trial of SaiLuoTong (SLT) for cognitive function in older adults with mild cognitive impairment. *Alzheimer's and Dementia Transl. Res. and Clin. Interventions* 9 (4), e12420. doi:10.1002/trc2.12420

Strandberg, T. E. (2019). Challenges of a statin trial in older people. *J. Am. Geriatr. Soc.* 67 (4), 856–857. doi:10.1111/jgs.15763

Sutalangka, C., Wattanathorn, J., Muchimapura, S., and Thukham-mee, W. (2013). *Moringa oleifera* mitigates memory impairment and neurodegeneration in animal model of age-related dementia. *Oxid. Med. Cell. Longev.* 2013, 695936–695939. doi:10.1155/2013/695936

Takizawa, C., Thompson, P. L., van Walsem, A., Faure, C., and Maier, W. C. (2014). Epidemiological and economic burden of Alzheimer's disease: a systematic literature review of data across Europe and the United States of America. *J. Alzheimer's Dis.* 43 (4), 1271–1284. doi:10.3233/JAD-141134

Tatulian, S. A. (2022). Challenges and hopes for Alzheimer's disease. *Drug Discov. Today* 27 (4), 1027–1043. doi:10.1016/j.drudis.2022.01.016

Tripathi, P., Lodhi, A., Rai, S., Nandi, N., Dumoga, S., Yadav, P., et al. (2024). Review of pharmacotherapeutic targets in Alzheimer's disease and its management using traditional medicinal plants. *Degener. Neurol. Neuromuscul. Dis.* 14, 47–74. doi:10.2147/DNND.S452009

Tuzimski, T., and Petruczynik, A. (2022). Determination of anti-alzheimer's disease activity of selected plant ingredients. *Molecules* 27 (10), 3222. doi:10.3390/molecules27103222

Ventola, C. L. (2010). Current issues regarding complementary and alternative medicine (CAM) in the United States: Part 1: the widespread use of CAM and the need for better-informed health care professionals to provide patient counseling. *P T* 35 (8), 461–468.

Wadhawan, S., Biswkarma, V. K., Chaudhary, A., and Masand, P. (2024). A critical review based on preclinical studies of medicinal plants for the management of Alzheimer's disease. *Curr. Bioact. Compd.* 20 (4). doi:10.2174/1573407219666230807150426

Wang, C., Zhang, X., Teng, Z., Zhang, T., and Li, Y. (2014a). Downregulation of PI3K/Akt/mTOR signaling pathway in curcumin-induced autophagy in APP/PS1 double transgenic mice. *Eur. J. Pharmacol.* 740, 312–320. doi:10.1016/j.ejphar.2014.06.051

Wang, J., Bi, W., Cheng, A., Freire, D., Vempati, P., Zhao, W., et al. (2014b). Targeting multiple pathogenic mechanisms with polyphenols for the treatment of Alzheimer's disease-experimental approach and therapeutic implications. *Front. Aging Neurosci.* 6, 6. doi:10.3389/fnagi.2014.00042

Webers, A., Heneka, M. T., and Gleeson, P. A. (2020). The role of innate immune responses and neuroinflammation in amyloid accumulation and progression of Alzheimer's disease. *Immunol. Cell. Biol.* 98 (1), 28–41. doi:10.1111/imcb.12301

Weerasinghe, P. (2024). Molecular targets of ashwagandha (Withania somnifera), Korean ginseng (panax ginseng), brahmi (Bacopa monneri) and gotu kola (Centella asiatica) in the treatment of autism spectrum disorder (asd): a global health perspective. *Adv. Complementary and Altern. Med.* 8 (3). doi:10.31031/acam.2024.08.000687

WHO (2024). WHO guidelines on good agricultural and collection practices (GACP) for medicinal plants.

Wright, K. M., Bollen, M., David, J., Speers, A. B., Brandes, M. S., Gray, N. E., et al. (2022). Pharmacokinetics and pharmacodynamics of key components of a standardized *Centella asiatica* product in cognitively impaired older adults: a phase 1, double-blind, randomized clinical trial. *Antioxidants* 11 (2), 215. doi:10.3390/antiox11020215

Wu, S., Zheng, L., Huang, J., Wang, S., Huang, Q., Guo, S., et al. (2024). Icariin, a natural flavonoid glucoside, inhibits neuroinflammation in mice with triple-transgenic Alzheimer's disease by regulating the Akt/GSK-3β signaling pathway. *J. Funct. Foods* 118, 106263. doi:10.1016/j.jff.2024.106263

Xie, L., Zhu, Q., and Lu, J. (2022). Can we use ginkgo biloba extract to treat Alzheimer's disease? Lessons from preclinical and clinical studies. *Cells* 11 (3), 479. doi:10.3390/cells11030479

Xin, G. Z., Qi, L. W., Shi, Z. Q., Li, P., Hao, H. P., Wang, G. J., et al. (2011). Strategies for integral metabolism profile of multiple compounds in herbal medicines: pharmacokinetics, metabolites characterization and metabolic interactions. *Curr. Drug Metab.* 12 (9), 809–817. doi:10.2174/138920011797470164

Xing, D., Yoo, C., Gonzalez, D., Jenkins, V., Nottingham, K., Dickerson, B., et al. (2022). Effects of acute ashwagandha ingestion on cognitive function. *Int. J. Environ. Res. Public Health* 19 (19), 11852. doi:10.3390/ijerph191911852

Yan, F., Liu, J., Chen, M. X., Zhang, Y., Wei, S. J., Jin, H., et al. (2023). Icariin ameliorates memory deficits through regulating brain insulin signaling and glucose transporters in 3×Tg-AD mice. *Neural Regen. Res.* 18 (1), 183–188. doi:10.4103/1673-5374.344840

Yang, G., Wang, Y., Sun, J., Zhang, K., and Liu, J. (2015). Ginkgo biloba for mild cognitive impairment and Alzheimer's disease: a systematic review and meta-analysis of randomized controlled trials. *Curr. Top. Med. Chem.* 16 (5), 520–528. doi:10.2174/1568026615666150813143520

Yassine, H. N., and Finch, C. E. (2020). APOE alleles and diet in brain aging and Alzheimer's disease. *Front. Aging Neurosci.* 12, 12. doi:10.3389/fnagi.2020.00150

Yiannopoulou, K. G., and Papageorgiou, S. G. (2020). Current and future treatments in alzheimer disease: an update. *J. Cent. Nerv. Syst. Dis.* 12, 117957352090739. doi:10.1177/1179573520907397

Yin, H., Zhang, J., Lin, H., Qiao, Y., Wang, R., Lu, H., et al. (2009). Effect of traditional Chinese medicine Shu-mai-tang on angiogenesis, arteriogenesis and cardiac function in rats with myocardial ischemia. *Phytotherapy Res.* 23 (1), 92–98. doi:10.1002/ptr.2565

Yu, J. T., Xu, W., Tan, C. C., Andrieu, S., Suckling, J., Evangelou, E., et al. (2020). Evidence-based prevention of Alzheimer's disease: systematic review and meta-analysis of 243 observational prospective studies and 153 randomised controlled trials. *J. Neurol. Neurosurg. Psychiatry* 91 (11), 1201–1209. doi:10.1136/jnnp-2019-321913

Zetterberg, H., and Bendlin, B. B. (2021). Biomarkers for Alzheimer's disease—preparing for a new era of disease-modifying therapies. *Mol. Psychiatry* 26 (1), 296–308. doi:10.1038/s41380-020-0721-9

Zhang, H. (2012). New insights into huperzine A for the treatment of Alzheimer's disease. *Acta Pharmacol. Sin.* 33 (9), 1170–1175. doi:10.1038/aps.2012.128

Zhang, J., Yang, C., Wei, D., Li, H., Leung, E. L. H., Deng, Q., et al. (2019). Long-term efficacy of Chinese medicine Bushen Capsule on cognition and brain activity in patients with amnestic mild cognitive impairment. *Pharmacol. Res.* 146, 104319. doi:10.1016/j.phrs.2019.104319

Zhao, Y., Deng, H., Li, K., Wang, L., Wu, Y., Dong, X., et al. (2019). Trans-cinnamaldehyde improves neuroinflammation-mediated NMDA receptor dysfunction and memory deficits through blocking NF-κB pathway in presenilin1/2 conditional double knockout mice. *Brain Behav. Immun.* 82, 45–62. doi:10.1016/j.bbi.2019.07.032

Zieneldien, T., Kim, J., and Cao, C. (2022). The multifaceted role of neuroprotective plants in Alzheimer's disease treatment. *Geriatrics* 7 (2), 24. doi:10.3390/geriatrics7020024

Zullino, D., and Borgeat, F. (2003). Hypertension induced by St. John's Wort - a case report. *Pharmacopsychiatry* 36, 32–37. doi:10.1055/s-2003-38086

frontiers | Frontiers in Pharmacology

TYPE Original Research
PUBLISHED 04 July 2025
DOI 10.3389/fphar.2025.1596728

Check for updates

OPEN ACCESS

EDITED BY
Bin Yu,
Nanjing University of Chinese Medicine, China

REVIEWED BY
Yuntao Li,
Wuhan University, China
Ming Ruan,
Nanjing Xiaozhuang University, China

*CORRESPONDENCE
Hong-He Xiao,
xiaohh89@163com
Hong-Jun Yang,
hongjun0420@vip.sina.com
Xian-Yu Li,
phd_xianyuli@foxmail.com

RECEIVED 20 March 2025
ACCEPTED 17 June 2025
PUBLISHED 04 July 2025

CITATION
Yang Z, Zhang F-R, Ren L, Bai J-M, Wang S-C, Li X-Y, Yang H-J and Xiao H-H (2025) Multi-omics reveal the neuroprotective mechanisms of Xinshubao tablet against scopolamine-induced cognitive dysfunction in mice. *Front. Pharmacol.* 16:1596728. doi: 10.3389/fphar.2025.1596728

COPYRIGHT
© 2025 Yang, Zhang, Ren, Bai, Wang, Li, Yang and Xiao. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

Multi-omics reveal the neuroprotective mechanisms of Xinshubao tablet against scopolamine-induced cognitive dysfunction in mice

Zhe Yang[1,2], Feng-Rong Zhang[3], Lu Ren[4], Jia-Ming Bai[1], Shi-Cong Wang[5], Xian-Yu Li[3]*, Hong-Jun Yang[3]* and Hong-He Xiao[1]*

[1]School of Pharmacy, Liaoning University of Traditional Chinese Medicine, Dalian, China, [2]Department of Oncology, Second Affiliated Hospital of Liaoning University of Traditional Chinese Medicine, Shenyang, China, [3]Beijing Key Laboratory of Traditional Chinese Medicine Basic Research on Prevention and Treatment for Major DisCMCeases, Experimental Research Center, China Academy of Chinese Medical Sciences, Beijing, China, [4]Mental Disorders Research Laboratory, Liaoning University of Traditional Chinese Medicine, Shenyang, China, [5]Fujian Pien Tze Huang Enterprise Key Laboratory of Natural Medicine Research and Development, Zhangzhou Pien Tze Huang Pharmaceutical Co., Ltd., Zhangzhou, China

Introduction: Alzheimer's disease (AD) is a progressive neurodegenerative disorder with limited treatments. Xinshubao tablet (XSB), a traditional Chinese medicine, contains several bioactive compounds with notable neuroprotective effects. Our previous studies have demonstrated that XSB can alleviate cognitive deficits in vascular dementia (VaD) models, suggesting its potential as a therapeutic candidate for AD.

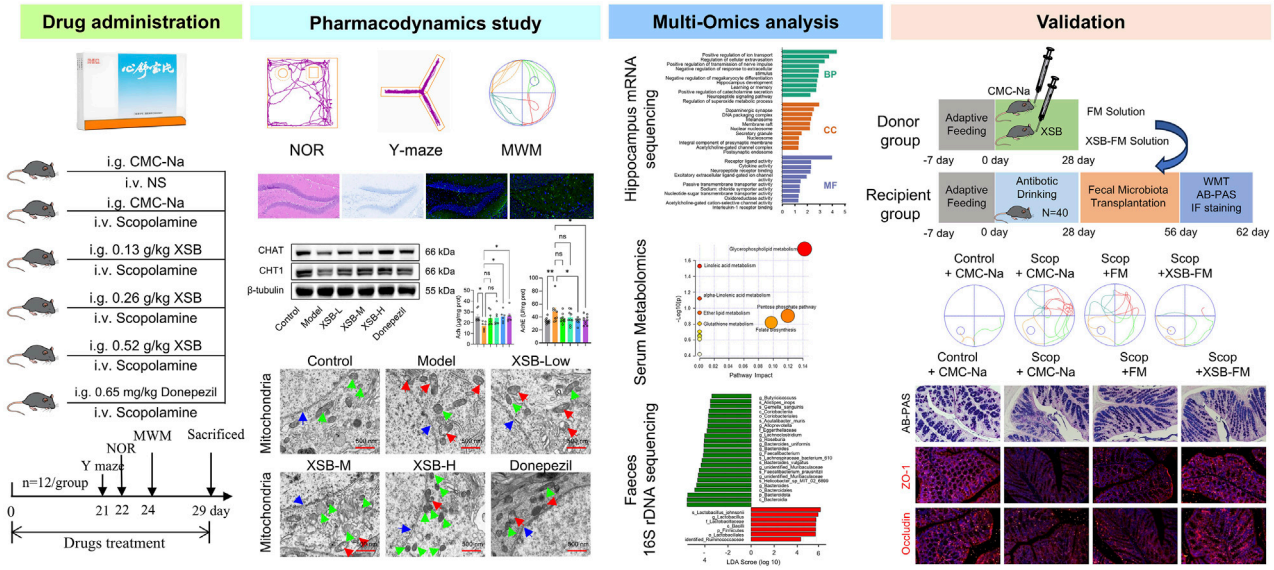
Methods: In this study, scopolamine-induced AD-like mice were orally administered with varying doses of XSB (0.13 g/kg, 0.26 g/kg and 0.52 g/kg) for 28 days. Behavior tests, H&E, Nissl, immunofluorescence staining, and Western blot assays were performed to evaluate the neuroprotection of XSB on AD-like mice. Then, fecal 16S rDNA sequencing, serum metabolomics, and hippocampal mRNA sequencing (mRNA-seq) analysis were performed to investigate the underlying mechanisms.

Results and discussion: The results revealed that oral administration of XSB improved cognitive function, mitigated neuropathological damage, and alleviated dysfunction in the cholinergic system in AD-like mice. XSB treatment also enhanced gut microbiota diversity, increased the abundance of *Enterococcus*, *Actinobacteriota*, *Coriobacteriales*, and *Eggerthellaceae*, but reduced the abundance of *Helicobacter rodentium* and *Lachnospiraceae*. Integrating mRNA-seq and metabolomics data highlighted key regulatory pathways including the biosynthesis of unsaturated fatty acids, tyrosine metabolism, and glycerophospholipid metabolism. Furthermore, XSB treatment reduced the expression of TNF-α, IL-1β, MPO, enhanced SOD, GSH activities, reduced malondialdehyde (MDA) levels, upregulated the expression of BDNF, SYN, PSD95, and improved synaptic density. Transformation of XSB derived fecal microbiota (XSB-FM) effectively alleviated cognitive dysfunction and intestinal barrier injures. In conclusion, XSB may exert its neuroprotective effects via the microbiota-metabolite-brain axis, thereby improving

neuroinflammation, neurotransmission, and synaptic integrity. These findings support the potential of XSB as a multifactorial therapeutic strategy for cognitive deficits in AD.

KEYWORDS

Alzheimer's disease, Xinshubao tablet, neuroinflammation, 16s rDNA sequencing, metabolomics analysis, mRNA sequencing



GRAPHICAL ABSTRACT

1 Introduction

Alzheimer's disease (AD) is a complex neurodegenerative disorder characterized by multiple pathological features, including extracellular amyloid-beta (Aβ) plaques, neurofibrillary tangles (NFTs) composed of hyperphosphorylated tau protein, neuroinflammation, synaptic dysfunction, neuronal loss, glial activation, and impaired neurogenesis. Due to the multifactorial etiology of AD, involving genetic, environmental, and lifestyle factors, its pathogenesis remains poorly understood, and effective treatments are still lacking (Golde et al., 2018; Jia et al., 2020). Therefore, there is an urgent need for the development of novel therapeutic strategies to more effectively address AD.

Traditional Chinese medicine (TCM), with its unique multi-component and multi-target characteristics, has attracted growing attention for its potential in treating multifactorial diseases like AD. Recent studies suggest that TCM-based interventions may regulate multiple pathological processes simultaneously, making them well-suited for managing complex diseases and offering new hope for AD treatment. Xinshubao tablet (XSB), a marketed Chinese patent medicine, is composed of five botanical drugs. These include the air-dried radix and rhizoma of *Salvia miltiorrhiza* Bunge. (Danshen in Chinese), air-dried radix of *Paeonia lactiflora* Pall. (Baishao in Chinese), air-dried radix *and* rhizome *Eleutherococcus senticosus* (Rupr. & Maxim.) Maxim (Ciwujia in Chinese), air-dried radix of *Curcuma wenyujin* Y. H. Chen et C. Ling (Yujin in Chinese), and air-dried mature fructus of *Crataegus oxyacantha var. pinnatifida* (Bunge) Regel (Shanzha in Chinese). The botanical names of these plants have been verified using resources from Medicinal Plant Names Services (https://mpns.science.kew.org/). The chemical profile of XSB has been characterized in our pervious study (Xiao et al., 2024). Clinically, XSB is primarily prescribed for the treatment of coronary heart disease, angina pectoris, chest tightness due to qi deficiency and blood stasis, as well as hypertension, hyperlipidemia, and atherosclerosis.

Numerous studies have demonstrated that the active components of XSB, including Tanshinones (Dong et al., 2017), Salvianolic acids (Liu et al., 2020), Eleutherosides (Huang et al., 2013), and flavonoids from Crataegus (Lee et al., 2019), exhibit significant neuroprotective effects. For example, Tanshinone IIA, a key metabolite of Danshen, has been shown to reduce amyloid deposition and neuroinflammation in APP/PS1 mice (Liu et al., 2024), as well as inhibit neuronal apoptosis induced by ischemia (Chien et al., 2016). Salvianolic Acid B has been demonstrated to rescue cognitive impairment by inhibiting neuroinflammation and decreasing Aβ level in mice (Liu et al., 2020). Eleutheroside E modulates PKA signaling and gut microbiota to alleviate cognitive impairment induced by radiation (Song et al., 2022). The fruit of Crataegus pinnatifida has been reported to ameliorate memory deficits in an AD mouse model induced by intracerebroventricular injection of Aβ (Lee et al., 2019). These neuroprotective effects are beneficial for cognitive recovery, but the efficacy of XSB in improving AD-related cognitive deficits remains unclear.

Our previous research has demonstrated that XSB can mitigate neuroinflammatory responses, alleviate white matter damage, promote hippocampal neurogenesis, and protect against mitochondrial dysfunction, leading to cognitive improvement in vascular dementia (VaD) mice (Xiao et al., 2024). Since AD and VaD are the two most common forms of dementia in clinical practice, often coexisting as mixed dementia. The multiple neuroprotective effects of XSB' compounds, coupled with its potent therapeutic effects on VaD, prompted us to investigate its potential therapeutic benefits in AD and to explore its underlying mechanisms.

In the present study, a scopolamine-induced amnesia model was established through intraperitoneal injection. Behavioral assessments and histopathological analyses were then employed to evaluate the anti-AD effects of XSB. To further elucidate the underlying pharmacological mechanisms, an integrative multi-omics approach was adopted, encompassing fecal 16S rDNA amplicon sequencing, serum metabolomics, and hippocampal transcriptomics.

2 Materials and methods

2.1 Materials

Primary antibodies including myeloperoxidase (MPO, 22225-1-AP), tumor necrosis factor-alpha (TNF-α, 60291-1-Ig), interleukin-1 beta (IL-1β, 16806-1-AP), brain-derived neurotrophic factor (BDNF, 66292-1-Ig), PSD95 (20665-1-AP), synaptophysin (SYN, 67864-1-Ig), choline acetyltransferase (CHAT, 20747-1-AP), choline transporter 1(CHT1, 21848-1-AP), zonula occludens-1 (ZO-1, 66452-1-Ig), Occludin (66378-1-Ig) and β-tubulin (80713-1-RR) were purchased from Proteintech Biotechnology Co., Ltd. (Wuhan, China). Commercial kits including total superoxide dismutase (SOD, A001-3-2), malonaldehyde (MDA, A003-1-2), reduced glutathione (GSH, A006-2-1), acetyl choline (ACh, A105-1-1), acetylcholinesterase (AChE, BC2025-100T/48S) were purchased from Nanjing Jiancheng Biotechnology Co., LTD. (Nanjing, China). AB-PAS staining assay (G1285) was purchased form Beijing Solarbio Science & Technology Co.,Ltd. Scopolamine (Scop, HY-N0296) was purchased from MedChemExpress LLC (China) (Shanghai, China). Xinshubao tablets (2011024) were obtained from Zhangzhou Pien Tze Huang Pharmaceutical Co., Ltd. (Zhangzhou, China). Donepezil hydrochloride tablets (2203026) were purchased from Eisai (China) Pharmaceutical Co., LTD.

2.2 Animals

Male C57BL/6J mice (6–8 weeks old) were procured from Beijing SPF Biotechnology Co., Ltd. (Certificate No.: SCXK (Beijing) 2019-0010). The animals were housed in a controlled environment with a temperature maintained at 23°C–25°C, relative humidity of 40%–60%, and a 12-h light/dark cycle. Standard laboratory chow and water were provided *ad libitum*. Following a week acclimatization period, the experimental procedures were initiated.

All the experiments were approved by the Ethical Committee of Experimental Animal Welfare of Experimental Research Center China Academy of Chinese Medicine Science (ERCCACMS21-2201-01), and conducted in accordance with the ARRIVE guidelines, and were carried out in accordance with Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

2.3 Animal grouping and drug administration

The experiment was divided into 6 groups: Control group, Model group, XSB low-dose, medium-dose, and high-dose groups, and a Positive Control group, with 12 mice in each group. Mice in the Control group received intraperitoneal injections of normal saline, while all other groups were administered scopolamine hydrobromide solution (3 mg/kg) via intraperitoneal injection once daily for 4 consecutive weeks to induce cognitive impairment (Xu et al., 2019). Simultaneously, the XSB-treated groups were orally administered XSB at doses of 0.13 g/kg, 0.26 g/kg and 0.52 g/kg once daily (Xiao et al., 2024). The Control and Model groups were given an equivalent volume of CMC-Na via oral gavage, whereas the Positive control group received 0.65 mg/kg donepezil hydrochloride suspension in CMC-Na by oral gavage (Xiao et al., 2020). n = 12 mice in each group.

2.4 Behavioral assessment of cognitive function in mice

Commencing on the 21st day of drug administration, the cognitive functions of the mice were systematically assessed through a series of three sequential behavioral paradigms: the novel object recognition (NOR) test, the spontaneous alternation Y-maze test, and the Morris water maze (MWM) test.

Spontaneous alternation Y-maze test: The spontaneous alternation Y-maze test was conducted to evaluate working memory in mice. Each mouse was placed at the distal end of one arm and allowed to explore freely for 5 min. Behavioral trajectories were recorded using a video tracking system, and the spontaneous alternation rate was calculated with the ANY-maze software.

NOR test: Following the Y-maze test, each mouse was placed individually in a chamber for 5 min to adapt to the environment. After 24 h, two identical objects were introduced, and mice were allowed to explore freely for 5 min (training phase). One hour later, in the testing phase, one of the identical objects was replaced with a novel object of similar size but different shape. The exploration time for the novel object (T1) and familiar object (T2) was recorded using ANY-maze software, and the recognition index (RI) was calculated as RI = T1/(T1 + T2).

MWM test: MWM test was conducted to evaluate spatial learning and memory. During the spatial acquisition phase, a hidden platform was placed 1 cm below the water surface (20°C–21°C). Mice were trained once daily for 5 days, with trials initiated from random quadrants. If the platform was not located within 60 s, mice were guided to it and allowed to remain for 20 s. On

the 6th day, the platform was removed, and a probe trial was performed. The escape latency, platform crossings, and time spent in the target quadrant were recorded to assess learning and memory.

2.5 Histologic examination

After the MWM test, 3 mice per group were randomly selected for histological analysis. Paraffin-embedded brain sections (4 μm) were prepared using a rotary microtome and stored at 4°C for histopathological analysis.

2.5.1 H&E staining

Brain sections were deparaffinized, and stained with hematoxylin (60°C, 1 min). Sections were differentiated in 1% acid ethanol, counterstained with 0.5% eosin, dehydrated in graded ethanol, cleared in xylene, and mounted with neutral resin. Histopathological changes were examined under a light microscope.

2.5.2 Nissl staining

Brain sections were deparaffinized, and stained with Nissl solution at 37°C for 10 min. After rinsing, sections were dehydrated in 95% ethanol, cleared in xylene, and mounted with neutral resin. Nissl bodies in the cortex, hippocampal CA1, CA3, and DG regions were imaged and quantified under a light microscope.

2.5.3 Immunofluorescence staining

Brain sections or colon secions were deparaffinized, and permeabilized with 0.5% Triton X-100 for 30 min. After blocking with 5% BSA for 1 h, sections were incubated overnight at 4°C with primary antibodies, including CHAT, CHT1, TNF-α, IL-1β, BDNF, PSD95, SYN, ZO-1 and Occludin (all 1:100). They were then incubated with Alexa Fluor-488 or Cy3-conjugated secondary antibodies for 1.5 h in the dark. Nuclei were counterstained with DAPI and mounted using an anti-fluorescent quencher. Fluorescence images were captured using a fluorescence microscope.

2.6 Quantification of SOD, MDA, GSH, ACh and AChE levels in brain tissue

Following the completion of the MWM test, 8 mice from each group were sacrificed under deep anesthesia, and their brains were rapidly harvested. The superoxide dismutase (SOD), malondialdehyde (MDA), glutathione (GSH), acetylcholine (ACh) and acetylcholinesterase (AChE) levels in brain tissue were quantified following the manufacturer's instructions.

2.7 Transmission electron microscopy (TEM) tests

Hippocampal tissues were collected from 3 mice per group. The hippocampal samples were rapidly dissected and fixed in 2% glutaraldehyde (pH 7.2). Tissue blocks from the CA1 region, measuring approximately 1.0 × 1.0 × 1.0 mm^3, were further fixed in 1% osmium tetroxide at room temperature for 2 h. After sequential dehydration, infiltration, embedding, and polymerization, ultrathin sections (70 nm) were prepared. These sections were stained with 2% uranyl acetate in ethanol and 2.6% lead citrate. Finally, the sections were visualized and imaged using a TEM (HT7700, Hitachi, Tokyo, Japan). Quantitative analysis of mitochondrial density and synaptic numbers was performed using ImageJ software.

2.8 Western blot assays

Hippocampal total proteins were separated by SDS-PAGE and subsequently transferred onto PVDF membranes via wet transfer at 250 mA for 80 min. The membranes were blocked with 5% BSA at room temperature for 1 h, followed by incubation with primary antibodies against CHAT, CHT1, BDNF, SYN, PSD95, TNF-α, IL-1β, and MPO at 4°C overnight. After thorough washing, membranes were incubated with the corresponding HRP-conjugated secondary antibodies. The protein bands were visualized using a gel imaging system, and grayscale intensity was quantified using ImageJ software. Protein expression levels were normalized to internal controls (n = 3 in each group).

2.9 Gut microbiota 16S rDNA amplicon sequencing analysis

The detailed information was included in Supplementary Material. In short, fecal samples from 6 mice per group were aseptically collected. Amplicon sequence variants (ASVs) were identified through exact sequence variants, and taxonomic classification was performed against the SILVA 16S rDNA gene reference database. Alpha diversity indices, such as Chao1, Shannon, and Simpson, were calculated to assess species richness and diversity. Beta diversity was evaluated using weighted UniFrac distances. Differentially abundant taxa were identified using linear discriminant analysis effect size (LEfSe), with an LDA score threshold of 3.0. Spearman correlation analysis was performed to investigate the associations between differentially abundant taxa and cognitive function-related indicators in the behavior tests, including escape latency, platform crossing number, time spent in the target quadrant, and spontaneous alternation. A correlation coefficient threshold of $|R| > 0.3$ and a significance level of $P < 0.05$ were used to identify potential associations.

2.10 Serum metabolomics analysis

The detailed information was included in Supplementary Material. In short, serum samples from 6 mice per group were collected. Metabolomics profiling was conducted using liquid chromatography coupled with mass spectrometry (LC-MS). Chromatographic separation was performed on an ACQUITY UPLC system, with mass spectrometric detection carried out on a Q Exactive Plus Orbitrap mass spectrometer. Data acquisition was

conducted in both positive and negative ion modes, and full-scan mass spectra were recorded across a specified m/z range. Differential metabolites (DMs) were determined based on the variable importance in projection (VIP) scores from the PLS-DA model, with a threshold of VIP >1.0. Pathway enrichment analysis was performed using KEGG database. Pearson correlation analysis was conducted to explore the relationships between DMs and cognitive function-related indicators. A correlation coefficient threshold of $|R| > 0.5$ and statistical significance of $P < 0.05$ were used to identify significant associations.

2.11 Hippocampal transcriptomic analysis

mRNA-seq was conducted to analyze transcriptomic variations in the hippocampal tissues. Total RNA was extracted using a commercial RNA extraction kit (RC101-01, Vazyme Biotech Co., Ltd., Nanjing, China). Gene expression levels were quantified by calculating the TPM value. Differentially expressed genes (DEGs) were identified using DESeq2 with an absolute $\log_2$ fold change ($\log_2$ FC) ≥ 1 and P value <0.05. Functional annotation of DEGs was conducted through Gene Ontology (GO) and KEGG pathway enrichment using DAVID database (https://davidbioinformatics.nih.gov/tools.jsp). The Volcano, Heatmap, GO and KEGG pathway enrichment plots were generated using the online tool available at https://www.bioinformatics.com.cn.

2.12 Fecal microbiota transplantation (FMT) experiments

2.12.1 Experiments design

Donors: Thirty male C57BL/6J mice were randomly divided into two donor groups (n = 15/group). One group received a vehicle control (0.3% CMC-Na solution), and the other was administered XSB (0.52 mg/kg) via oral gavage once daily for 4 weeks. Fresh fecal samples were collected from each group to prepare standardized fecal microbiota suspensions: FM (from the vehicle-treated group) and XSB-FM (from the XSB-treated group). The samples were suspended in sterile PBS, homogenized using a vortex mixer, and centrifuged at 600 *g* for 3 min. The resulting supernatant was used to prepare a bacterial suspension at a concentration of 100 g/L.

Recipients: Forty recipient mice were administered a broad-spectrum antibiotic cocktail *ad libitum* for 4 weeks. Based on previous studies, the antibiotic mixture was prepared at the following concentrations: ampicillin (1 g/L), neomycin (1 g/L), metronidazole (1 g/L), and vancomycin (0.5 g/L) (Wang et al., 2021). Then, the 40 mice was randomized into 4 groups (n = 10/group): (1) Control + CMC-Na: Normal saline (i.p.) + CMC-Na 0.2 mL, oral gavage); (2) Scop + CMC-Na: Scopolamine (3 mg/kg, i.p.) + CMC-Na (0.2 mL, oral gavage); (3) Scop + FM: Scopolamine (3 mg/kg, i.p.) + FM (0.2 mL, oral gavage); (4) Scop + XSB-FM: Scopolamine (3 mg/kg, i.p.) + XSB-FM (0.2 mL, oral gavage). Each mouse received 0.2 mL of the corresponding treatment by oral gavage twice daily for 4 weeks. Cognitive function was assessed using the MWM test. Intestinal barrier integrity was evaluated via Alcian blue-periodic acid-Schiff (AB-PAS) staining, and colonic expression of the tight junction proteins ZO-1 and Occludin was determined by immunofluorescence.

2.12.2 AB-PAS staining

Colon tissue sections were deparaffinized, rehydrated, and sequentially stained with Alcian blue, periodic acid, Schiff's reagent, and hematoxylin. After dehydration and mounting, the sections were examined under a microscope. The colonic epithelium showed positive staining, with AB-PAS-positive cells appearing blue. n = 3 mice/group.

2.13 Statistical analysis

All data were presented as mean ± standard error of the mean (mean ± SEM). Statistical analyses were performed using GraphPad Prism 9.4 software. Escape latency data in the MWM test, collected across multiple trials, were analyzed using repeated-measures two-way ANOVA followed by Bonferroni post-hoc test. For comparisons among multiple groups, one-way ANOVA with Tukey's post-hoc test was applied. A p-value of less than 0.05 was considered statistically significant. Fluorescence intensity quantification was conducted using ImageJ software.

3 Results

3.1 XSB improves cognitive function in model mice

The NOR test results showed treatment with XSB-M (Figures 1A–C, P <0.05 vs. Model group), and XSB-H (Figures 1A–C, P < 0.01 vs. Model group) significantly improved the recognition index, with effects similar to the positive control drug donepezil (Figures 1A–C P < 0.01 vs. Model group). Similarly, in the Y-maze test, XSB-H significantly improved spontaneous alternation behavior (Figures 1D–F, P < 0.05 vs. Model group). In the MWM test, the typical swimming track diagrams of mice were as shown in Figure 1G. XSB treatment decreased escape latency over time, test, XSB treatment decreased escape latency over time, with significant differences observed on days 4 and 5 compared to the Model group (Figure 1H, P < 0.001 on day 5). Additionally, in the spatial probe trial, XSB treatment increased platform crossings (Figure 1I, P < 0.01 vs. Model group) and time spent in the target quadrant compared (Figure 1J, P < 0.01 vs. Model group) to the Model group. These results suggest that XSB can enhance short-term memory, working memory, and spatial learning and memory in model mice.

3.2 XSB alleviates cortical and hippocampal structural damage in model mice

H&E staining results revealed that the XSB-L, XSB-M, and XSB-H treatment groups exhibited well-organized and compact neuronal arrangements, with round nuclei and evenly distributed staining in both the cytoplasm and nuclei (Figure 2A). Nissl staining further demonstrated that in the XSB-treated groups, neurons were more

10.3389/fphar.2025.1596728

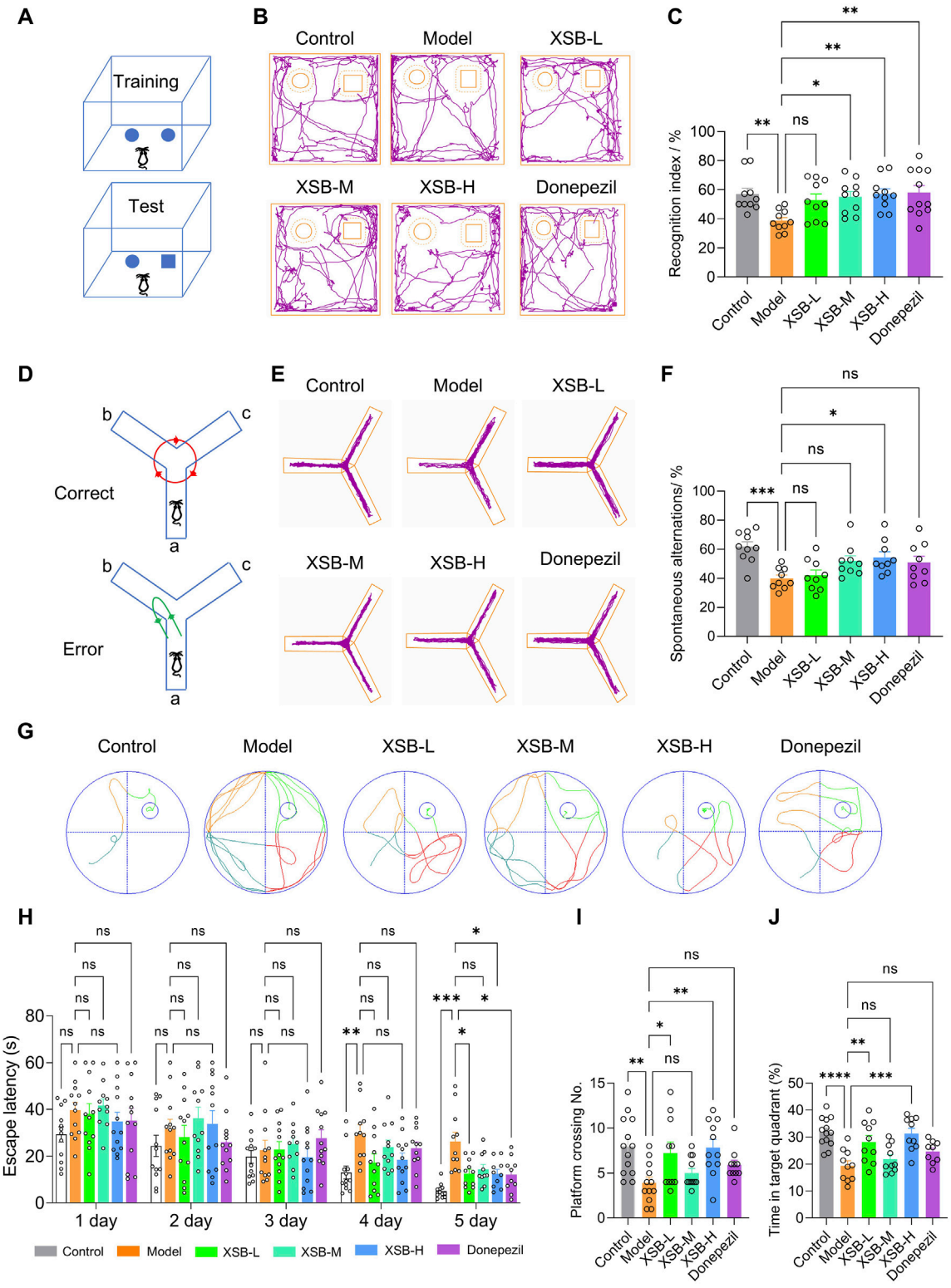


FIGURE 1
XSB improves cognitive function in AD model mice. **(A)**: Schematic diagram of the NOR test; **(B)**: Representative trajectory of mouse movement in the NOR test; **(C)**: Statistical graph of the recognition index in the NOR test for each group of mice; **(D)**: Schematic diagram of the Y-maze test; **(E)**: Representative trajectory of mouse movement in the Y-maze test; **(F)**: Statistical graph of spontaneous alternation counts in the Y-maze test; **(G)**: Typical swimming track diagrams of mice in the MWM test; **(H-J)**: Statistical graph of escape latency **(H)**, the number of platform crossings **(I)** and the percentage of time spent in the target quadrant **(J)**. Data are presented as mean ± SEM; *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$, ****: $P < 0.0001$, ns: $P > 0.05$. n = 12 mice/group.

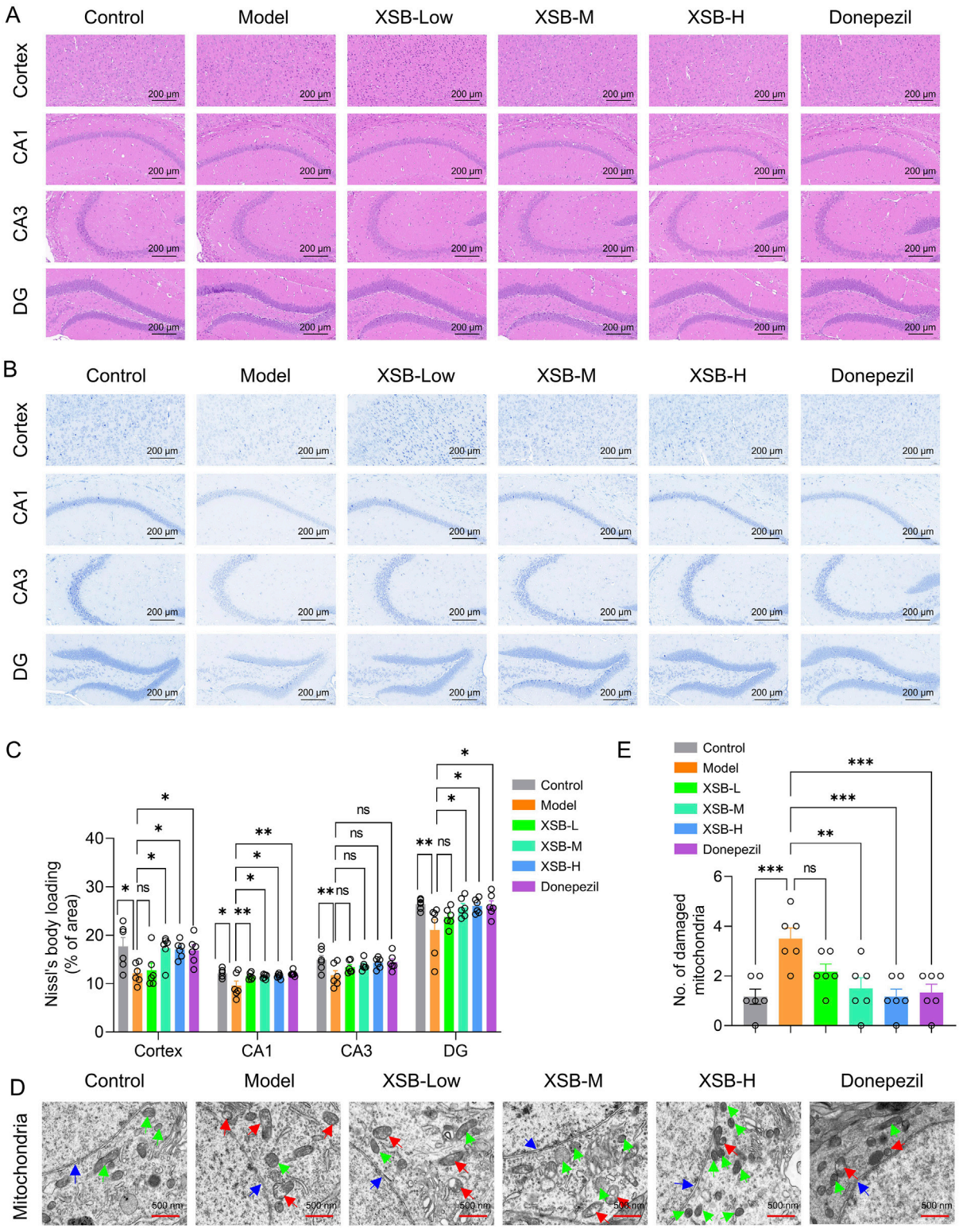


FIGURE 2
XSB alleviates cortical and hippocampal structural damage in AD model mice. **(A)**: Representative images of H&E staining; **(B)**: Representative images of Nissl staining; **(C)**: Quantitative analysis of Nissl bodies; **(D)**: Representative images of TEM; **(E)**: Quantitative analysis of damaged mitochondria based on D; Green arrows indicate healthy mitochondria, red arrows indicate damaged mitochondria, and blue arrows indicate nuclear envelopes. Data are presented as mean ± SEM; *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$, ****: $P < 0.0001$, ns: $P > 0.05$. n = 3 mice/group.

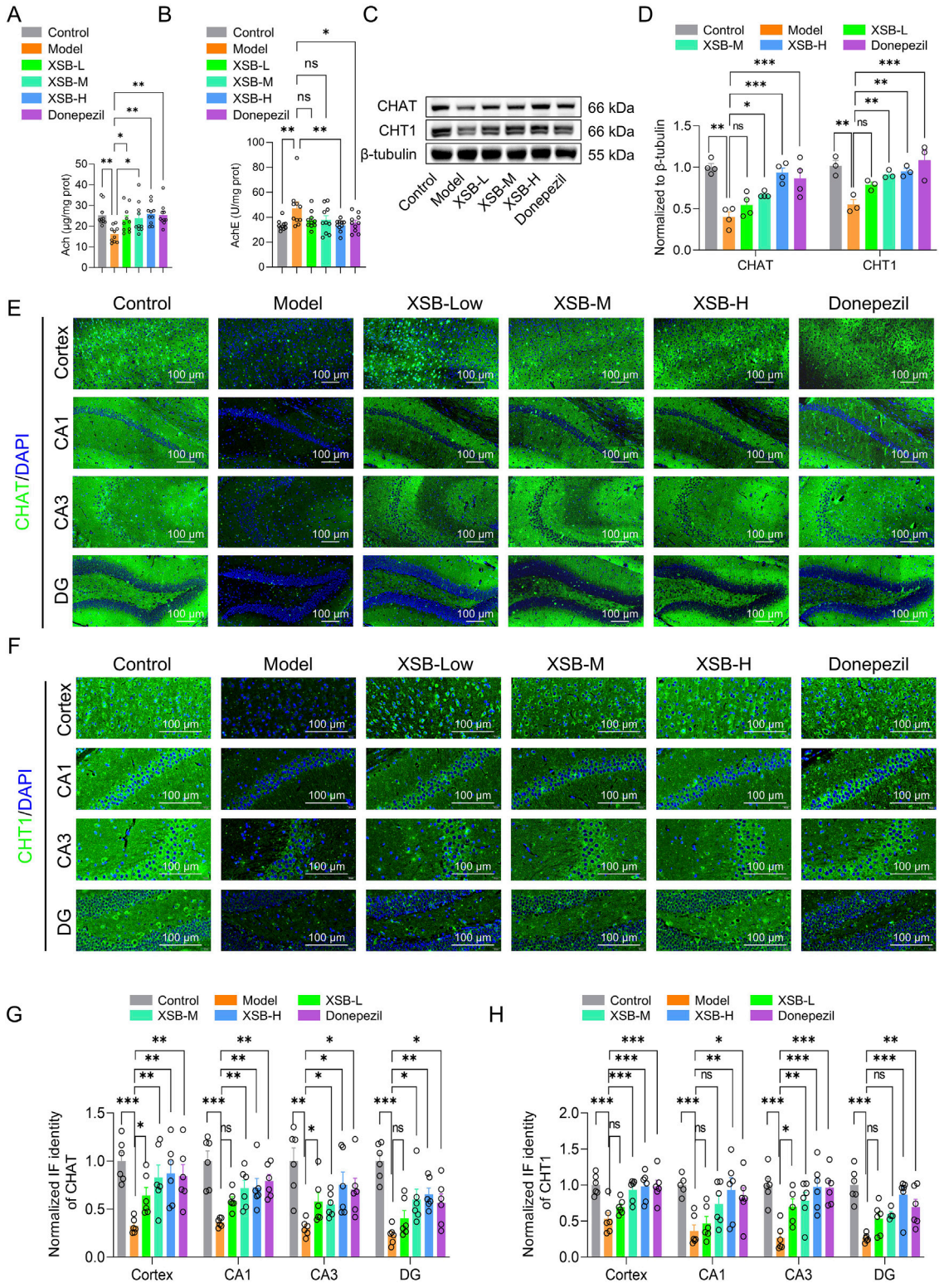


FIGURE 3
XSB improves the cholinergic system in the brain of AD model mice. **(A)**: ACh levels in the brain of each group; **(B)**: AChE activity; **(C)**: Representative Western blot images of ChAT and CHT1 protein expression in the brain of each group; **(D)**: Quantification of ChAT and CHT1 protein expression based on C; **(E,F)**: Representative immunofluorescence images of ChAT **(E)** and CHT1 **(F)** expressions in the cortex and hippocampus; **(G,H)**: Statistical analysis of ChAT **(G)** and CHT1 **(H)** expressions in the cortex and hippocampal subregions based on **(E,F)**. Data are presented as mean ± SEM; *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$, ****: $P < 0.0001$, ns: $P > 0.05$. n = 8 mice/group in assays of ACh and AChE, and n = 8 mice/group in Western blot and immunofluorescence assays.

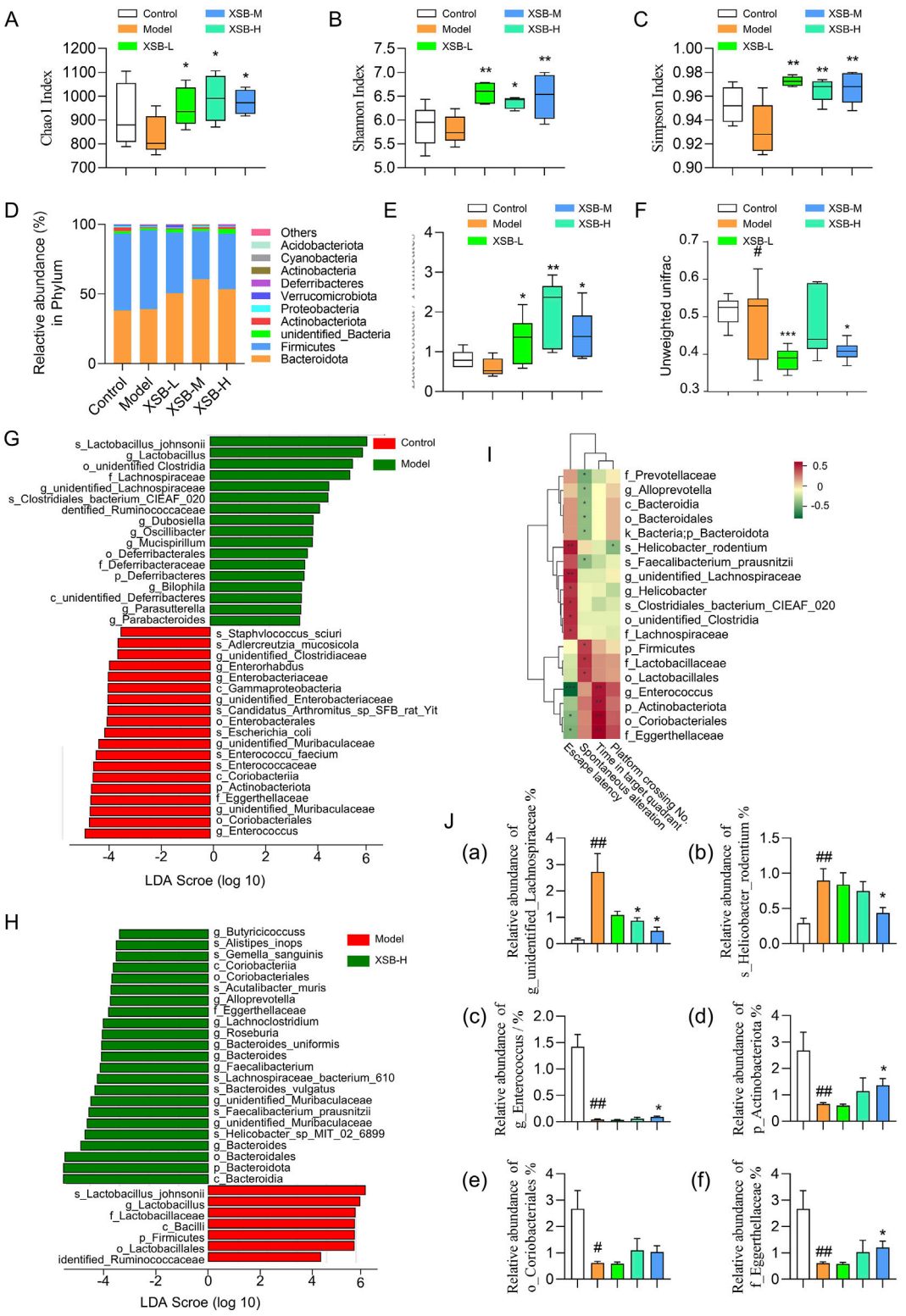


FIGURE 4
XSB rebalances gut microbiota composition in AD model mice. **(A)**: Chao1 index diversity; **(B)**: Shannon index diversity; **(C)**: Simpson index diversity; **(D)**: Relative abundance of species at the phylum level; **(E)**: ratio of Bacteroidota to Firmicutes relative abundance; **(F)**: Comparison of beta diversity among different groups based on Unweighted UniFrac distance. **(G,H)**: LEfSe analysis revealed significant bacterial differences in fecal microbiota between Model vs. Control **(G)**, and XSB-H vs. Model **(H)**; **(I)**: Spearman correlation analysis between differentially abundant taxa and cognitive function-related indicators. **(J)**: Relative abundance of bacteria associated with cognitive function. (a) g_unidentified_Lachnospiraceae, (b) s_*Helicobacter*_rodentium; (c): g_*Enterococcus*, (d) p_Actinobacteriota, (e) o_Coriobacteriales, (f) f_Eggerthellaceae. Data are presented as mean ± SEM; *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$ vs. Model group; #: $P < 0.05$, ##: $P < 0.01$, ###: $P < 0.001$ vs. Control group. n = 6 mice/group.

densely packed and more intense staining than that in Model group. Notably, the XSB-M and XSB-H groups showed a significant increase in the number of Nissl bodies in the cortex (Figures 2B,C, $P < 0.05$) and hippocampal CA1 (Figures 2B,C, $P < 0.05$) and DG (Figures 2B,C, $P < 0.05$) regions compared to the Model group, with effects comparable to the positive control drug donepezil.

TEM analysis revealed that following XSB treatment, mitochondrial morphology was restored to typical elongated, fusiform shapes, with well-defined mitochondrial cristae and an intact nuclear membrane structure. Furthermore, the number of injured mitochondria was markedly decreased (Figures 2D,E, $P < 0.001$ vs. Model group), with effects similar to the positive control drug donepezil (Figures D,E, $P < 0.001$ vs. Model group). These results demonstrate that XSB effectively alleviates both pathological and ultrastructural damage in the cortex and hippocampus of model mice.

3.3 XSB enhances cholinergic system function in the brain of model mice

As shown in Figure 3, treatment with XSB-H significantly increased ACh levels (Figure 3A, $P < 0.05$ vs. Model group) and decreased AChE activity (Figure 3B, $P < 0.05$ vs. Model group) in the Model mice. Moreover, both XSB-M and XSB-H treatment significantly elevated the expression of ChAT and CHT1 in the cortex and hippocampus evidenced by western bolt and immunofluorescence data (Figures 3C–H), suggesting that XSB can effectively improve the cholinergic system in the brain of model mice.

3.4 XSB rebalances gut microbiota composition in model mice

As shown in Figure 4, treatment with XSB, particularly XSB-M and XSB-H, significantly improved microbial diversity, as reflected by lower Chao 1 (Figure 4A), Shannon (Figure 4B) and Simpson indices (Figure 4C). At the phylum level, XSB significantly decreased the relative abundance of *Firmicutes* while increasing *Bacteroidota* (Figures 4D,E), suggesting a shift towards a more balanced microbiota in these groups. The UniFrac distance analysis (Figure 4F) showed that the gut microbiota composition in XSB-treated groups was significantly closer to that of the Control group compared to the Model group.

Additionally, LEfSe analysis identified that *s-Lactobacillus-johnsonii*, g-*Lactobacillus*, *o-unidentified Clostridia*, *f-Lachnospiraceae*, *g-unidentified-Lachnospiraceae*, *s-Clostridiales-bacterium-CIEAF-020*, and *dentified-Ruminococcaceae* were enriched in Model group, while *g-Enterococcus*, *o-Coriobacteriales*, *s-Enterococcaceae*, *g-unidentified-Muribaculaceae*, *p-Actinobacteriota*, *f-Eggerthellaceae* which were identified beneficial for cognitive function were enriched in Control group (Figure 4G). Compared to Model group, *Bacteroidia*, *Helicobacter*-sp-*MIT-02-6899*, *g-unidentified-Muribaculaceae*, *s-Faecalibacterium-prausnitzii* and *s-Lachnospiraceae-bacterium-610*, associated with gut health and cognitive function, were enriched in XSB-H group (Figure 4H).

Moreover, correlation analysis between cognitive function-related indicators and beneficial bacteria revealed several taxa significantly associated with cognitive performance. For instance, *g_Enterococcus*, *p_Actinobacteriota*, *o_Coriobacteriales*, and *f_Eggerthellaceae* exhibited a positive correlation with cognitive function (Figure 4I, $P < 0.001$), whereas *s_Helicobacter_rodentium* and *g_unidentified_Lachnospiraceae* showed a negative correlation (Figure 4I, $P < 0.01$). Furthermore, in the XSB-treated groups, the abundance of the cognitively harmful taxa, including *s_Helicobacter_rodentium* and *g_unidentified_Lachnospiraceae*, was slightly decreased, while the cognitively beneficial taxa, including *g_Enterococcus*, *p_Actinobacteriota*, *o_Coriobacteriales*, and f_*Eggerthellaceae*, was slightly elevated (Figure 4J). These findings suggest that scopolamine-induced cognitive impairment leads to gut microbiota dysbiosis, while XSB administration partially restores the abundance of beneficial bacteria, thereby re-establishing microbial balance and subsequently improving cognitive function in the model mice.

3.5 XSB restores metabolic homeostasis in model mice

As shown in Figure 5A, we identified 439 DMs between the Model and Control groups (218 upregulated and 221 downregulated), 127 DMs between the XSB-L and Model groups (37 upregulated and 90 downregulated), 158 DMs between the XSB-M and Model groups (88 upregulated and 70 downregulated), and 256 DMs between the XSB-H and Model groups (141 upregulated and 115 downregulated). The clustering heatmap in Figure 5B and the PLS-DA score plot in Figure 5C reveal that the XSB-H and XSB-M groups cluster more closely with the Control group. This suggests that intraperitoneal injection of scopolamine induces alterations in serum metabolites, and that XSB treatment can partially restore these metabolic disturbances. Figure 5D shows the top 30 metabolites with VIP >1 from the PLS-DA analysis. Pearson correlation analysis of these metabolites with cognitive function-related indicators indicated several significant correlations. Specifically, HMDB0000748 (L-3-Phenyllactic acid), HMDB0040891 (3′,4′,5′-Trimethoxycinnamyl alcohol acetate), HMDB0114073 (PE-NMe2 (18:2 (9Z, 12Z)/18:2 (9Z, 12Z))), and HMDB0052711 (TG (18:2 (9Z, 12Z)/22:4 (7Z,10Z,13Z, 16Z)/18:2 (9Z, 12Z))) were negatively correlated with cognitive function, whereas HMDB0015673 (Carglumic acid), HMDB0005772 (Postin), HMDB0000755 (Hydroxyphenyllactic acid), and HMDB0005781 (Glycitein) exhibited positive correlations (Figure 5E). Additionally, the abnormal levels of these metabolites were partially corrected by XSB treatment (Figure 5F).

KEGG enrichment analysis of the DMs with VIP >1 further revealed that XSB primarily restores metabolic balance in model mice by modulating key metabolic pathways, including glycerophospholipid metabolism, linoleic acid metabolism, alpha-Linolenic acid metabolism, ether lipid metabolism, glutathione metabolism, pentose phosphate pathway and folate biosynthesis (Figure 5G).

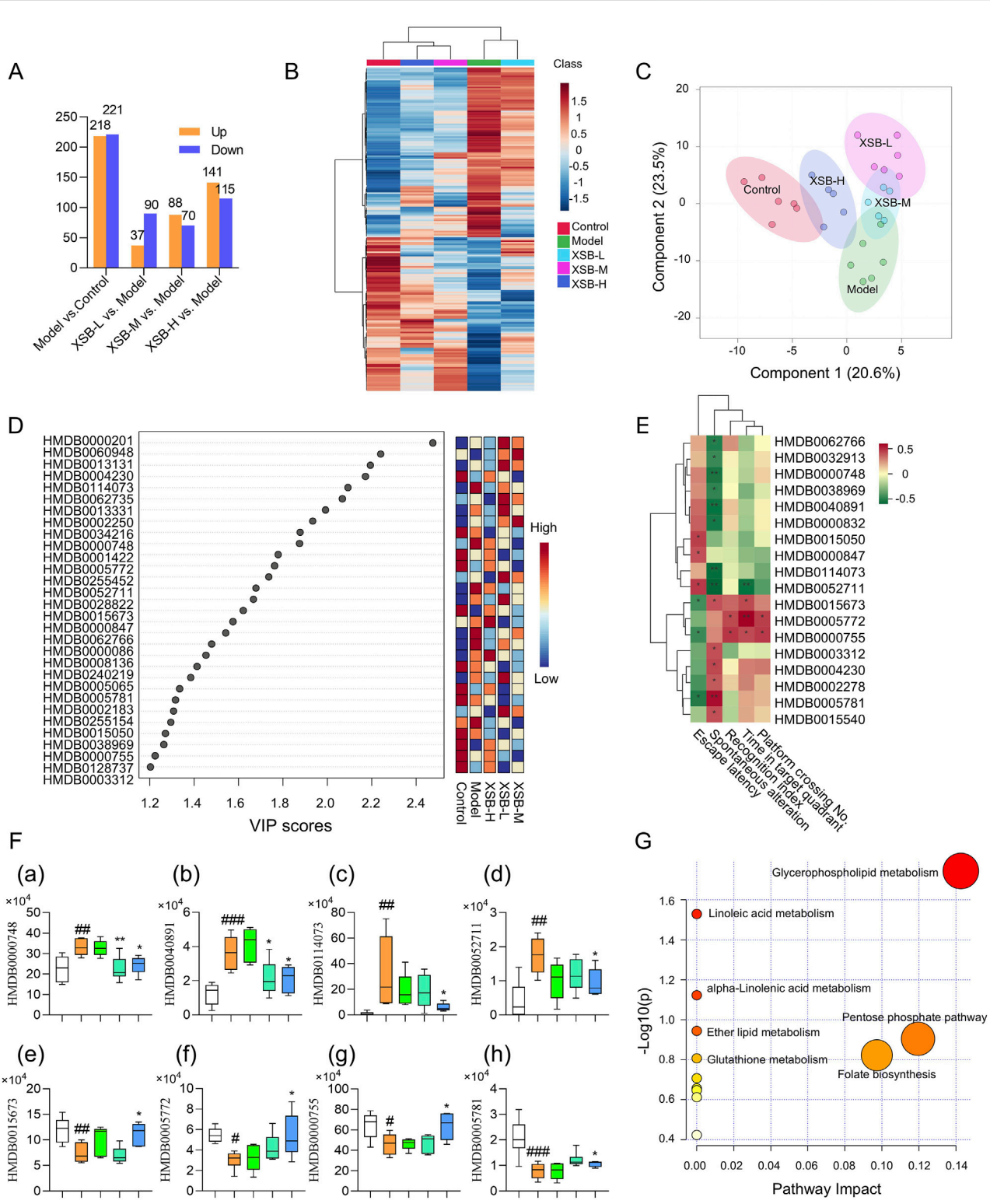


FIGURE 5
XSB restores metabolic homeostasis in AD model mice. **(A)**: Statistical analysis of the number of differential metabolites among different comparison groups; **(B)**: Heatmap of hierarchical clustering of differential metabolites; **(C)**: PLS-DA score plot of differential metabolites; **(D)**: Heatmap of the top 30 metabolites by VIP values; **(E)**: Pearson correlation analysis between DMs and cognitive function; **(F)**: Relative peak area of metabolites significantly associated with cognitive function, (a) HMDB0000748 (L-3-Phenyllactic acid), (b) HMDB0040891 (3′,4′,5′-Trimethoxycinnamyl alcohol acetate), (c) HMDB0114073 (PE-NMe2 (18:2 (9Z, 12Z)/18:2 (9Z, 12Z))), (d) HMDB0052711 (TG (18:2 (9Z, 12Z)/22:4 (7Z,10Z,13Z, 16Z)/18:2 (9Z, 12Z))), (e) HMDB0015673 (Carglumic acid), (f) HMDB0005772 (Postin), (g) HMDB0000755 (Hydroxyphenyllactic acid); (h) HMDB0005781 (Glycitein); **(G)**: KEGG enrichment analysis of DMs. Data are presented as mean ± SEM; *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$ vs. Model group; #: $P < 0.05$, ##: $P < 0.01$, ###: $P < 0.001$ vs. Control group. n = 6 mice/group.

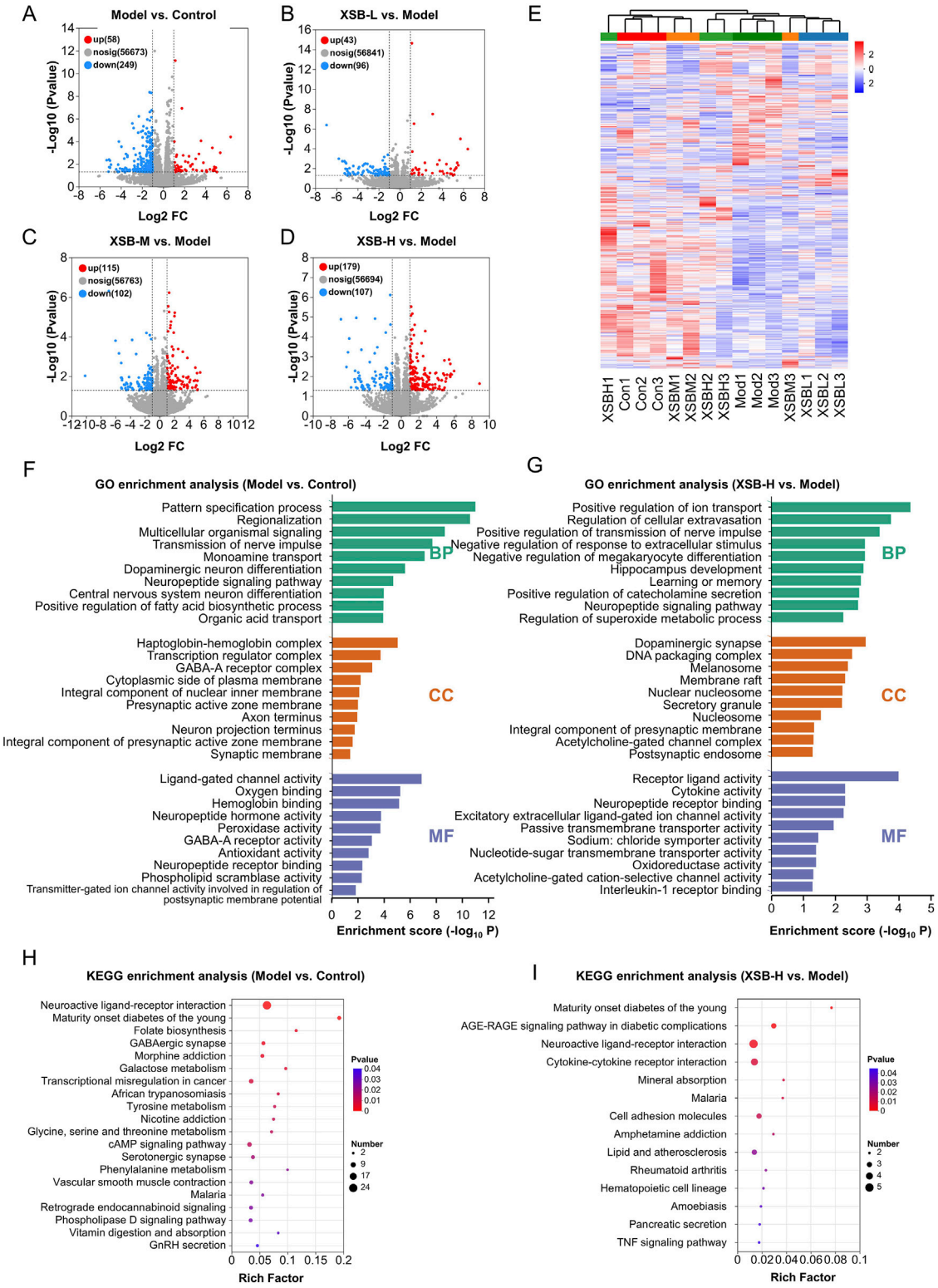


FIGURE 6
XSB restores hippocampal transcriptomic homeostasis in AD model mice. **(A)**: Volcano maps depicting DEGs in Model vs. Control **(A)**, XSB-L vs. Model **(B)**, XSB-M vs. Model **(C)**, and XSB-H vs. Model **(D)** groups; **(E)** Cluster analysis heat maps of DEGs among different groups; **(F,G)**: GO enrichment analysis of DEGs in the Model vs. Control group **(F)**, and Model XSB-H group **(G)**; **(H,I)**: KEGG enrichment analysis of DEGs in the Model vs. Control group **(H)**, and Model XSB-H group **(I)**. n = 3 mice/group.

3.6 XSB restores hippocampal transcriptomic homeostasis in model mice

As illustrated in Figures 6A–D, the Model group exhibited 307 DEGs compared to the Control group, with 58 genes upregulated and 249 downregulated. In contrast, the XSB-L group demonstrated 139 DEGs relative to the Model group, comprising 43 upregulated and 96 downregulated genes. The XSB-M group exhibited 217 DEGs, with 115 upregulated and 102 downregulated, while the XSB-H group displayed 286 DEGs, including 179 upregulated and 107 downregulated, when compared to the Model group. Subsequently, these DEGs underwent hierarchical clustering analysis. As shown in Figure 6E, the XSB-H and XSB-M groups clustered closely with the Control group, indicating a potential reversal of gene expression patterns by XSB treatment.

The GO functional annotation revealed that the DEGs in the XSB-H vs. Model comparison were predominantly enriched in cellular components such as the dopaminergic synapse, DNA packaging complex, membrane raft, integral component of the presynaptic membrane, and acetylcholine-gated channel complex. Additionally, these genes were associated with diverse molecular functions, including receptor-ligand activity, cytokine activity, neuropeptide receptor binding, passive transmembrane transporter activity, oxidoreductase activity, and acetylcholine-gated cation-selective channel activity. Functionally, these DEGs were implicated in key biological processes such as the regulation of nerve impulse transmission, response to extracellular stimuli, megakaryocyte differentiation, hippocampal development, learning and memory, neuropeptide signaling pathways, superoxide metabolic processes, and interleukin-1 receptor binding (Figure 6G). Furthermore, KEGG pathway enrichment analysis indicated that the DEGs in the XSB vs. Model group were significantly associated with neuroactive ligand-receptor interactions, cytokine-cytokine receptor interactions, cell adhesion molecules, and the TNF signaling pathway (Figure 6I). The GO and KEGG enrichment analysis results of Model vs. Control group were as shown in Figures 6F,H. These findings suggest that intraperitoneal scopolamine injection disrupts hippocampal gene expression, while XSB treatment partially restores homeostasis through the regulation of neuroactive ligand-receptor interactions and modulation of neuroinflammatory pathways, both of which are critical for maintaining cognitive function.

3.7 Multi-omics integration: transcriptomics, metabolomics, and gut microbiota-metabolome analysis

Using MetaboAnalyst, joint pathway analysis was performed to integrate DEGs and DMs Figure 7A. The results indicated that XSB primarily regulates metabolic pathways, including linoleic acid metabolism, retinol metabolism, biosynthesis of unsaturated fatty acids, tyrosine metabolism, and glycerophospholipid metabolism (Figure 7B). To explore potential associations between DMs and gut microbiota, Spearman correlation analysis was performed. The results revealed significant associations between specific bacterial species and DMs (Figure 7C). For instance, bacteria beneficial to cognitive function, such as *g_Enterococcus*, *p_Actinobacteriota*, *o_Coriobacteriales*, and *f_Eggerthellaceae*, exhibited negative correlations with metabolites detrimental to cognitive function, including HMDB0114073, HMDB0040891, and HMDB0000748, while showing positive correlations with metabolites beneficial to cognition, such as HMDB0005781 and HMDB0015673. In contrast, bacteria detrimental to cognitive function, such as *g_unidentified_Lachnospiraceae* and *s_Helicobacter_rodentium*, demonstrated positive correlations with HMDB0114073, HMDB0040891, and HMDB0000748, but negative correlations with HMDB0005781 and HMDB0015673. These results highlight the critical role of XSB in modulating key metabolic pathways and the interplay between gut microbiota and metabolites, suggesting a potential mechanism through which XSB improves cognitive function by restoring microbial and metabolic homeostasis.

3.8 XSB inhibits neuroinflammation in model mice

Based on the enrichment of inflammatory-related signaling pathways, such as the TNFα signaling pathway and arachidonic acid metabolism, identified in the KEGG analysis, we further assessed the levels of key inflammatory mediators-TNF-α, IL-1β, and MPO-in the hippocampus using Western blot analysis. As shown in Figures 8A,B, XSB treatment significantly reduced the protein expression levels of TNF-α ($P < 0.01$ vs. Model group), IL-1β ($P < 0.05$ vs. Model group), and MPO ($P < 0.05$ vs. Model group) in the hippocampus. Similar results were observed in immunofluorescence experiments (Figures 8C–E), further confirming the anti-neuroinflammatory effects of XSB. Moreover, XSB treatment increased the activity of SOD ($P < 0.01$ vs. Model group), elevated the levels of GSH ($P < 0.01$ vs. Model group), and reduced MDA levels ($P < 0.01$ vs. Model group), thereby alleviating oxidative stress damage in the brains of model mice (Figures 8F–H).

3.9 XSB enhances synaptic protein expression and synaptic density

Given that KEGG enrichment analysis revealed the involvement of neuroactive ligand-receptor interaction signaling pathways, which are crucial for synaptic signaling, we then evaluated the expression of critical synaptic proteins-BDNF, SYN, and PSD95-using Western blot and immunofluorescence methods. As shown in Figures 9A,B, XSB treatment notably increased the expression of BDNF ($P < 0.05$), SYN ($P < 0.001$), and PSD95 ($P < 0.001$) compared to Model group. Immunofluorescence results (Figures 9C,D) supported these findings, further confirming the beneficial effects of XSB on synaptic damage. Additionally, XSB treatment significantly increased synaptic density (Figures 9E,F, $p < 0.05$ vs. Model group). These results suggest that XSB promotes the expression of BDNF, SYN, and PSD95 and enhances synaptic density, potentially contributing to the improvement of cognitive function in model mice.

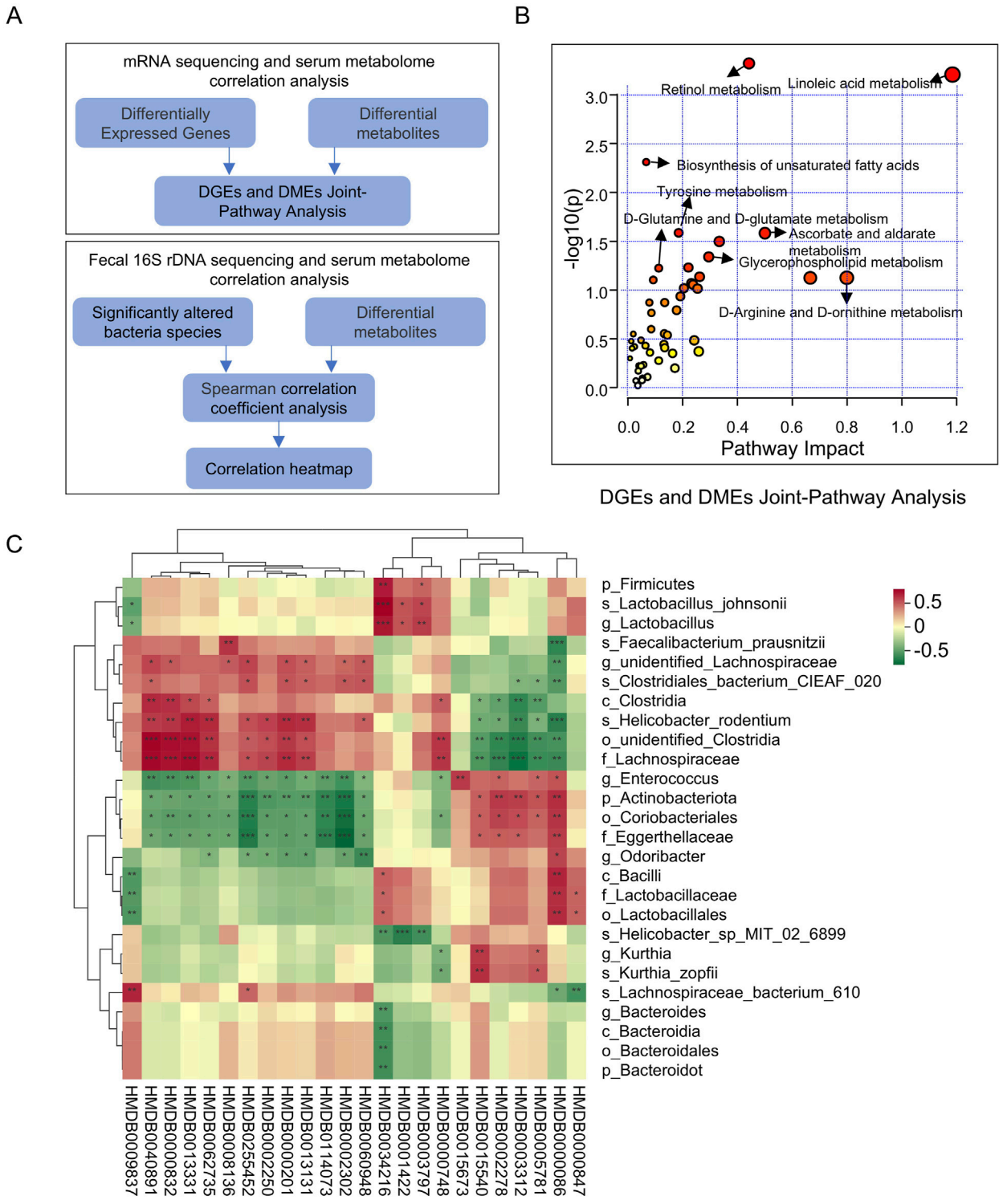


FIGURE 7
Multi-Omics integration: transcriptomics, metabolomics, and gut microbiota-metabolome analysis. **(A)** Schematic of Multi-Omics Integration Analysis; **(B)** DGEs and DMEs Joint-Pathway Analysis; **(C)** Spearman correlation coefficient analysis of DMs and Significantly altered bacteria species.

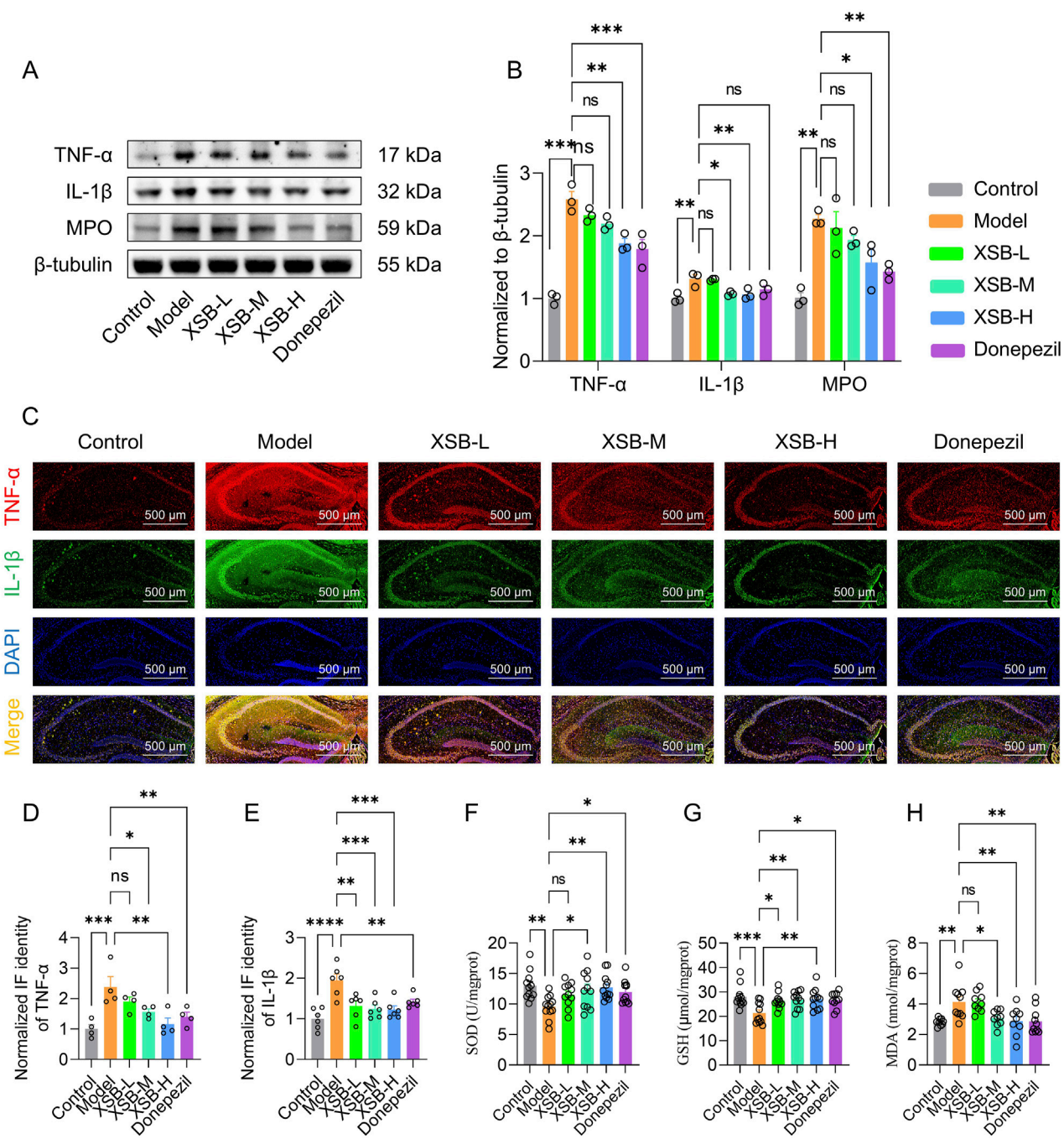


FIGURE 8
XSB inhibits neuroinflammation in AD model mice. **(A)**: Representative immunoblot images of TNF-α, IL-1β, MPO and β-tubulin. **(B)**: quantitative analysis of TNF-α, IL-1β and MPO expression based on A; **(C)**: Representative immunofluorescence images of TNF-α and IL-1β. **(D,E)**: Quantitative analysis of TNF-α **(D)** and IL-1β **(E)** based on C; **(F-H)**: SOD activity **(F)**, GSH content **(G)** and MDA content **(H)** in brains in different groups. Data are presented as mean ± SEM; *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$, ns: $P > 0.05$, n = 3 mice/group in Western blot and immunofluorescence assays, n = 9–12 mice/group in SOD, GSH and MDA assays.

3.10 Transplantation of XSB-FM rescues cognitive dysfunction and intestinal barrier injures in AD model mice

The results demonstrated that transplantation of XSB-FM significantly decreased the escape latency (Figures 10B,C, $p < 0.05$ vs. Scop-FM group on 5th day), increased the platform crossing number (Figure 10D, $P < 0.05$ vs. Scop-FM group) and the time spent in target square (Figure 10E, $P < 0.05$ vs. Scop-FM group). Moreover, compared with Scop-FM group, transplantation of XSB-FM markedly boosted the AB-PAS positive cells (Figure 10F) in the intestinal mucosa, and upregulated ZO-1 (Figures 10G,I, $p < 0.05$) and Occludin (Figures

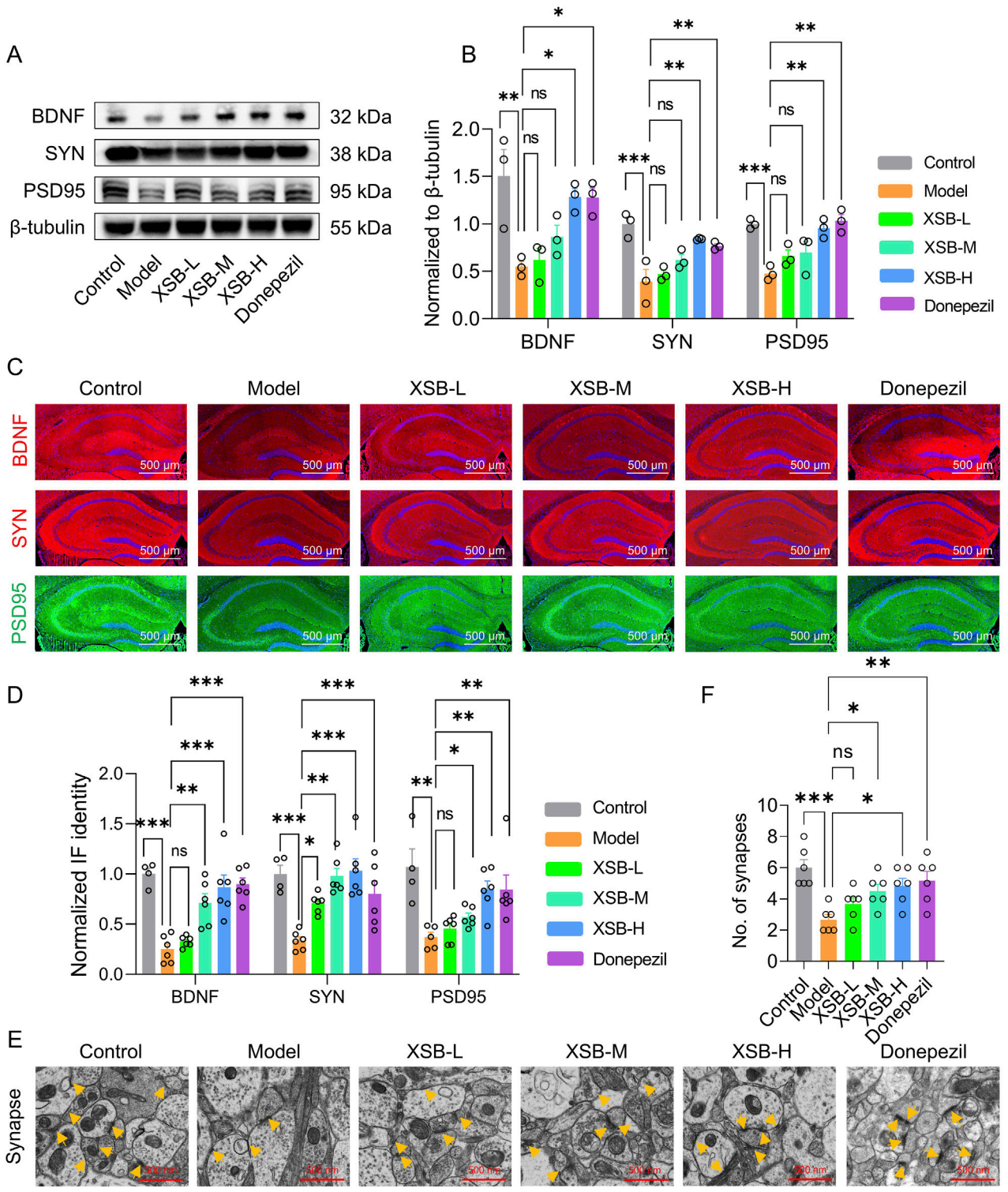


FIGURE 9
XSB enhances synaptic protein expression and synaptic density. **(A)**: Representative immunoblot images of BNDF, SYN, PSD95 and β-tubulin. **(B)**: Quantitative analysis of BNDF, SYN and PSD95 expression based on A; **(C)**: Representative immunofluorescence images of BNDF, SYN and PSD95; **(D)**: Quantitative analysis of BNDF, SYN and PSD95 expression based on C; **(E)**: Representative TEM images of synapses in neurons in hippocampal CA1 region; yellow arrows indicate synapses; **(F)**: Quantitative analysis of synapse number in different groups. Data are presented as mean ± SEM; *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$, ns: $P > 0.05$, n = 3 mice/group.

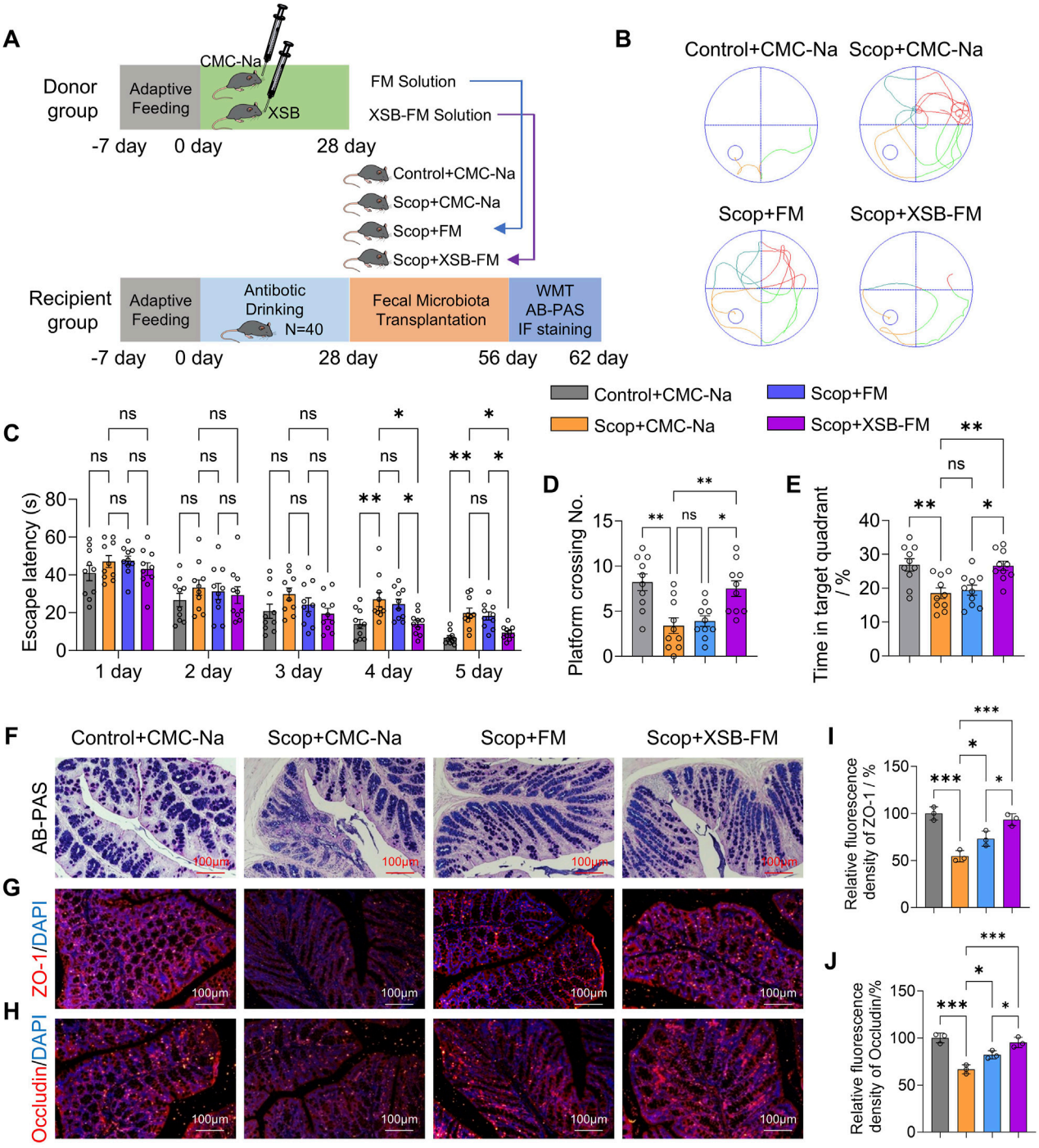


FIGURE 10
Transplantation of XSB-FM rescues cognitive dysfunction and intestinal barrier injures in AD model mice. **(A)**: Schematic diagram of the FMT experiment; **(B)**: Representative swimming track diagrams of mice in the MWM test; **(C-E)**: Statistical graph of escape latency **(C)**, the number of platform crossings **(D)** and the percentage of time spent in the target quadrant **(E)**. **(F)**: Representative images of AB-PAS staining; **(G,H)**: Representative immunofluorescence images of ZO-1 **(G)** and Occludin **(H)**. **(I,J)**: Statistical graph of ZO-1 **(I)** and Occludin **(J)** based on **(G,H)**, respectively, n = 3 mice/group. Data are presented as mean ± SEM; *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$, ns: $P > 0.05$, n = 10 mice/group in MWM test, n = 3 mice/group in AB-PAS, ZO-1 and Occludin staining tests.

10H,J, $p < 0.05$) protein expressions in dementia mice induced by scopolamine. These findings indicated that transplantation of XSB-FM rescues cognitive dysfunction and intestinal barrier injures in AD model mice, which further confirmed that gut microbiota remodeling is essential for XSB's therapeutic efficacy.

4 Discussion

AD is a prevalent and devastating neurodegenerative disorder characterized by progressive cognitive decline, synaptic dysfunction, and pathological features such as neuroinflammation and

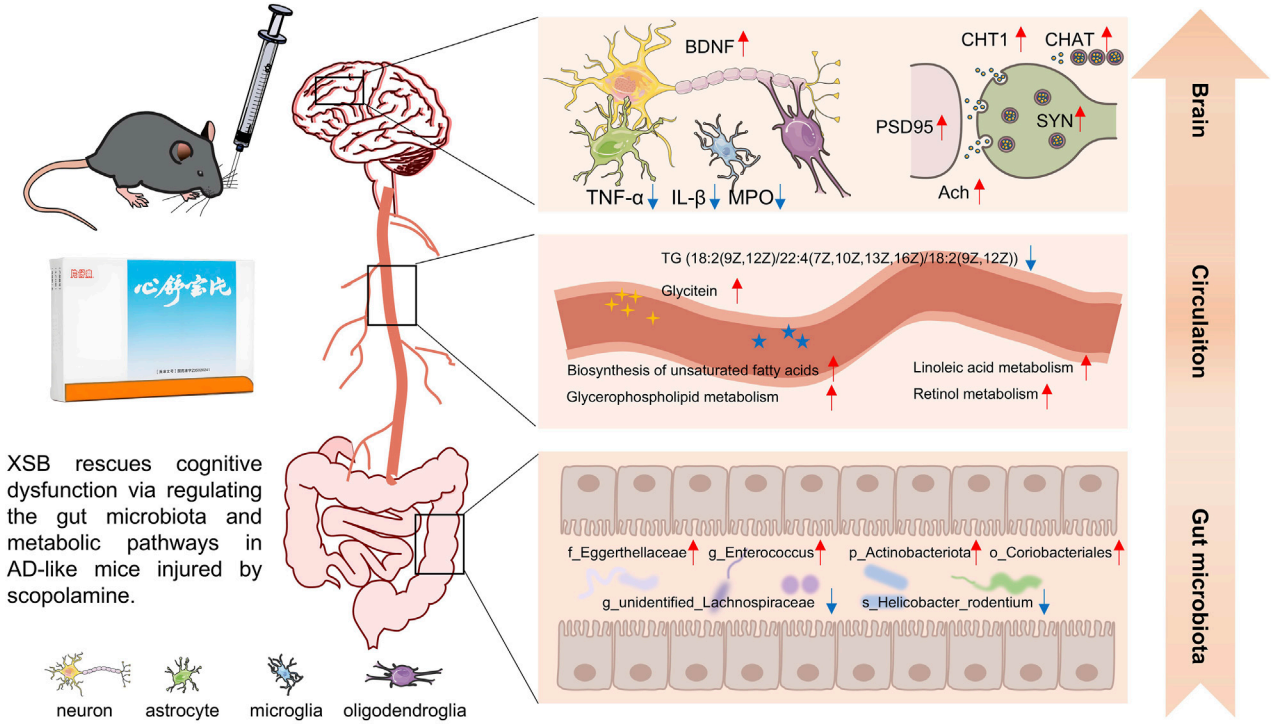


FIGURE 11
Overview of multiple regulations of XSB on AD model mice induced by scopolamine.

cholinergic system impairment. TCM has been recognized as a promising approach for managing neurodegenerative diseases, with various natural products showing potential therapeutic benefits.

In the present study, we explored the potential therapeutic effects of XSB, a traditional Chinese patent medicine, in an AD mouse model induced by scopolamine. Our findings demonstrated that XSB significantly alleviated cognitive impairments, ameliorated hippocampal neuronal damage, and mitigated key neuropathological hallmarks, including synaptic dysfunction, dysregulation of the cholinergic nervous system, neuroinflammation, and oxidative stress in AD mice. Moreover, XSB increased the abundance of beneficial bacteria (e.g., *g_Enterococcus*, *p_Actinobacteriota*, *o_Coriobacteriales*, and *f_Eggerthellaceae*), while decreasing the abundance of harmful bacteria (e.g., *s_Helicobacter_rodentium* and *g_unidentified_Lachnospiraceae*), effectively modulating the gut microbiota balance. XSB also regulated metabolic pathways related to tyrosine metabolism, glycerophospholipid metabolism, and unsaturated fatty acid metabolism, leading to a reduction in harmful metabolites such as HMDB0000748 (L-3-Phenyllactic acid), HMDB0040891 (3′,4′,5′-Trimethoxycinnamyl alcohol acetate), HMDB0114073 (PE-NMe2 (18:2 (9Z, 12Z)/18:2 (9Z, 12Z))), and HMDB0052711 (TG (18:2 (9Z, 12Z)/22:4 (7Z,10Z,13Z, 16Z)/18:2 (9Z, 12Z))), while increasing beneficial metabolites such as HMDB0015673 (Carglumic acid), HMDB0005772 (Postin), HMDB0000755 (Hydroxyphenyllactic acid), and HMDB0005781 (Glycitein) (Figure 11). In addition, XSB-FM transformation successfully alleviated cognitive and intestinal barrier damages. These findings collectively underscore the multifaceted protective effects of XSB in mitigating key pathological processes associated with AD, highlighting its potential as a therapeutic intervention.

The cholinergic system, particularly in the basal forebrain, cortex, and hippocampus, plays a critical role in memory formation and cognition. Dysfunction of this system is regarded as one of the key pathogenic mechanisms underlying AD (Hampel et al., 2018). Cholinergic neurons are primarily located in the basal nucleus and the diagonal band of Broca in the basal forebrain, which provide the main cholinergic innervation to the hippocampus and cerebral cortex (Chen et al., 2018). Neurotransmitters transmit signals from the neurons of the basal forebrain to hippocampal neurons, a process that involves the synthesis, packaging, secretion, and clearance of the neurotransmitters. ACh is synthesized by the enzyme ChAT through the combination of choline and acetyl-CoA. ACh is then transported into synaptic vesicles from the cytoplasm by the vesicular acetylcholine transporter (VAChT). Upon neuronal firing, ACh stored in the vesicles is released into the synaptic cleft, thereby completing signal transduction. Subsequently, some of the ACh is hydrolyzed by AChE into choline and acetate. Choline is then reabsorbed by the high-affinity choline transporter (CHT) located on the presynaptic membrane and is recycled by ChAT to synthesize more ACh (Chen et al., 2018). Any disruption in these processes may lead to cholinergic dysfunction, which in turn causes cognitive impairment.

Scopolamine, a non-selective muscarinic acetylcholine receptor antagonist, induces central cholinergic dysfunction, thereby impairing learning ability and short-term memory (Lee et al., 2014). Moreover, scopolamine has been shown to induce neurotrophic deficits, oxidative stress, neuroinflammation, and

increased Aβ accumulation, which closely resemble the pathological features observed in AD patients (Xiao et al., 2020; Tang, 2019; Yun et al., 2022). Thus, scopolamine-induced cognitive impairment models are widely employed for evaluating the anti-dementia efficacy of potential therapeutic agents. In this study, we found that XSB treatment effectively restored the cholinergic dysfunction induced by scopolamine, as evidenced by reduced AChE activity, increased ACh levels, and upregulation of ChAT and CHT1 protein expression (Figure 3).

Gut microbiota plays a crucial role in the development and progression of AD and other neurodegenerative disorders. Dysbiosis, characterized by an imbalance in the gut microbial composition, has been linked to neuroinflammation, oxidative stress, and neuronal dysfunction, all of which contribute to cognitive decline in AD. Our findings demonstrated that treatment with XSB effectively restored microbial diversity, increasing the abundance of beneficial taxa-including *Enterococcus*, *Actinobacteriota*, *Coriobacteriales*, and *Eggerthellaceae* (Figure 4). *Enterococcus* has been reported to be significantly reduced in both APP/PS1 transgenic mice and Aβ-induced mouse models (Jung et al., 2022; Qian et al., 2023). The phylum *Actinobacteriota*, which includes beneficial genera such as *Bifidobacterium*, has demonstrated neuroprotective effects by stimulating BDNF production and reducing pro-inflammatory cytokines such as TNF-α and IL-1β (Wang et al., 2025), thereby alleviating neuroinflammation and supporting cognitive function (Yin et al., 2024). Although elevated levels of *Coriobacteriales_Incertae_Sedis* have been observed in obese individuals and are thought to be negatively associated with cognitive performance (Zhao T. et al., 2023), it is important to recognize that the *Coriobacteriales* order comprises diverse genera and strains with potentially distinct biological functions. Members of the *Eggerthellaceae* family have been positively associated with white matter integrity and memory performance (Hammond et al., 2023), although their abundance tends to decline with aging and in APP/PS1 mice (Qian et al., 2023). These bacteria are capable of converting daidzein, an isoflavone found in soy products, into equol-an estrogen-like compound with neuroprotective properties (Hammond et al., 2023).

Meanwhile, XSB significantly suppressed the abundance of detrimental taxa including *Helicobacter rodentium* and *Lachnospiraceae* (Figure 4). *Helicobacter rodentium* has been associated with intestinal inflammation (Zhao B. et al., 2023), which may exacerbate neuroinflammation and cognitive deficits. Similarly, *Lachnospiraceae UCG-001* has been identified as a hub and keystone bacterium in APP/PS1 mice due to its role in quinolinic acid synthesis, a neurotoxic metabolite implicated in AD pathology (Li et al., 2023). Therefore, XSB-mediated regulation of the gut microbiota not only restores microbial homeostasis but also potentially reduces the production of pro-inflammatory metabolites while increasing neuroprotective metabolite levels.

mRNA-seq analysis revealed that XSB treatment modulates neuroactive ligand-receptor interactions, cytokine-cytokine receptor interactions, and the TNF signaling pathway, thereby restoring the disrupted hippocampal transcriptome. Additionally, Western blot and IF analyses demonstrated that XSB treatment significantly reduced TNF-α and IL-1β protein levels (Figure 8) while increasing BDNF, SYN, and PSD95 expression in the hippocampus of AD model mice (Figure 9). BDNF, a neurotrophin essential for the survival and function of serotonergic, hippocampal, and cortical neurons (Allen et al., 2013) is often reduced in AD, contributing to Aβ accumulation, tau phosphorylation, neuroinflammation, and neuronal apoptosis (Gao et al., 2022; Wang et al., 2023; Wang et al., 2019). Moreover, BDNF plays a crucial role in maintaining synaptic plasticity and cognitive function by promoting the expression of SYN and PSD95, two key proteins vital for synaptic integrity (Wang et al., 2022). Notably, XSB treatment significantly increased BDNF levels and enhanced synaptic density in the hippocampal CA1 region (Figure 9), suggesting that XSB may mitigate synaptic dysfunction and cognitive decline in AD by restoring synaptic integrity and neurotrophic support.

The integration of transcriptomics and metabolic analysis revealed that glycerophospholipid metabolism, linoleic acid metabolism, and the biosynthesis of unsaturated fatty acids are the key metabolic pathways regulated by XSB treatment. Glycerophospholipid metabolism is essential for maintaining neuronal membrane integrity, regulating inflammatory responses, and supporting mitochondrial function (Frisardi et al., 2011). Abnormalities in glycerophospholipid metabolism have been observed in APP/PS1 mice (Qian et al., 2023), where they directly exacerbate AD progression by promoting Aβ deposition and tau protein tangles (Tong et al., 2024), potentially linked to dysregulated gut microbiota and neuroinflammation (Qian et al., 2023; Tian et al., 2022). Linoleic acid, an essential omega-6 polyunsaturated fatty acid, is metabolized into various bioactive molecules, including arachidonic acid and eicosanoids, which can drive inflammatory responses (Alarcon-Gil et al., 2022). In AD, chronic neuroinflammation is a key driver of neuronal damage, and dysregulated fatty acid metabolism-particularly elevated levels of pro-inflammatory metabolites-further exacerbates this pathology. Correlation analysis revealed a significant association between these metabolites and gut microbiota composition (Figure 9), reinforcing the role of XSB in restoring both microbial and metabolic homeostasis. To determine whether the therapeutic effects of XSB are mediated through modulation of the gut microbiota, FMT experiments were performed. The results showed that XSB-FM significantly improved cognitive function (Figures 10B–E) and ameliorated intestinal barrier damage (Figures 10F–J) in AD model mice. These findings highlight gut microbiota remodeling as a critical mechanism underlying the therapeutic efficacy of XSB.

In summary, we systematically explored the potential mechanisms of XSB intervention in AD from three complementary omics perspectives-gut microbiota, metabolomics, and transcriptomics. The modulation of gut microbial composition, characterized by the enrichment of *Actinobacteriota* and *Eggerthellaceae* and the reduction of pathogenic bacteria such as *Helicobacter rodentium*, suggests that XSB may exert regulatory effects on the intestinal microenvironment. These microbial changes were accompanied by significant alterations in metabolites related to amino acid metabolism, bile acid metabolism, and neuroinflammation, including increased levels of Glycitein and Carglumic acid and decreased levels of pro-inflammatory metabolites such as L-3-Phenyllactic acid. These shifts in the metabolic landscape may, in turn, influence central nervous

system function, as evidenced by transcriptomic findings showing the involvement of key signaling pathways such as TNFα and neuroactive ligand-receptor interaction. Notably, the upregulation of BDNF, SYN, and PSD95 in the hippocampus indicates enhanced synaptic function and neuroplasticity. By integrating insights from these three omics layers, our findings suggest that XSB may ameliorate AD-related cognitive decline through a microbiota-metabolite-brain axis, ultimately improving neuroinflammatory status, neurotransmission, and synaptic integrity.

5 Conclusion

In conclusion, XSB significantly ameliorates scopolamine-induced cognitive impairment in AD model mice through a network of interconnected mechanisms. These include attenuating neuropathological damage, inhibiting neuroinflammation and oxidative stress, restoring cholinergic function, enhancing synaptic activity, and modulating gut microbiota composition. Notably, XSB affects key metabolic pathways, including the biosynthesis of unsaturated fatty acids, tyrosine metabolism, and glycerophospholipid metabolism. Integrated analyses of the gut microbiota, metabolomics, and transcriptomics indicate that XSB may exert its neuroprotective effects via the microbiota-metabolite-brain axis, thereby improving neuroinflammation, neurotransmission, and synaptic integrity. Collectively, these findings underscore the therapeutic potential of XSB as a multifactorial intervention for alleviating cognitive deficits in AD.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding authors.

Ethics statement

The animal study was approved by the Ethical Committee of Experimental Animal Welfare of Experimental Research Center China Academy of Chinese Medicine Science (ERCCACMS21-2201-01). The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

ZY: Methodology, Writing – original draft, Writing – review and editing. F-RZ: Data curation, Methodology, Writing – original draft, Writing – review and editing. LR: Data curation, Visualization, Writing – original draft, Writing – review and editing. J-MB: Data curation, Methodology, Writing – original draft, Writing – review and editing. S-CW: Data curation, Validation, Writing – original draft, Writing – review and editing. X-YL: Data curation, Software, Supervision, Writing – original draft, Writing – review and editing. H-JY: Project administration, Supervision, Writing – original draft, Writing – review and editing. H-HX: Conceptualization, Funding acquisition, Project administration, Supervision, Writing – original draft, Writing – review and editing.

Funding

The author(s) declare that financial support was received for the research and/or publication of this article. This work was financially supported by the National Natural Science Foundation of China (No. 82204648), Key Research and Development Project of Liaoning Province (2024JH2/102500064), Project Funded by China Postdoctoral Science Foundation (No. 2022M710706), Natural Science Foundation of Liaoning University of Traditional Chinese Medicine (2021LZY046), and the Natural Science Foundation of Liaoning Provincial Department of Education (LJ222410162067).

Conflict of interest

Author S-CW was employed by Zhangzhou Pien Tze Huang Pharmaceutical Co., Ltd.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declare that no Generative AI was used in the creation of this manuscript.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2025.1596728/full#supplementary-material

References

Alarcon-Gil, J., Sierra-Magro, A., Morales-Garcia, J. A., Sanz-SanCristobal, M., Alonso-Gil, S., Cortes-Canteli, M., et al. (2022). Neuroprotective and anti-inflammatory effects of linoleic acid in models of Parkinson's disease: the implication of lipid droplets and lipophagy. *Cells* 11, 2297. doi:10.3390/cells11152297

Allen, S. J., Watson, J. J., Shoemark, D. K., Barua, N. U., and Patel, N. K. (2013). GDNF, NGF and BDNF as therapeutic options for neurodegeneration. *Pharmacol. Ther.* 138, 155–175. doi:10.1016/j.pharmthera.2013.01.004

Chen, B. H., Park, J. H., Kim, D. W., Park, J., Choi, S. Y., Kim, I. H., et al. (2018). Melatonin improves cognitive deficits via restoration of cholinergic dysfunction in a mouse model of scopolamine-induced amnesia. *ACS Chem. Neurosci.* 9, 2016–2024. doi:10.1021/acschemneuro.7b00278

Chien, M. Y., Chuang, C. H., Chern, C. M., Liou, K. T., Liu, D. Z., Hou, Y. C., et al. (2016). Salvianolic acid A alleviates ischemic brain injury through the inhibition of inflammation and apoptosis and the promotion of neurogenesis in mice. *Free Radic. Biol. Med.* 99, 508–519. doi:10.1016/j.freeradbiomed.2016.09.006

Dong, M., Zhao, W., Hu, D., Ai, H., and Kang, B. (2017). N-terminus binding preference for either tanshinone or analogue in both inhibition of amyloid aggregation and disaggregation of preformed amyloid fibrils-toward introducing a kind of novel anti-Alzheimer compounds. *ACS Chem. Neurosci.* 8, 1577–1588. doi:10.1021/acschemneuro.7b00080

Frisardi, V., Panza, F., Seripa, D., Farooqui, T., and Farooqui, A. A. (2011). Glycerophospholipids and glycerophospholipid-derived lipid mediators: a complex meshwork in Alzheimer's disease pathology. *Prog. Lipid Res.* 50, 313–330. doi:10.1016/j.plipres.2011.06.001

Gao, L., Zhang, Y., Sterling, K., and Song, W. (2022). Brain-derived neurotrophic factor in Alzheimer's disease and its pharmaceutical potential. *Transl. Neurodegener.* 11, 4. doi:10.1186/s40035-022-00279-0

Golde, T. E., DeKosky, S. T., and Galasko, D. (2018). Alzheimer's disease: the right drug, the right time. *Science* 362, 1250–1251. doi:10.1126/science.aau0437

Hammond, T. C., Green, S. J., Jacobs, Y., Chlipala, G. E., Xing, X., Heil, S., et al. (2023). Gut microbiome association with brain imaging markers, APOE genotype, calcium and vegetable intakes, and obesity in healthy aging adults. *Front. Aging Neurosci.* 15, 1227203. doi:10.3389/fnagi.2023.1227203

Hampel, H., Mesulam, M. M., Cuello, A. C., Farlow, M. R., Giacobini, E., Grossberg, G. T., et al. (2018). The cholinergic system in the pathophysiology and treatment of Alzheimer's disease. *Brain* 141, 1917–1933. doi:10.1093/brain/awy132

Huang, D., Hu, Z., and Yu, Z. (2013). Eleutheroside B or E enhances learning and memory in experimentally aged rats. *Neural Regen. Res.* 8, 1103–1112. doi:10.3969/j.issn.1673-5374.2013.12.005

Jia, L., Du, Y., Chu, L., Zhang, Z., Li, F., Lyu, D., et al. (2020). Prevalence, risk factors, and management of dementia and mild cognitive impairment in adults aged 60 years or older in China: a cross-sectional study. *Lancet Public Health* 5, e661–e671. doi:10.1016/S2468-2667(20)30185-7

Jung, J. H., Kim, G., Byun, M. S., Lee, J. H., Yi, D., Park, H., et al. (2022). Gut microbiome alterations in preclinical Alzheimer's disease. *PLoS One* 17, e0278276. doi:10.1371/journal.pone.0278276

Lee, S., Kim, J., Seo, S. G., Choi, B. R., Han, J. S., Lee, K. W., et al. (2014). Sulforaphane alleviates scopolamine-induced memory impairment in mice. *Pharmacol. Res.* 85, 23–32. doi:10.1016/j.phrs.2014.05.003

Lee, J., Cho, E., Kwon, H., Jeon, J., Jung, C. J., Moon, M., et al. (2019). The fruit of Crataegus pinnatifida ameliorates memory deficits in β-amyloid protein-induced Alzheimer's disease mouse model. *J. Ethnopharmacol.* 243, 112107. doi:10.1016/j.jep.2019.112107

Li, Y., Chen, Y., Fan, Y., Chen, Y., and Chen, Y. (2023). Dynamic network modeling of gut microbiota during Alzheimer's disease progression in mice. *Gut Microbes* 15, 2172672. doi:10.1080/19490976.2023.2172672

Liu, J., Wang, Y., Guo, J., Sun, J., and Sun, Q. (2020). Salvianolic Acid B improves cognitive impairment by inhibiting neuroinflammation and decreasing Aβ level in Porphyromonas gingivalis-infected mice. *Aging* 12, 10117–10128. doi:10.18632/aging.103306

Liu, X. Q., Hu, T., Wu, G. L., Qiao, L. J., Cai, Y. F., Wang, Q., et al. (2024). Tanshinone IIA, the key compound in Salvia miltiorrhiza, improves cognitive impairment by upregulating Aβ-degrading enzymes in APP/PS1 mice. *Int. J. Biol. Macromol.* 254, 127923. doi:10.1016/j.ijbiomac.2023.127923

Qian, X., Hai, W., Chen, S., Zhang, M., Jiang, X., and Tang, H. (2023). Multi-omics data reveals aberrant gut microbiota-host glycerophospholipid metabolism in association with neuroinflammation in APP/PS1 mice. *Gut Microbes* 15, 2282790. doi:10.1080/19490976.2023.2282790

Song, C., Duan, F., Ju, T., Qin, Y., Zeng, D., Shan, S., et al. (2022). Eleutheroside E supplementation prevents radiation-induced cognitive impairment and activates PKA signaling via gut microbiota. *Commun. Biol.* 5, 680. doi:10.1038/s42003-022-03602-7

Tang, K. S. (2019). The cellular and molecular processes associated with scopolamine-induced memory deficit: a model of Alzheimer's biomarkers. *Life Sci.* 233, 116695. doi:10.1016/j.lfs.2019.116695

Tian, T., Mao, Q., Xie, J., Wang, Y., Shao, W. H., Zhong, Q., et al. (2022). Multi-omics data reveals the disturbance of glycerophospholipid metabolism caused by disordered gut microbiota in depressed mice. *J. Adv. Res.* 39, 135–145. doi:10.1016/j.jare.2021.10.002

Tong, B., Ba, Y., Li, Z., Yang, C., Su, K., Qi, H., et al. (2024). Targeting dysregulated lipid metabolism for the treatment of Alzheimer's disease and Parkinson's disease: current advancements and future prospects. *Neurobiol. Dis.* 196, 106505. doi:10.1016/j.nbd.2024.106505

Wang, Z. H., Xiang, J., Liu, X., Yu, S. P., Manfredsson, F. P., Sandoval, I. M., et al. (2019). Deficiency in BDNF/TrkB neurotrophic activity stimulates δ-secretase by upregulating C/EBPβ in Alzheimer's disease. *Cell Rep.* 28, 655–669.e5. doi:10.1016/j.celrep.2019.06.054

Wang, R., Chen, T., Wang, Q., Yuan, X. M., Duan, Z. L., Feng, Z. Y., et al. (2021). Total flavone of abelmoschus manihot ameliorates stress-induced microbial alterations drive intestinal barrier injury in DSS colitis. *Drug Des. Devel Ther.* 15, 2999–3016. doi:10.2147/DDDT.S313150

Wang, C. S., Kavalali, E. T., and Monteggia, L. M. (2022). BDNF signaling in context: from synaptic regulation to psychiatric disorders. *Cell* 185, 62–76. doi:10.1016/j.cell.2021.12.003

Wang, W., Li, Y., Ma, F., Sheng, X., Chen, K., Zhuo, R., et al. (2023). Microglial repopulation reverses cognitive and synaptic deficits in an Alzheimer's disease model by restoring BDNF signaling. *Brain Behav. Immun.* 113, 275–288. doi:10.1016/j.bbi.2023.07.011

Wang, L., Cao, L., Yu, Q., Liang, M., Yang, Z., Wang, G., et al. (2025). Bifidobacterium bifidum CCFM1359 alleviates intestinal motility disorders through the BDNF-TrkB pathway. *Food Funct.* 16, 437–451. doi:10.1039/d4fo03710c

Xiao, H., Li, H., Song, H., Kong, L., Yan, X., Li, Y., et al. (2020). Shenzao jiannao oral liquid, an herbal formula, ameliorates cognitive impairments by rescuing neuronal death and triggering endogenous neurogenesis in AD-like mice induced by a combination of Aβ42 and scopolamine. *J. Ethnopharmacol.* 259, 112957. doi:10.1016/j.jep.2020.112957

Xiao, H. H., Zhang, F. R., Li, S., Guo, F. F., Hou, J. L., Wang, S. C., et al. (2024). Xinshubao tablet rescues cognitive dysfunction in a mouse model of vascular dementia: involvement of neurogenesis and neuroinflammation. *Biomed. Pharmacother.* 172, 116219. doi:10.1016/j.biopha.2024.116219

Xu, Y. M., Wang, X. C., Xu, T. T., Li, H. Y., Hei, S. Y., Luo, N. C., et al. (2019). Kai Xin san ameliorates scopolamine-induced cognitive dysfunction. *Neural Regen. Res.* 14, 794–804. doi:10.4103/1673-5374.249227

Yin, X., Liu, W., Feng, H., Huang, J., Wang, Q., Zhang, Q., et al. (2024). Bifidobacterium animalis subsp. lactis A6 attenuates hippocampal damage and memory impairments in an ADHD rat model. *Food Funct.* 15, 2668–2678. doi:10.1039/d3fo04665f

Yun, D., Wang, Y., Zhang, Y., Jia, M., Xie, T., Zhao, Y., et al. (2022). Sesamol attenuates scopolamine-induced cholinergic disorders, neuroinflammation, and cognitive deficits in mice. *J. Agric. Food Chem.* 70, 13602–13614. doi:10.1021/acs.jafc.2c04292

Zhao, B., Osbelt, L., Lesker, T. R., Wende, M., Galvez, E. J. C., Hönicke, L., et al. (2023). Helicobacter spp. are prevalent in wild mice and protect from lethal Citrobacter rodentium infection in the absence of adaptive immunity. *Cell Rep.* 42, 112549. doi:10.1016/j.celrep.2023.112549

Zhao, T., Huang, H., Li, J., Shen, J., Zhou, C., Xiao, R., et al. (2023). Association between erythrocyte membrane fatty acids and gut bacteria in obesity-related cognitive dysfunction. *Amb. Express* 13, 148. doi:10.1186/s13568-023-01655-3

Glossary

AD	Alzheimer's Disease
VaD	Vascular Dementia
XSB	Xinshubao Tablet
Aβ	Amyloid-Beta
NFTs	Neurofibrillary Tangles
H&E	Hematoxylin-Eosin
NOR	Novel Object Recognition
MWM	Morris Water Maze
TCM	Traditional Chinese Medicine
TEM	Transmission Electron Microscopy
mRNA-seq	mRNA Sequencing
ACh	Acetylcholine
AChE	Acetylcholinesterase
CHAT	Choline Acetyltransferase
CHT1	Choline Transporter 1
BDNF	Brain-Derived Neurotrophic Factor
PSD95	Postsynaptic Density Protein 95
AB-PAS	Alcian blue-periodic acid-Schiff
SYN	Synaptophysin
TNF-α	Tumor Necrosis Factor-Alpha
IL-1β	Interleukin-1 Beta
MPO	Myeloperoxidase
SOD	Superoxide Dismutase
GSH	Reduced Glutathione
MDA	Malondialdehyde
DEGs	Differentially Expressed Genes
DMs	Differential Metabolites
GO	Gene Ontology
KEGG	Kyoto Encyclopedia of Genes and Genomes
LC-MS	Liquid Chromatography-Mass Spectrometry
LEfSe	Linear Discriminant Analysis Effect Size
PLS-DA	Partial Least Squares Discriminant Analysis
SEM	Standard Error of the Mean
ANOVA	Analysis of Variance.
ZO-1	Zonula Occludens-1

frontiers | Frontiers in Pharmacology

TYPE Original Research
PUBLISHED 10 July 2025
DOI 10.3389/fphar.2025.1582644

Check for updates

OPEN ACCESS

EDITED BY
Bin Yu,
Nanjing University of Chinese Medicine, China

REVIEWED BY
Mamunur Rashid,
University of Nebraska Medical Center, United States
Serban Moldoveanu,
Independent Researcher, Greensboro, United States

*CORRESPONDENCE
Hong Ma,
redlemononly@icloud.com
Ling Yang,
yling@shutcm.edu.cn

†These authors have contributed equally to this work

RECEIVED 24 February 2025
ACCEPTED 26 June 2025
PUBLISHED 10 July 2025

CITATION
Hu C, Wang Y, Liu Y, Wang X, Song P, Ma H and Yang L (2025) Pharmacokinetics and tissue distribution analysis of ginsenoside Rh_3 in rats using a novel LC-MS/MS quantification strategy. *Front. Pharmacol.* 16:1582644. doi: 10.3389/fphar.2025.1582644

COPYRIGHT
© 2025 Hu, Wang, Liu, Wang, Song, Ma and Yang. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

Pharmacokinetics and tissue distribution analysis of ginsenoside Rh_3 in rats using a novel LC-MS/MS quantification strategy

Cong Hu[1,2†], Yuheng Wang[3†], Yu Liu[1], Xiaojing Wang[1], Peifang Song[1], Hong Ma[1*] and Ling Yang[1,2*]

[1]Key Laboratory of Basic Pharmacology of Ministry of Education and Joint International Research Laboratory of Ethnomedicine of Ministry of Education, Zunyi Medical University, Zunyi, China, [2]Institute of Interdisciplinary Integrative Medicine Research, Shanghai University of Traditional Chinese Medicine, Shanghai, China, [3]Department of Neurology, The First Affiliated Hospital, Dalian Medical University, Dalian, China

Introduction: Ginsenoside Rh3 (GRh_3), a rare ginsenoside, demonstrates diverse pharmacological activities *in vitro;* however, the lack of pharmacokinetic and tissue distribution data has limited its translation to *in vivo* applications. This study aimed to develop and validate a novel liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for quantifying GRh_3 in rat biological matrices and to characterize its pharmacokinetic profile and tissue distribution following oral administration.

Methods: A validated LC-MS/MS method was established for the quantification of GRh_3 in rat plasma and tissues. Male Sprague-Dawley rats received an oral dose of GRh_3 (100 mg/kg), and plasma samples were collected up to 72 h post-dose for pharmacokinetic analysis. Tissue samples (intestine, stomach, liver, brain, etc.) were collected at the time corresponding to the maximum plasma concentration for distribution analysis.

Results: The LC-MS/MS method showed excellent precision, accuracy, and extraction recovery (≥ 85%), with minimal matrix effects. GRh_3 exhibited a prolonged elimination half-life (14.7 ± 1.7 h), a low clearance rate (13.0 ± 3.8 L/h/kg), and a high volume of distribution (280.4 ± 109.3 L/kg). Tissue distribution analysis revealed the highest GRh_3 concentrations in the intestine (15445.2 ng/g), followed by the stomach (2906.7 ng/g) and liver (1930.8 ng/g). Notably, GRh_3 was able to cross the blood-brain barrier, with significant accumulation observed in the hippocampus (520.0 ng/g).

Discussion: The prolonged elimination and extensive tissue distribution of GRh_3, particularly its ability to penetrate the brain, indicate potential therapeutic benefits or neurotoxic risks involving the central nervous system. The mechanism underlying its blood-brain barrier permeability warrants further investigation, potentially involving transporter-mediated uptake or modulation of barrier integrity. These findings provide a foundation for optimizing GRh_3 dosing regimens and guiding future preclinical studies.

KEYWORDS

GRh_3, pharmacokinetics, tissue distribution, blood-brain barrier, LC-MS/MS

1 Introduction

Ginseng (*Panax ginseng* C. A. Meyer) is a quintessential traditional medicinal plant. It has held pivotal roles in both the traditional Chinese medicine theory of "invigorating qi for relieving desertion" and modern medical systems, owing to its multifaceted therapeutic properties (Yun, 2001). As one of the most commercially valuable herbs globally, *ginseng* is also widely utilized in dietary supplements, functional foods, and alternative medicine (Baeg and So, 2013; Eom et al., 2017). Ginsenosides, characterized by their extensive pharmacological activities, are deemed the key bioactive compounds in ginseng (Hao et al., 2025). These can be classified into two major categories, prototype ginsenosides and rare ginsenosides, based on their natural abundance and the number of glycosyl substitutions (Gan et al., 2024). Among these, rare ginsenosides, which undergo structural modifications such as deglycosylation or hydroxylation, exhibit enhanced multi-target activity. These modifications contribute to their significant advantages in antitumor effects, neuroprotective effects, metabolic regulation, and organ-protective functions (Shah et al., 2023; Shan et al., 2023; Shang et al., 2025; Zare-Zardini et al., 2024).

Ginsenoside Rh_3 (GRh_3), as a secondary metabolite of ginsenoside Rg_5, is an important member of the rare ginsenoside family (Lee et al., 2015). Recent studies have progressively revealed its multifaceted pharmacological effects. Experimental evidence demonstrates that GRh_3 protects endometrial cells against oxygen-glucose deprivation-reperfusion (OGDR)-induced oxidative damage by activating the Nrf2 signaling pathway, and attenuating reactive oxygen species (ROS) generation and lipid peroxidation while preserving mitochondrial membrane potential stability (Wang et al., 2020). In metabolic regulation, GRh_3 exerts systemic protective effects on hepatic function by modulating critical targets including epidermal growth factor receptor (EGFR), steroid receptor coactivator (SRC) and mitogen activated protein kinase (MAPK) 1 to ameliorate hepatic insulin resistance, while concurrently influencing forkhead box O (FOXO), peroxisome proliferator activated receptor (PPAR), and interleukin-17 (IL-17) signaling pathways (Wang et al., 2024). In the neuroprotective domain, GRh_3 significantly alleviates memory dysfunction by upregulating hippocampal brain-derived neurotrophic factor (BDNF) expression and enhancing cAMP response element-binding protein (CREB) phosphorylation (Wu et al., 2023). Besides, GRh_3 exhibits substantial anticancer potential through induction of pyroptosis and ferroptosis in colorectal cancer cells via the Stat3/p53/NRF2 axis (Kim et al., 2013).

Although numerous *in vitro* studies have confirmed the multifaceted pharmacological effects of GRh_3, the absence of comprehensive data on its pharmacokinetics (PK) and tissue distribution has hindered the effective translation of these pharmacological findings into *in vivo* efficacy research. To date, only one study has reported the plasma PK profile of GRh_3 in rats (Yang et al., 2022). However, the extraction recovery rate of the analytical method used in that study failed to meet bioanalytical regulatory standards (EMA, 2011; FDA, 2018), and the short sampling duration (with the last time-point blood concentration exceeding 10% of C_{max}) in PK analysis compromised the accuracy of PK parameters (CDE, 2005). These limitations necessitate the redevelopment of a validated analytical method and subsequent reinvestigation of GRh_3's PK characteristics. Furthermore, the tissue distribution of GRh_3 has not been investigated, resulting a significant gap in our understanding. Both PK and tissue distribution data are essential for advancing GRh_3 development in pharmacodynamic exposure correlation analysis, toxicity risk assessment, and optimization of dosing strategies.

To address these challenges, this study successfully developed and validated a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for detecting GRh_3 concentration in rat plasma and tissues. Using this method, a comprehensive investigation was conducted on the PK and tissue distribution of GRh_3 following oral administration in rats. This work provides a critical foundation for the *in vivo* research and pharmaceutical development of GRh_3.

2 Materials and methods

2.1 Chemicals and reagents

GRh_3 and GRh_4 (internal standard, IS) were obtained from Alfa Biotechnology Co., Ltd. (Chengdu, China). Both compounds had a purity greater than 98% and were of analytical grade. Acetonitrile and formic acid (HPLC grade) were obtained from Merck (Darmstadt, Germany). Ultrapure water was generated using a Millipore water purification system (Billerica, United States). Isoflurane was obtained from RWD Life Science Co., Ltd. (Shenzhen, China).

2.2 Animals

Male Sprague-Dawley rats (SPF grade, 200 ± 20 g) were procured from Charles River Laboratories (Zhejiang, China). The rats were acclimatized for 1 week under a 12 h light-dark cycle and were fasted for 12 h prior to GRh_3 administration while being allowed free access to water. The study was approved by the Animal Experimentation Committee of Zunyi Medical University (Approval No. ZMU21-2403-453).

2.3 Pharmacokinetics experiment

Seven male Sprague-Dawley rats were orally administered GRh_3 (100 mg/kg) suspended in 0.5% carboxymethyl cellulose sodium (CMC-Na). Blood samples were collected from the orbital sinus at the following time points: 0, 0.083, 0.249, 0.498, 0.747, 1, 2, 4, 6, 8, 12, 24, 36, 48, 60, and 72 h post-dose into polypropylene tubes containing K_2EDTA. The samples were subsequently centrifuged at 3000 × *g* for 20 min at 4°C, and the supernatant (plasma) was collected for PK analysis.

2.4 Tissue distribution experiment

Seven male Sprague-Dawley rats were orally administered GRh_3 (100 mg/kg) suspended in 0.5% CMC-Na. At T_{max} (the time corresponding to maximum plasma GRh_3 concentration), the

rats were euthanized and perfused with physiological saline via cardiac perfusion to minimize blood contamination in tissue samples. Subsequently, various tissues, including the liver, intestine, stomach, kidney, lung, heart, spleen, hippocampus, cerebral cortex, and brainstem were excised. Among these, the brain tissue sampling protocol followed previously published methods (Aboghazleh et al., 2024): target regions, including the cortex, hippocampus, and brainstem, were precisely dissected according to standard anatomical landmarks on a low-temperature dissection platform. To avoid cross-contamination between different brain regions, independent surgical instruments were used for each region, and all tools were disinfected and cleaned before and after sampling. The excised tissues were weighed and homogenized in PBS (0.1 M, pH 7.4) at 4°C using an automated tissue homogenizer (Shanghai Jingxin Industrial Development Co., Ltd., Shanghai, China). Tissue homogenates were prepared at a final concentration of 0.2 g/mL.

2.5 Preparation of calibration and quality control samples

Stock solutions of GRh_3 and IS were prepared at 2.0 mg/mL in acetonitrile. The GRh_3 stock solution was serially diluted with acetonitrile to produce standard calibration and quality control (QC) working solutions. 200 ng/mL IS working solution was obtained by diluting the IS stock solution with acetonitrile. 190 μL of blank rat plasma or blank liver homogenate was spiked with 10.0 μL GRh_3 working solution to acquire 25 ng/mL (LLOQ), 50 ng/mL, 125 ng/mL, 250 ng/mL, 500 ng/mL, 2,000 ng/mL, 4,000 ng/mL, and 5000 ng/mL (ULOQ) for standard calibration samples, and 25 ng/mL (LLOQ QC), 100 ng/mL (LQC), 400 ng/mL (MQC), and 3000 ng/mL (HQC) for the QC samples.

2.6 Sample processing

40 μL of calibration samples, QC samples, or test samples were mixed with 160 μL of the IS working solution (200 ng/mL) and vortexed. The mixture was then centrifuged at 12,000 × *g* for 10 min at 4°C. Subsequently, 100 μL of the supernatant was collected and mixed with an equal volume of purified water. A 10 μL aliquot of this mixture was then injected into the LC-MS/MS system for analysis.

2.7 LC-MS/MS analysis

Sample analysis was conducted using an LC-MS/MS system, which consisted of a Shimadzu LC 20A liquid chromatograph (Kyoto, Japan) coupled with an Applied Biosystems Sciex Qtrap 4500 mass spectrometer (Massachusetts, United States). Chromatographic separation was performed on a Shimadzu Shim-pack GIST-HP C18 column (2.1 mm × 50 mm, 3 μm). The mobile phase consisted of (A) 0.1% formic acid in water and (B) acetonitrile. The gradient elution program was as follows: 0.0–1.0 min, 30%–98% B; 1.0–3.5 min, 98% B; 3.5–3.6 min, 98%–30% B; 3.6–5.0 min, 30% B, with a flow rate of 0.4 mL/min.

Mass spectrometric analysis was conducted in negative ion mode using multiple reaction monitoring (MRM). The ion source temperature was set to 550°C, with an ion spray voltage of −4500 V. Nebulizer gas (GAS1) and auxiliary heating gas (GAS2) were both set to 60 psi, while the curtain gas was set to 20 psi, all using nitrogen. Quantification was performed using the following ion transitions: m/z 649.6 > 603.1 for GRh_3, with a declustering potential (DP) of −50.0 V and collision energy (CE) of −26.0 eV. For the IS, the ion transition was 665.4 > 619.4, with DP at −50.0 V and CE at −30.0 eV.

2.8 Method validation

The LC-MS/MS method for quantifying GRh_3 in rat plasma and tissues was validated according to the guidelines for *Bioanalytical Method Validation* issued by the FDA and the European Medicines Agency (EMEA) (EMA, 2011; FDA, 2018). Considering the liver's prominent metabolic activity and pronounced matrix effects, it was selected as the representative tissue for methodological validation in this study. The validation parameters encompassed selectivity, carryover, linearity, precision, accuracy, matrix effects, extraction recovery, and sample stability.

2.8.1 Selectivity

Selectivity was assessed based on matrix selectivity (endogenous interference), interference of the GRh_3 with the IS, and interference of the IS with GRh_3.

2.8.1.1 Matrix selectivity

This was evaluated using double blank samples (without GRh_3 and IS) and LLOQ samples prepared from blank matrices of six individual rats. The peak areas for GRh_3 and IS at their respective retention times in the double blank samples should not exceed 20.0% and 5.0%, respectively, of the corresponding peak areas in the LLOQ samples.

2.8.1.2 Interference of GRh_3 with IS

Six samples containing only GRh_3 (ULOQ without IS) were prepared. The average peak area at the IS retention time in these samples should not exceed 5.0% of the average peak area of the IS in standard calibration and QC samples from the same analytical batch.

2.8.1.3 Interference of IS with GRh3

Six samples containing only the IS (QC0) were prepared. The average peak area at GRh_3 retention time in these samples should not exceed 20.0% of the average peak area of GRh_3 in LLOQ samples from the same analytical batch.

2.8.2 Carryover

Carryover was evaluated by injecting double blank samples after ULOQ samples (carryover samples). The peak area of GRh_3 in carryover samples should not exceed 20.0% of the mean peak area in LLOQ samples. Similarly, the peak area of IS in carryover samples should not exceed 5.0% of the mean peak area in standard calibration and QC samples.

2.8.3 Linearity

The calibration curve was constructed by performing linear regression of the GRh_3-to IS peak area ratios (y) against the nominal concentrations of GRh_3 (x), using a $1/x^2$ weighting factor. The measured concentrations at each calibration samples should deviate from the theoretical values by ± 15.0% (±20.0% for LLOQ), and the correlation coefficient (r) should be ≥ 0.99. A minimum of six concentration points, including LLOQ and ULOQ, were used to construct the calibration curve.

2.8.4 Precision and accuracy

Precision and accuracy were assessed at four levels (LLOQ QC, LQC, MQC, and HQC), with six replicates per level, prepared independently over three consecutive days. Precision was evaluated by calculating the relative standard deviation (R.S.D%) of the measured concentrations for the replicate samples. Accuracy was assessed by comparing the ratio of the measured to the theoretical concentrations (%). The ratio should fall within 85.0%–115.0% (80.0%–120.0% for LLOQ QC), and the R.S.D% should not exceed 15.0% (20.0% for LLOQ QC).

2.8.5 Extraction recovery and matrix effect

Extraction recovery and matrix effect were evaluated at three levels (LQC, MQC, and HQC), with six replicates. For extraction recovery evaluating, the QC samples prepared for precision and accuracy assessments were used as test samples, while blank plasma and blank tissue homogenate extracts spiked with GRh_3 and IS served as the basic samples. For matrix effect assessment, basic samples for extraction recovery were used as test samples, and PBS (as a surrogate matrix) spiked with GRh_3 and IS post-extraction served as the basic samples. The absolute extraction recovery and absolute matrix effect for GRh_3 and IS should have an R.S.D% not exceeding 15.0%. The IS-normalized extraction recovery and IS-normalized matrix effect should be within 85%–115%, with an R.S.D % not exceeding 15.0%.

2.8.6 Sample stability

Sample stability was assessed under the following conditions: 3 h at room temperature under white light, 30 days at −80°C, and three freeze-thaw cycles (from −80°C to room temperature). This assessment was performed at three concentration levels (LQC, MQC, and HQC), with six replicates. The acceptance criteria are that the relative error (R.E%) between the measured and nominal concentrations should be within ± 15.0%, and the R.S.D% should not exceed 15.0%.

2.8.7 Post-preparation sample stability

Post-preparation stability was assessed at three levels (LQC, MQC, and HQC), with six replicates, under the following conditions: 3 h at room temperature under white light, 12 h in an autosampler at 8°C, and 2 days in a refrigerator at −20°C. The acceptance criteria for this evaluation are consistent with those applied in the sample stability assessments.

2.8.8 Statistical analysis

LC-MS/MS data acquisition and peak integration were performed using Analyst™ software (AB Sciex, version 1.6.3). The concentrations of GRh_3 in plasma and tissues were calculated based on the calibration curve. PK parameters, including the area under the concentration-time curve (AUC), mean residence times (MRT), elimination half-life ($T_{1/2}$), peak plasma concentration (C_{max}), volume of distribution (V_d), T_{max}, and clearance rate (CL) were calculated using Phoenix WinNonlin 8.1 (Certara, New Jersey, United States). Data were presented as mean ± standard deviation (SD) and visualized using GraphPad Prism software (version 9.5.1, San Diego, CA, United States).

3 Results and discussion

3.1 Selectivity

Double-blank plasma and liver samples were analyzed, showing no detectable GRh_3 or IS in either matrix. In Figure 1, MRM chromatograms at the LLOQ level for both matrices display well-defined peaks for GRh_3 and IS, with no evidence of matrix interference. Figure 2 shows MRM chromatograms of ULOQ without IS samples in plasma and liver homogenate, where no IS peaks were detected. Figure 3 presents QC0 samples in the same matrices, confirming the absence of GRh_3. These findings collectively illustrate that the analytical method is no matrix interference, and mutual non-interference between GRh_3 and IS. Overall, this method demonstrates excellent selectivity.

3.2 Carryover

Carryover samples from plasma and liver were analyzed. Neither GRh_3 nor GRh_4 were detected, indicating no carryover interference in this analytical method.

3.3 Linearity

The deviation between calculated and nominal concentrations of standard calibration samples was within ± 15.0% (±20.0% for LLOQ). Figure 4 presents representative calibration curves for plasma and tissue, while Table 1 summarizes linearity assessment results from three analytical batches for precision and accuracy evaluations. GRh_3 showed excellent linearity within the concentration range of 25.0–5000 ng/mL, with correlation coefficients (r) exceeding 0.99.

3.4 Precision and accuracy

The intra-day and inter-day precision and accuracy results are detailed in Table 2. At four QC levels, the intra-day accuracies for plasma and liver were within 89.3%–112.3% and 85.9%–112.5%, respectively, with intra-day precisions (R.S.D%) being less than 10.7% and 12.8%, respectively. The inter-day accuracies for plasma and liver were within 90.2%–102.0% and 89.3%–105.9%, respectively, with inter-day precisions (R.S.D%) being less than 10.6% and 12.2%, respectively. These results indicate that the

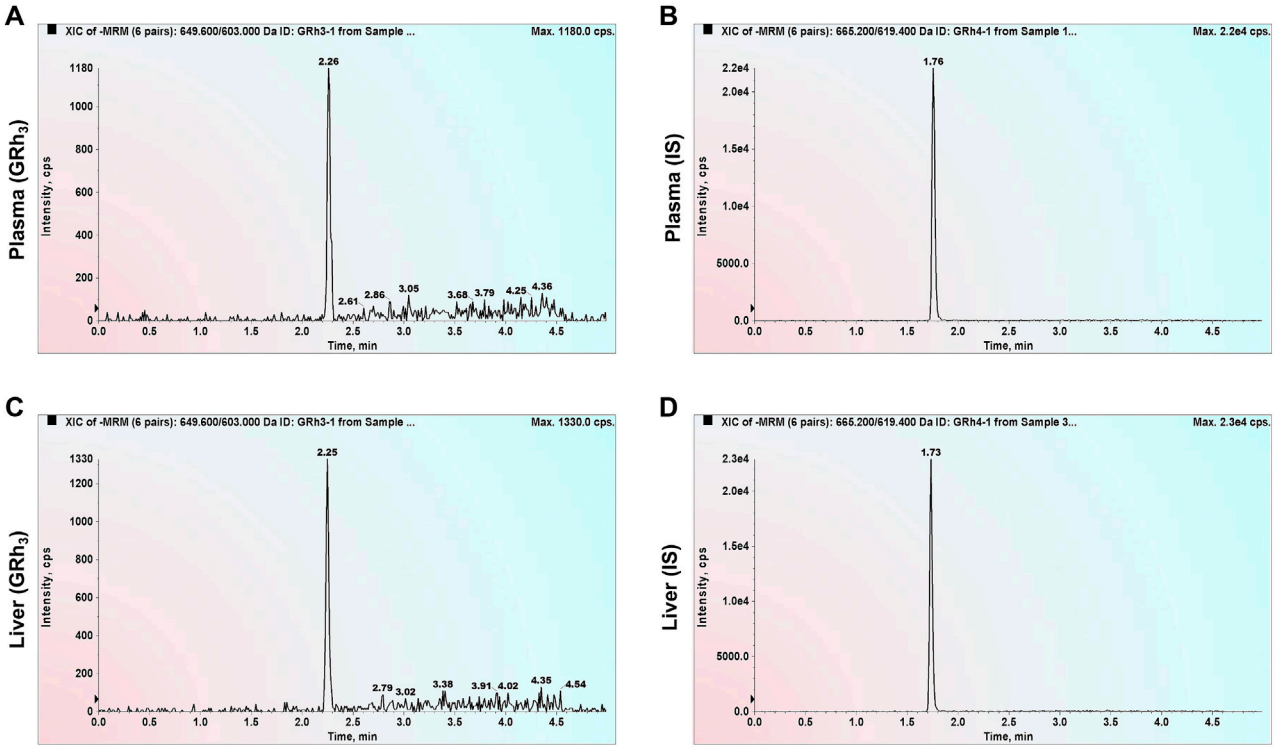


FIGURE 1
MRM chromatograms of LLOQ samples. **(A)** GRh_3 in plasma. **(B)** IS in plasma. **(C)** GRh_3 in liver. **(D)** IS in liver.

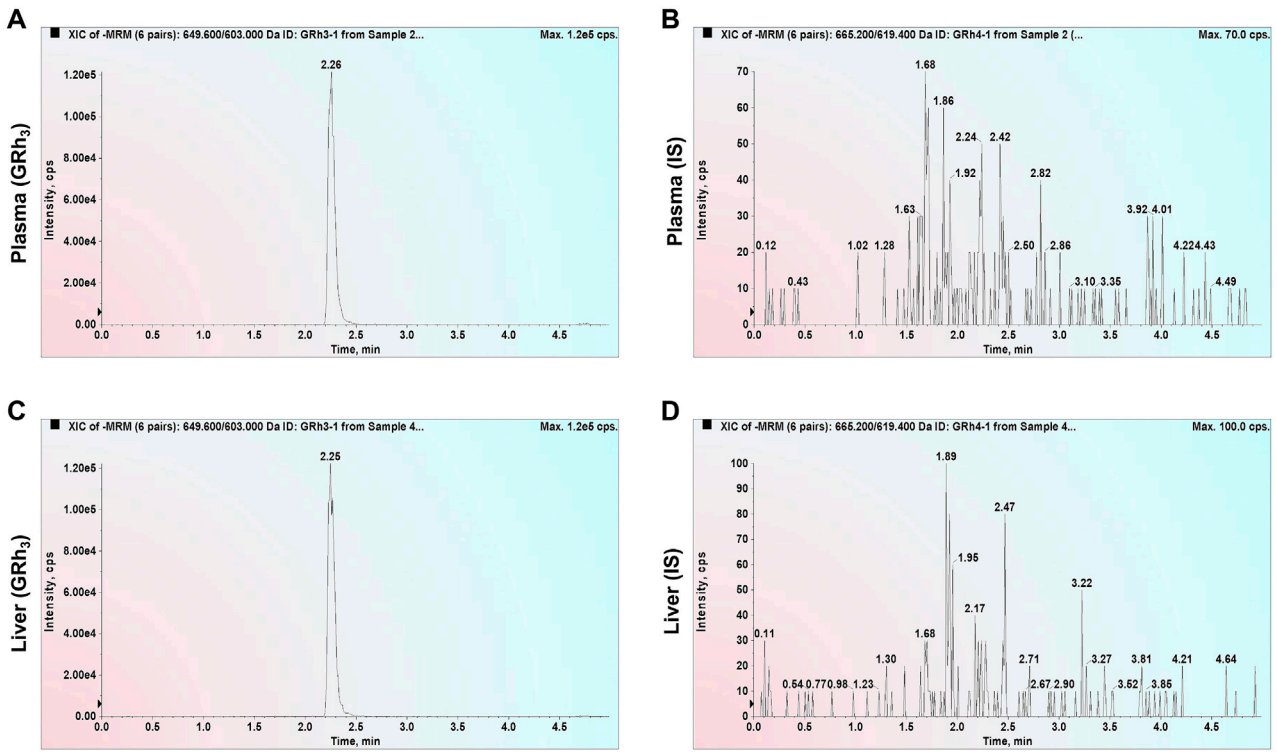


FIGURE 2
MRM chromatograms of ULOQ without IS samples. **(A)** GRh_3 in plasma. **(B)** IS in plasma. **(C)** GRh_3 in liver. **(D)** IS in liver.

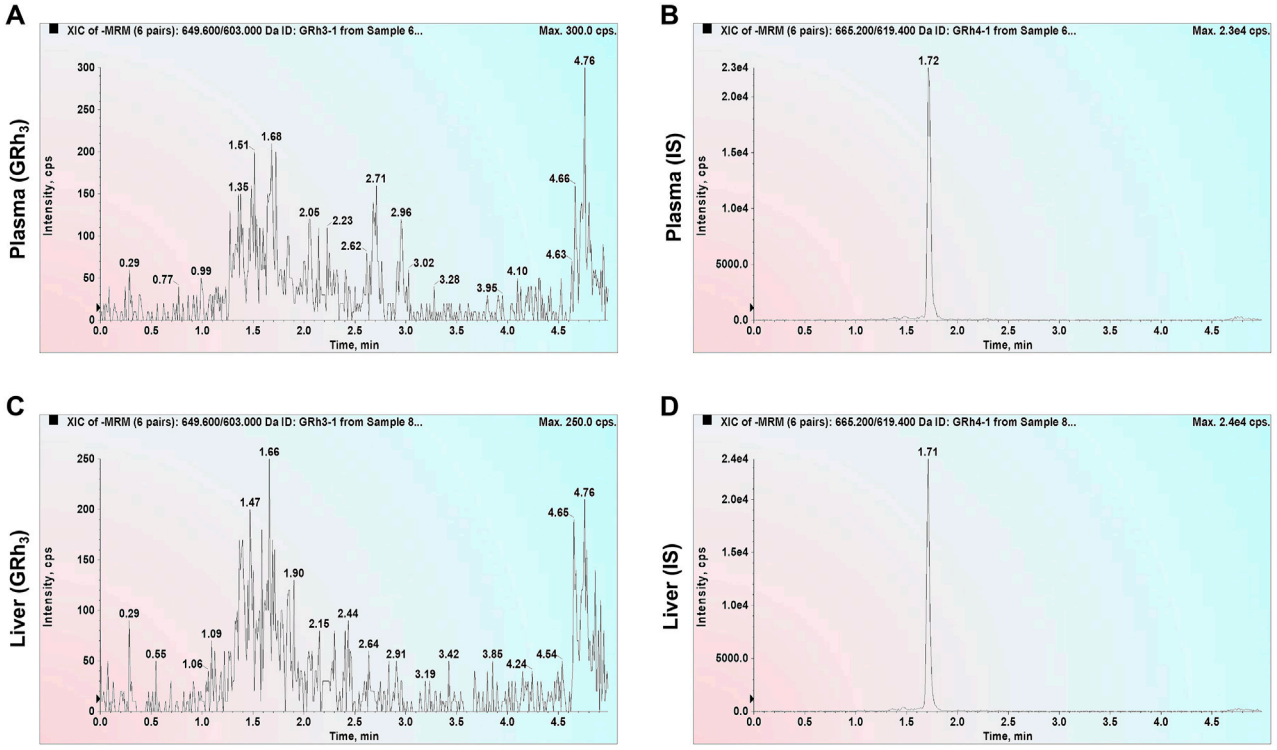


FIGURE 3
MRM chromatograms of QC0 samples. **(A)** GRh_3 in plasma. **(B)** IS in plasma. **(C)** GRh_3 in liver. **(D)** IS in liver.

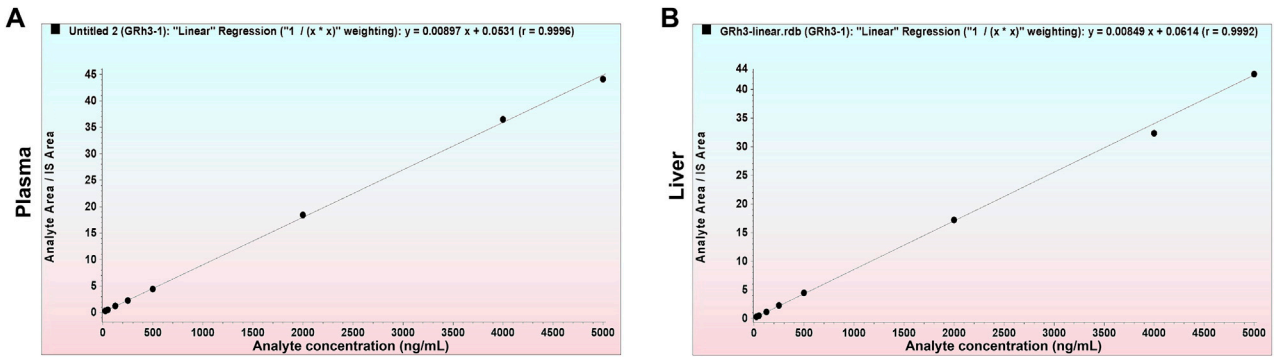


FIGURE 4
Representative calibration curves. **(A)** GRh_3 in plasma. **(B)** GRh_3 in liver.

TABLE 1 Linear parameters for precision and accuracy evaluation batches.

Matrix	Range (ng/mL)	Batch	a (slope)	b (intercept)	*r*
Plasma	25.0–5000	A&P-1	0.008971	0.05312	0.9996
		A&P-2	0.008651	0.05693	0.9989
		A&P-3	0.008748	0.05010	0.9992
Liver	25.0–5000	A&P-1	0.008493	0.06136	0.9992
		A&P-2	0.008812	0.04989	0.9994
		A&P-3	0.007643	0.03514	0.9983

TABLE 2 Intra-day and inter-day accuracy and precision of GRh_3.

Matrix	Nominal concentration (ng/mL)	Intra-day (n = 6)		Inter-day (n = 18)	
		Accuracy (%)	Precision R.S.D. (%)	Accuracy (%)	Precision R.S.D. (%)
Plasma	25	89.6–112.3	4.3–10.7	90.2	10.6
	100	90.1–101.1	2.9–8.4	101.4	8.7
	400	93.4–103.2	5.8–6.4	102.0	6.3
	3000	89.3–111.0	3.9–7.0	98.2	8.3
Liver	25	85.9–109.7	6.4–12.8	89.3	12.2
	100	91.6–102.6	7.1–9.9	97.7	7.3
	400	94.0–110.2	4.5–11.0	100.6	8.8
	3000	90.3–112.5	3.7–9.7	105.9	9.2

TABLE 3 Extraction recoveries and matrix effects of GRh_3 and GRh_4 (IS) in plasma and liver homogenate (n = 6).

Matrix	Nominal concentration (ng/mL)	Absolutely				IS normalization	
		Extraction recovery (%)	R.S.D. (%)	Matrix effect (%)	R.S.D. (%)	Extraction recovery (%)	Matrix effect (%)
Plasma	100	93.2	10.0	98.7	8.2	96.4	97.9
	400	95.6	5.8	101.6	6.7	98.9	101.3
	3000	97.1	7.2	103.5	7.6	100.4	103.5
Liver	100	92.4	9.5	100.3	8.8	98.1	102.2
	400	93.6	8.5	94.3	7.6	99.4	96.1
	3000	98.7	6.3	97.6	4.2	104.8	99.5
Plasma-IS	50000	96.7	9.6	97.9	6.7	—	—
Liver-IS	50000	94.2	11.3	98.1	8.3	—	—

method provides excellent precision and accuracy for the quantification of GRh_3 in plasma and tissue samples.

3.5 Extraction recovery and matrix effect

The extraction recovery and matrix effect were assessed using three QC concentrations, as detailed in Table 3. For plasma, GRh_3 demonstrated an extraction recovery ranging from 93.2% to 97.1% (R.S.D% < 10.0%), whereas the IS exhibited an extraction recovery of 96.7% (R.S.D% = 9.6%). Following IS normalization, the corrected extraction recoveries for GRh_3 were between 96.4% and 100.4%. In liver tissue, GRh_3 showed an extraction recovery range of 92.4%–98.7% (R.S.D% < 9.5%), with the IS displaying an extraction recovery of 94.2% (R.S.D% = 11.3%). Post-IS correction, the adjusted extraction recoveries for GRh_3 were between 98.1% and 104.8%. Regarding matrix effects in plasma, GRh_3 values spanned 98.7%–103.5% (R.S.D% < 8.2%), while the IS matrix effect was 97.9% (R.S.D% = 6.7%). After IS correction, the adjusted matrix effects for GRh_3 were between 97.9% and 103.5%. In liver tissue, GRh_3 matrix effects ranged from 94.3% to 100.3% (R.S.D% < 8.8%), with the IS matrix effect being 98.1% (R.S.D% = 8.3%). Post-IS correction, the adjusted matrix effects for GRh_3 were between 96.1% and 102.2%.

3.6 Sample stability

Collected samples are not immediately processed for analysis but are temporarily stored at −80°C until needed, at which point they are thawed and brought to room temperature for sample preparation. To ensure the reliability and precision of analytical outcomes, it is crucial to assess the stability of these samples under various conditions. The assessment results, as detailed in Table 4, reveal that plasma and liver homogenate with three QC concentrations maintain their stability under diverse conditions: when kept at room temperature for 3 h (R.E%: −4.3%–5.7%, R.S.D % < 9.2%), stored at −80°C for up to 30 days (R.E%: −6.9% to −4.2%, R.S.D% < 9.7%), and subjected to three freeze-thaw cycles (R.E %: −12.3% to −2.3%, R.S.D% < 9.7%). These findings underscore the robust stability of the samples across all tested conditions, confirming that they meet stringent stability criteria.

10.3389/fphar.2025.1582644

TABLE 4 Sample stability of GRh_3 in plasma and liver homogenate (n = 6).

Matrix	Nominal concentration (ng/mL)	3 h at room temperature		30 days at refrigerator (−80°C)		Three freeze-thaw cycles	
		Accuracy R.E (%)	Precision R.S.D. (%)	Accuracy R.E (%)	Precision R.S.D. (%)	Accuracy R.E (%)	Precision R.S.D. (%)
Plasma	100	4.5	4.2	−4.2	8.4	−12.3	9.7
	400	−3.0	8.5	−5.4	6.4	−4.1	6.1
	3000	−4.3	7.1	−6.9	8.9	−2.7	8.8
Liver	100	−1.8	9.2	−5.7	9.7	−9.3	7.5
	400	4.4	3.4	−6.9	5.3	−2.6	6.5
	3000	5.7	6.2	−6.8	6.2	−2.3	2.5

TABLE 5 Post-preparation sample stability (n = 6).

Analytes	Nominal concentration (ng/mL)	3 h at room temperature		12 h at auto-sampler (8°C)		2 days at refrigerator (−20°C)	
		Accuracy R.E (%)	Precision R.S.D. (%)	Accuracy R.E (%)	Precision R.S.D. (%)	Accuracy R.E (%)	Precision R.S.D. (%)
Plasma	100	2.7	3.3	−4.2	4.8	−7.6	8.8
	400	−2.2	7.1	−3.5	7.2	−5.3	7.0
	3000	−6.0	10.2	−2.7	7.3	−8.2	5.9
Liver	100	−2.8	5.0	−8.5	9.0	−8.2	10.1
	400	6.6	4.8	−7.7	7.1	−4.9	6.9
	3000	6.3	3.6	−4.9	3.9	−7.4	5.7

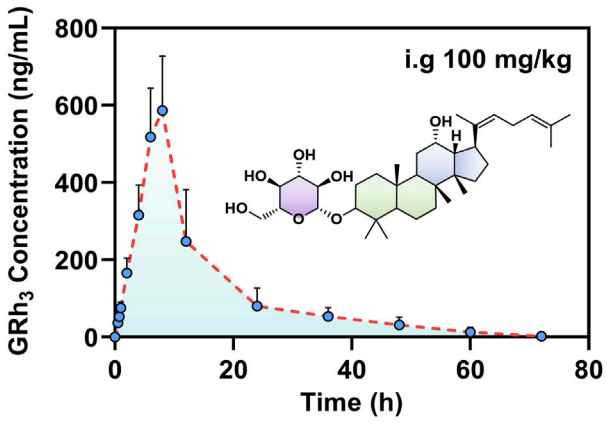


FIGURE 5
The plasma concentration-time profile of GRh_3 after oral administration of 100 mg/kg in rats (n = 7).

TABLE 6 PK parameters of GRh_3 after oral administration of 100 mg/kg in rats (n = 7).

Parameter	Unit	Mean ± SD
$AUC_{(0-t)}$	μg/L·h	7742.9 ± 2441.4
$AUC_{(0-\infty)}$	μg/L·h	8272.9 ± 2348.0
$MRT_{(0-t)}$	h	14.4 ± 2.8
$MRT_{(0-\infty)}$	h	18.5 ± 1.8
$T_{1/2}$	h	14.7 ± 1.7
T_{max}	h	8.0 ± 0.0
V_d	L/kg	280.4 ± 109.3
CL	L/h/kg	13.0 ± 3.8
C_{max}	μg/L	586.6 ± 140.5

3.7 Post-preparation sample stability

Post-preparation samples are not immediately analyzed but are instead subjected to periods of storage at room temperature and in an autosampler (8°C). Additionally, in this study, some samples are temporarily stored in a refrigerator at −20°C until analysis. To ensure the reliability and precision of analytical outcomes, it is crucial to assess the stability of post-preparation samples under various conditions: at room temperature, in an autosampler (8°C), and in a refrigerator at −20°C. As detailed in Table 5, post-

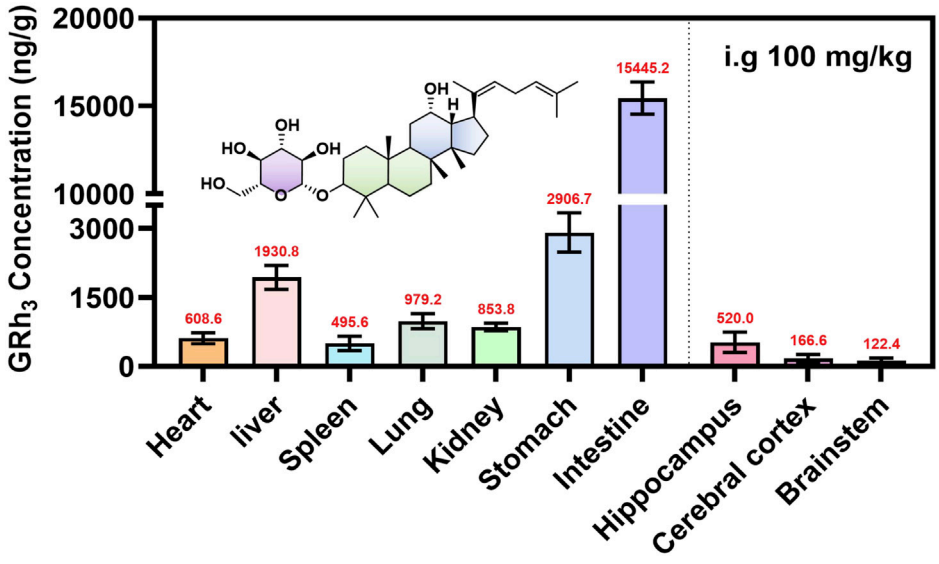


FIGURE 6
The tissue distribution of GRh_3 after oral administration of 100 mg/kg in rats (n = 7).

preparation samples exhibit satisfactory stability when kept at room temperature for 3 h (R.E%: −6.0%–6.6%, R.S.D% < 10.2%), in an autosampler (8°C) for 12 h (R.E%: −8.5% to −2.7%, R.S.D% < 9.0%), and in a refrigerator at −20°C for up to 2 days (R.E%: −8.2% to −4.9%, R.S.D% < 10.1%). These findings confirm that the samples meet the necessary stability criteria, thereby ensuring the validity and accuracy of subsequent analyses. These results underscore the stability of post-preparation samples across all conditions. Consequently, this method ensures the validity and accuracy of subsequent analyses.

3.8 Pharmacokinetics analysis

After intragastric administration of GRh_3, the plasma concentration-time profile is illustrated in Figure 5, while the PK parameters derived from non-compartmental analysis are summarized in Table 6. The findings reveal a T_{max} of 8.0 h for GRh_3, with a $T_{1/2}$ of 14.7 ± 1.7 h. MRT were calculated as 14.4 ± 2.8 h for $MRT_{(0\text{-}t)}$ and 18.5 ± 1.8 h for $MRT_{(0\text{–}\infty)}$. Moreover, the CL was determined to be 13.0 ± 3.8 L/h/kg, and the V_d was estimated at 280.4 ± 109.3 L/kg.

3.9 Tissue distribution study

To comprehensively characterize the tissue distribution of GRh_3 in rats, tissues were harvested at T_{max} (8.0 h) post-intragastric administration, and the GRh_3 content in each tissue was quantitatively determined. The results reveal extensive distribution of GRh_3 across multiple tissues, with notably higher concentrations in certain organs compared to plasma levels (Figure 6). Specifically, the highest concentrations were observed in the intestines (15445.2 ng/g), followed by the stomach (2906.7 ng/g) and liver (1930.8 ng/g). Remarkably, GRh_3 demonstrated substantial brain penetration, accumulating prominently in the hippocampus, where concentrations reached up to 520.0 ng/g. These findings highlight the broad tissue distribution and significant brain transmissivity of GRh_3.

4 Discussion

This study developed and validated a highly sensitive and selective method based on LC-MS/MS for the precise quantification of GRh_3 in rat plasma and various tissues. During method development, Q1 full scans revealed that GRh_3 exhibited the highest intensity in the $[M+HCOO]^-$, which directed subsequent product ion scanning (Figure 7A). The two most intense product ions were paired with $[M+HCOO]^-$ to establish the MRM quantitative ion transitions. Systematic optimization of key parameters (DP, CE, ion source temperature, spray voltage, and gas settings) maximized signal intensity. The optimization of column selection and mobile phase conditions revealed that the Shimadzu Shim-pack GIST-HP C18 column (2.1 mm × 50 mm, 3 μm), paired with a water (0.1% formic acid)/acetonitrile system, provided optimal chromatographic separation and maximized analytical sensitivity. The ion transition 649.6 > 603.1 was ultimately selected for GRh_3 quantification based on peak symmetry, sensitivity, and minimal interference. As no isotopically labeled IS for GRh_3 was available, GRh_4 was selected as the IS due to its similar properties. The optimization for GRh_4 followed the same procedure. The GRh_4 product ion scan was conducted (Figure 7B), and the ion transition 665.5 > 619.4 was chosen for detection.

The PK data demonstrate that GRh_3 exhibits a "prolonged retention-low clearance" sustained-release profile. The extended time to reach peak plasma concentration (T_{max} = 8.0 h) suggests a delayed absorption phenomenon, which may be attributed to gastrointestinal retention effects or significant first-pass elimination

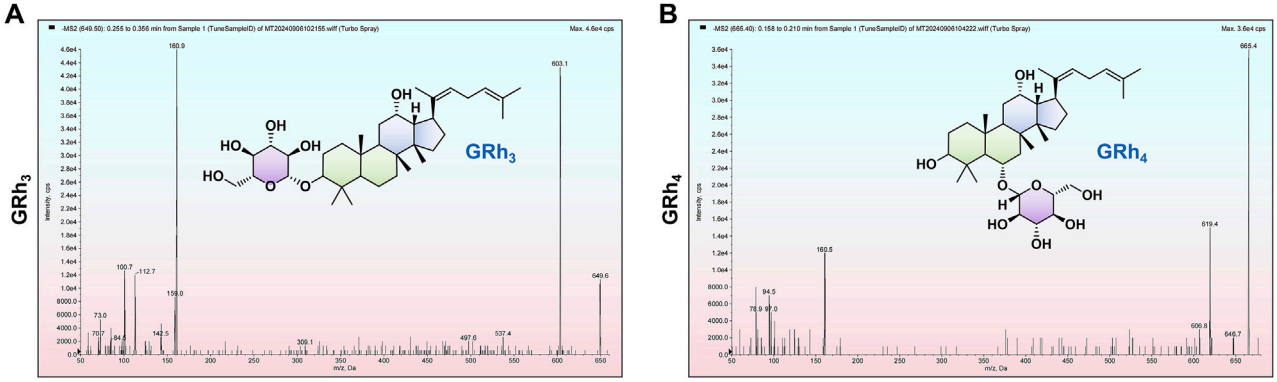


FIGURE 7
The $[M+HCOO]^-$ product ion scans. **(A)** GRh_3. **(B)** GRh_4.

(Omeh et al., 2022; Pond and Tozer, 1984). Consequently, this delays the entry of GRh_3 into the systemic circulation. From the perspective of elimination half-life and mean residence time, GRh_3 exhibits relatively high $T_{1/2}$ (14.7 ± 1.7 h), $MRT_{(0\text{-}t)}$ (14.4 ± 2.8 h), and $MRT_{(0-\infty)}$ (18.5 ± 1.8 h). These results suggest that GRh_3 may undergo tissue redistribution. In terms of apparent volume of distribution and clearance, GRh_3 exhibited a significantly larger V_d (280.4 ± 109.3 L/kg) compared to conventional drugs, suggesting that GRh_3 may be widely distributed in peripheral tissues (Hoch et al., 2024). Its low CL (13.0 ± 3.8 L/h/kg) was associated with the "reservoir effect" caused by tissue accumulation. This "reservoir effect" reduce the free drug concentration, thereby decreasing systemic clearance and ultimately prolonging the GRh_3's $T_{1/2}$ (Eusébio et al., 2024). Although this study is the first to reveal the sustained-release pharmacokinetic characteristics of GRh_3, some limitations exist. Future research could employ radiolabeling techniques to trace GRh_3's tissue distribution pathways or develop population pharmacokinetic models based on transporter gene polymorphisms. These approaches could help optimize the clinical application of GRh_3, particularly in chronic disease treatment, leveraging its long-acting properties.

Tissue distribution data indicated that the highest concentrations of GRh_3 in the intestines, followed by the stomach and liver, indicative of a pronounced first-pass effect (Hervieu et al., 2025). These findings are consistent with the high V_d, prolonged $T_{1/2}$, and extended MRT observed in the PK data. Notably, GRh_3 also exhibits significant brain distribution, particularly in the hippocampus, where its concentrations reached up to 520.0 ng/g, exceeding levels observed in the spleen (495.6 ng/g). The classical BBB theory posits that compounds with high lipophilicity and molecular weight below 500 Da can passively diffuse through the BBB (Xie et al., 2019). However, tissue distribution study has shown orally administered GRh_3 accumulates in rat brain parenchyma despite its molecular weight of 604 Da, which deviates significantly from the BBB permeability criteria.

This finding suggests that passive diffusion alone cannot fully explain the observed central nervous system (CNS) distribution of GRh_3, implying the involvement of active transporter-mediated mechanisms. The BBB expresses a diverse array of transporters that regulate the movement of substances into and out of the brain. These transporters are broadly categorized into influx and efflux systems (Parvez et al., 2023; Ronaldson and Davis, 2015). Influx transporters, including organic anion transporting polypeptides (OATPs), organic cation transporters (OCTs), monocarboxylate transporters (MCTs), L-type amino acid transporter 1 (LAT1), and glucose transporters (GLUTs), facilitate the uptake of nutrients and drugs from the bloodstream into the brain (Parvez et al., 2023; Tamai and Tsuji, 2000). Conversely, efflux transporters such as P-glycoprotein (P-gp), breast cancer resistance protein (BCRP), and multidrug resistance-associated proteins (MRPs) actively expel substances from the brain, helping to maintain CNS homeostasis (Cox et al., 2023; Parvez et al., 2023). Previous studies have reported that GRb_1 can cross the BBB through interactions with GLUT1 (Wang et al., 2018), and that GRg_3 has been shown to act as a P-gp inhibitor at the BBB (Xu et al., 2021). Ginsenosides share a similar structural backbone, and GRh_3 is likely to retain critical molecular characteristics that support interactions with transporters in a manner similar to its analogs, potentially enabling its transport across the BBB into the brain parenchyma.

In addition to potential active transport, the integrity of the BBB may also contribute to GRh_3's accumulation in the CNS. Our previous *in vitro* studies demonstrated that GRh_3 induces oxidative stress and neuronal apoptosis via the IP3R-Ca^{2+}/NOX2/NF-κB pathway (Wang et al., 2025). Both oxidative stress and inflammation have been implicated in BBB disruption (Fang et al., 2024; Gao et al., 2023), suggesting that GRh_3 may impair BBB function and thereby facilitate its own entry into the brain. This is supported by the notably high concentration of GRh_3 in the hippocampus, a brain region highly susceptible to oxidative damage and neuroinflammation. To verify this possibility, future studies should assess BBB integrity following GRh_3 administration using classical methods such as Evans Blue dye extravasation and immunohistochemical detection of tight junction proteins, including Claudin-5 and Occludin. Overall, elucidating both active transport mechanisms and structural BBB alterations will be critical for fully understanding the brain pharmacokinetics and potential neurotoxicity of GRh_3.

5 Conclusion

This study developed and validated a LC-MS/MS method for detecting GRh_3 concentrations in rat plasma and tissues. Subsequently, the PK result indicated that oral administration of GRh_3 exhibited a prolonged T_{max} (8.0 h) and an extended $T_{1/2}$ (14.7 ± 1.7 h), indicating relatively slow absorption and excretion processes. The tissue distribution data showed that GRh_3 significantly accumulated in the intestine, stomach, and liver, and was also able to penetrate the BBB to accumulate in brain tissues, particularly in the hippocampus. However, the mechanisms underlying its BBB penetration remain to be further investigated. This study provides essential support for the further research and development of GRh_3.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

Ethics statement

The animal study was approved by Animal Experimentation Committee of Zunyi Medical University. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

CH: Conceptualization, Data curation, Formal Analysis, Methodology, Validation, Writing – original draft, Writing – review and editing, Project administration, Software, Visualization. YW: Formal Analysis, Visualization, Writing – review and editing, Conceptualization, Software. YL: Data curation, Methodology, Visualization, Writing – review and editing, Validation. XW: Data curation, Visualization, Writing – review and editing, Validation. PS: Writing – review and editing, Data curation, Software. HM: Conceptualization, Supervision, Writing – review and editing, Formal Analysis, Software. LY: Conceptualization, Funding acquisition, Supervision, Writing – review and editing.

Funding

The author(s) declare that financial support was received for the research and/or publication of this article. This study was supported by National Natural Science Foundation of China (8227131503).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declare that no Generative AI was used in the creation of this manuscript.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

Aboghazleh, R., Boyajian, S. D., Atiyat, A., Udwan, M., Al-Helalat, M., and Al-Rashaideh, R. (2024). Rodent brain extraction and dissection: a comprehensive approach. *MethodsX* 12, 102516. doi:10.1016/j.mex.2023.102516

Baeg, I. H., and So, S. H. (2013). The world ginseng market and the ginseng (korea). *J. Ginseng Res.* 37 (1), 1–7. doi:10.5142/jgr.2013.37.1

CDE. (2005). Technical guidelines for non-clinical pharmacokinetics studies of chemical drugs: CFDA.

Cox, B., Nicolaï, J., and Williamson, B. (2023). The role of the efflux transporter, P-glycoprotein, at the blood-brain barrier in drug discovery. *Biopharm. Drug Dispos.* 44 (1), 113–126. doi:10.1002/bdd.2331

EMA (2011). *Guideline on bioanalytical method validation (EMEA/CHMP/EWP/192217/2009)*. London, United Kingdom: European Medicines Agency EMA.

Eom, S. J., Hwang, J. E., Kim, K. T., and Paik, H. D. (2017). Antibacterial effects against various foodborne pathogens and sensory properties of yogurt supplemented with Panax ginseng marc extract. *Korean J. Food Sci. Anim. Resour.* 37 (5), 787–791. doi:10.5851/kosfa.2017.37.5.787

Eusébio, S., Soares, A. R., Fiúza, P., and Garcia, T. (2024). Amiodarone-induced interstitial pneumonia: a cause of respiratory failure. *Cureus* 16 (11), e74253. doi:10.7759/cureus.74253

Fang, M., Yu, Q., Ou, J., Lou, J., Zhu, J., and Lin, Z. (2024). The neuroprotective mechanisms of PPAR-γ: inhibition of microglia-mediated neuroinflammation and oxidative stress in a neonatal mouse model of hypoxic-ischemic white matter injury. *CNS Neurosci. Ther.* 30 (11), e70081. doi:10.1111/cns.70081

FDA (2018). *Bioanalytical method validation guidance for industry*. Rockville, MD: FDA.

Gan, Y., Li, Z., Fan, B., Ji, Z., Yang, L., Wu, Y., et al. (2024). *De novo* Biosynthesis of a Polyene-Type Ginsenoside Precursor Dammaradienol in *Saccharomyces cerevisiae*. *ACS Synth. Biol.* 13 (12), 4015–4026. doi:10.1021/acssynbio.4c00396

Gao, H. M., Chen, H., Cui, G. Y., and Hu, J. X. (2023). Damage mechanism and therapy progress of the blood-brain barrier after ischemic stroke. *Cell Biosci.* 13 (1), 196. doi:10.1186/s13578-023-01126-z

Hao, L., Li, S., Li, C., Zhang, Z., Hu, X., and Yan, H. (2025). A review of the therapeutic potential of ginseng and its bioactive components in nonalcoholic fatty liver disease. *Drug Des. Devel Ther.* 19, 83–96. doi:10.2147/dddt.S500719

Hervieu, L., Groo, A. C., Bellien, J., Guerrot, D., and Malzert-Fréon, A. (2025). Glucuronidation of orally administered drugs and the value of nanocarriers in strategies for its overcome. *Pharmacol. Ther.* 266, 108773. doi:10.1016/j.pharmthera.2024.108773

Hoch, M., Huth, F., Manley, P. W., Loisios-Konstantinidis, I., Combes, F. P., Li, Y. F., et al. (2024). Clinical pharmacology of asciminib: a review. *Clin. Pharmacokinet.* 63 (11), 1513–1528. doi:10.1007/s40262-024-01428-6

Kim, E. J., Jung, I. H., Van Le, T. K., Jeong, J. J., Kim, N. J., and Kim, D. H. (2013). Ginsenosides Rg5 and Rh3 protect scopolamine-induced memory deficits in mice. *J. Ethnopharmacol.* 146 (1), 294–299. doi:10.1016/j.jep.2012.12.047

Lee, Y. Y., Park, J. S., Lee, E. J., Lee, S. Y., Kim, D. H., Kang, J. L., et al. (2015). Anti-inflammatory mechanism of ginseng saponin metabolite Rh3 in lipopolysaccharide-stimulated microglia: critical role of 5'-adenosine monophosphate-activated protein kinase signaling pathway. *J. Agric. Food Chem.* 63 (13), 3472–3480. doi:10.1021/jf506110y

Omeh, R., Ugwueze, M., Offiah, R., Mbah, C., Momoh, A., Onyishi, I., et al. (2022). Oral drug delivery: gastrointestinal tract adaptations, barriers and strategies for delivery enhancement - a review. *Bio-Research* 20, 1685–1698. doi:10.4314/br.v20i3.6

Parvez, M. M., Sadighi, A., Ahn, Y., Keller, S. F., and Enoru, J. O. (2023). Uptake transporters at the blood-brain barrier and their role in brain drug disposition. *Pharmaceutics* 15 (10), 2473. doi:10.3390/pharmaceutics15102473

Pond, S. M., and Tozer, T. N. (1984). First-pass elimination. Basic concepts and clinical consequences. *Clin. Pharmacokinet.* 9 (1), 1–25. doi:10.2165/00003088-198409010-00001

Ronaldson, P. T., and Davis, T. P. (2015). Targeting transporters: promoting blood-brain barrier repair in response to oxidative stress injury. *Brain Res.* 1623, 39–52. doi:10.1016/j.brainres.2015.03.018

Shah, M. A., Abuzar, S. M., Ilyas, K., Qadees, I., Bilal, M., Yousaf, R., et al. (2023). Ginsenosides in cancer: targeting cell cycle arrest and apoptosis. *Chem. Biol. Interact.* 382, 110634. doi:10.1016/j.cbi.2023.110634

Shan, M., Bai, Y., Fang, X., Lan, X., Zhang, Y., Cao, Y., et al. (2023). American ginseng for the treatment of alzheimer's disease: a review. *Molecules* 28 (15), 5716. doi:10.3390/molecules28155716

Shang, S., Yang, H., Qu, L., Fan, D., and Deng, J. (2025). Ginsenoside, a potential natural product against liver diseases: a comprehensive review from molecular mechanisms to application. *Crit. Rev. Food Sci. Nutr.*, 1–25. doi:10.1080/10408398.2025.2451761

Tamai, I., and Tsuji, A. (2000). Transporter-mediated permeation of drugs across the blood-brain barrier. *J. Pharm. Sci.* 89 (11), 1371–1388. doi:10.1002/1520-6017(200011)89:11<1371::aid-jps1>3.0.co;2-d

Wang, X. M., She, C., Li, Q., Zhang, D., Xu, J. X., Li, M. H., et al. (2020). Ginsenoside Rh3 activates Nrf2 signaling and protects endometrial cells from oxygen and glucose deprivation-reoxygenation. *Aging (Albany NY)* 12 (7), 6109–6119. doi:10.18632/aging.103009

Wang, Y., Chen, J., Li, S., and Cai, Z. (2025). Ginsenoside Rh3-induced neurotoxicity involving the IP3R-Ca2+/NOX2/NF-κB signaling pathways. *J. Nat. Med.* doi:10.1007/s11418-025-01912-8

Wang, Y., Wu, D., Wang, Y., Sun, J., Wang, X., Huang, Y., et al. (2024). Bioinformatics study of the potential therapeutic effects of ginsenoside Rh3 in reversing insulin resistance. *Front. Mol. Biosci.* 11, 1339973. doi:10.3389/fmolb.2024.1339973

Wang, Y. Z., Xu, Q., Wu, W., Liu, Y., Jiang, Y., Cai, Q. Q., et al. (2018). Brain transport profiles of ginsenoside Rb(1) by glucose transporter 1: *in vitro* and *in vivo*. *Front. Pharmacol.* 9, 398. doi:10.3389/fphar.2018.00398

Wu, Y., Pi, D., Zhou, S., Yi, Z., Dong, Y., Wang, W., et al. (2023). Ginsenoside Rh3 induces pyroptosis and ferroptosis through the Stat3/p53/NRF2 axis in colorectal cancer cells. *Acta Biochim. Biophys. Sin. (Shanghai)* 55 (4), 587–600. doi:10.3724/abbs.2023068

Xie, J., Shen, Z., Anraku, Y., Kataoka, K., and Chen, X. (2019). Nanomaterial-based blood-brain-barrier (BBB) crossing strategies. *Biomaterials* 224, 119491. doi:10.1016/j.biomaterials.2019.119491

Xu, J. F., Wan, Y., Tang, F., Chen, L., Yang, Y., Xia, J., et al. (2021). Emerging significance of ginsenosides as potentially reversal agents of chemoresistance in cancer therapy. *Front. Pharmacol.* 12, 720474. doi:10.3389/fphar.2021.720474

Yang, T., Xu, W., Wei, X., Zhang, Z., Sun, Y., Liu, H., et al. (2022). Determination of ginsenoside Rh3 in rat plasma by LC-MS/MS and its application to a pharmacokinetic study. *Biomed. Chromatogr.* 36 (2), e5268. doi:10.1002/bmc.5268

Yun, T. K. (2001). Brief introduction of Panax ginseng C.A. meyer. *J. Korean Med. Sci.* 16 (Suppl. l), S3–S5. doi:10.3346/jkms.2001.16.S.S3

Zare-Zardini, H., Hedayati-Goudarzi, M. T., Alizadeh, A., Sadeghian-Nodoushan, F., and Soltaninejad, H. (2024). A review of cardioprotective effect of ginsenosides in chemotherapy-induced cardiotoxicity. *Biomed. Eng. Online* 23 (1), 128. doi:10.1186/s12938-024-01322-z

frontiers | Frontiers in Pharmacology

TYPE Review
PUBLISHED 18 July 2025
DOI 10.3389/fphar.2025.1603417

Check for updates

OPEN ACCESS

EDITED BY
Bin Yu,
Nanjing University of Chinese Medicine, China

REVIEWED BY
Hong-he Xiao,
Liaoning University of Traditional Chinese Medicine, China
Xiaokai Li,
Inner Mongolia Agricultural University, China

*CORRESPONDENCE
Liqin Li,
liliqin@hzhospital.com
Yuntao Li,
lyta722@live.com

†These authors have contributed equally to this work

RECEIVED 31 March 2025
ACCEPTED 08 July 2025
PUBLISHED 18 July 2025

CITATION
Niu P, Zhang Y, Wang B, Qiu S, Dong Q, Li L and Li Y (2025) Modulation of neuroinflammation by natural plant compounds: a promising approach for ischemic stroke.
Front. Pharmacol. 16:1603417.
doi: 10.3389/fphar.2025.1603417

COPYRIGHT
© 2025 Niu, Zhang, Wang, Qiu, Dong, Li and Li. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

Modulation of neuroinflammation by natural plant compounds: a promising approach for ischemic stroke

Pingping Niu[1,2†], Yonggang Zhang[1†], Binghao Wang[1], Sheng Qiu[1], Quanming Dong[2,3], Liqin Li[2*] and Yuntao Li[1*]

[1]Department of Neurosurgery, Huzhou Central Hospital, Fifth School of Clinical Medicine of Zhejiang Chinese Medical University, Huzhou, China, [2]Scientific Research Department, Huzhou Central Hospital, Fifth School of Clinical Medicine of Zhejiang Chinese Medical University, Huzhou, China, [3]Scientific Research Department, Zhejiang Chinese Medical University, Hangzhou, China

Ischemic stroke remains a leading global cause of mortality and long-term disability, imposing substantial health and economic burdens on society. Although interventions such as intravenous thrombolysis and mechanical thrombectomy have proven effective, their narrow therapeutic time window restricts accessibility for many patients. Post-ischemic brain injury is significantly driven by a cascade involving inflammatory cells and mediators, culminating in an "inflammatory storm" that damages neuronal cells. Consequently, targeting neuroinflammation following ischemic stroke to explore potential therapeutic strategies is of paramount importance. Natural plant compounds, bioactive constituents derived from plants, demonstrate considerable promise for ischemic stroke treatment, with many exhibiting potent neuroinflammation-modulating activities. This review synthesizes current research on natural plant compounds targeting post-ischemic stroke neuroinflammation and elucidates their underlying mechanisms of action. It aims to offer insights for developing plant-derived therapeutics specifically targeting neuroinflammation after ischemic stroke.

KEYWORDS

ischemic stroke, natural plant compounds, neuroinflammation, neuroprotective, anti-inflammation

1 Introduction

Ischemic stroke remains a global health challenge characterized by disproportionately high rates of disability and mortality, posing significant threats to patient survival and quality of life. Although medical advancements have expanded therapeutic options, early pharmacological thrombolysis and mechanical thrombectomy persist as the primary evidence-based interventions. However, the narrow therapeutic time window severely limits clinical accessibility, with only a limited proportion of patients deriving benefit from these time-sensitive treatments (Feigin et al., 2025; Potter et al., 2022; Hilkens et al., 2024). This underscores the critical need to elucidate the pathophysiological mechanisms underlying post-ischemic neuronal injury and develop novel therapeutic strategies.

Neuroinflammatory cascades constitute a pivotal mechanism exacerbating cerebral tissue damage following ischemic stroke (DeLong et al., 2022). The ischemic insult triggers substantial production and release of inflammatory mediators, which initiate tissue injury and recruit peripheral immune cells. Subsequent immune cell infiltration establishes a self-perpetuating

cycle: infiltrating leukocytes secrete additional proinflammatory cytokines that amplify tissue destruction while promoting further immune cell recruitment, culminating in a cytokine storm (Jayaraj et al., 2019; Wang H. et al., 2023; Zietz et al., 2024). Furthermore, inflammatory processes compromise blood-brain barrier integrity, facilitating cerebral edema formation and perpetuating neuroinflammation through enhanced peripheral immune cell trafficking (Chen et al., 2021). These mechanisms collectively position anti-inflammatory interventions as promising therapeutic targets for improving neurological outcomes in ischemic stroke.

Naturally derived compounds demonstrate considerable therapeutic potential in cerebral ischemia management (Ahsan et al., 2021). Current evidence indicates that specific botanical metabolites exhibit multi-target pharmacological activities: Gastrodia elata polysaccharides mitigate oxidative stress and ferroptosis pathways (Zhang Y. et al., 2024); Ginkgolide K modulates oxidative stress, neuroinflammation, and autophagy while promoting angiogenesis (Feng et al., 2019); Salidroside demonstrates dual anti-inflammatory and antioxidant properties (Fan et al., 2020). These natural plant compounds collectively target key pathological processes in ischemic stroke, including inflammation regulation, oxidative stress mitigation, ferroptosis inhibition, angiogenesis promotion, and autophagy modulation, thereby representing promising candidates for drug development.

This comprehensive review systematically evaluates the anti-inflammatory efficacy of naturally derived compounds in ischemic stroke, critically appraising current experimental evidence to inform the development of phytochemical-based therapeutics for post-stroke neuroinflammation management.

2 Literature search strategy

A literature search was conducted using the Web of Science and PubMed databases. The search covered the period from January 2005 to January 2025 to encompass research progress on natural plant compounds modulating neuroinflammation in ischemic stroke, including *in vivo*, *in vitro*, and clinical studies. We utilized a combination of Medical Subject Headings (MeSH) terms and free-text keywords, connected with Boolean operators, such as ("Ischemic Stroke" OR "Cerebral Ischemic "OR "Cerebral Ischemia-Reperfusion Injury") AND ("Inflammation" OR "Neuroinflammation") AND ("Natural plant compounds" OR "Natural Drugs" OR "Natural Medicine" OR "Herbal medicine" OR "Chinese Medicine"). Only peer-reviewed English articles were included, excluding case reports, editorials, and conference abstracts. The screening process involved an initial title and abstract review by two independent reviewers, with exclusions based on poor quality, outdated data, or lack of originality. Full texts of potentially eligible studies were then assessed. Any discrepancies arising during screening were resolved through discussion with a third reviewer or consensus.

3 Neuroinflammation in ischemic stroke

Acute cerebral ischemia is caused by reduced or obstructed blood flow, typically attributable to cardioembolism, small vessel occlusion, or atherosclerosis (Zhao et al., 2022). This impairment disrupts energy metabolism and initiates multiple pathophysiological processes, including secondary inflammatory responses. The neuroinflammatory mechanisms following ischemic stroke involve intricate interactions across several dimensions: (1) Glial cell activation and polarization post-ischemia: Microglia, the predominant immune cells in brain tissue, recognize damage-associated molecular patterns (DAMPs) through pattern recognition receptors (e.g., Toll-like receptor 4, TLR4), initiating M1 pro-inflammatory polarization and releasing substantial inflammatory mediators. Concurrently, ischemia induces astrocyte transformation into A1-type pro-inflammatory phenotypes, upregulating complement C3 expression to establish a neurotoxic microenvironment (Li X. et al., 2022). (2) Pro-inflammatory signaling activation: The NF-κB signaling cascade becomes activated following cerebral ischemia, characterized by IKK complex phosphorylation and subsequent IκBα degradation. This facilitates NF-κB nuclear translocation, driving transcriptional activation of COX-2, iNOS, and cytokine genes, thereby amplifying inflammatory responses and promoting blood-brain barrier disruption (Xu et al., 2024). (3) NLRP3 inflammasome assembly: Post-ischemic reactive oxygen species (ROS) accumulation and potassium efflux activate NLRP3, which recruits pro-caspase-1 via ASC adaptor proteins. This catalytic process facilitates IL-1β/IL-18 precursor maturation, inducing pyroptosis and promoting peripheral immune cell infiltration (Yang K. et al., 2024; Han et al., 2023). (4) Chemokine network regulation: Ischemic injury stimulates cerebral chemokine production and release, mediating peripheral immune cell recruitment. Specifically, the CXCL8/CXCR2 axis facilitates neutrophil transendothelial migration, while the CCL2/CCR2 pathway drives monocyte aggregation in injured areas, establishing a pro-inflammatory feedback loop between immune cells and inflammatory mediators (Wang et al., 2022). (5) Complement cascade activation: Post-ischemic C3a/C5a activation through G protein-coupled receptors upregulates endothelial adhesion molecules (ICAM-1/VCAM-1), promoting leukocyte rolling and extravasation. Simultaneously, C5b-9 membrane attack complexes directly induce neuronal membrane lysis (Széplaki et al., 2009; Si et al., 2019).

Collectively, the mechanisms underlying neuroinflammatory exacerbation of cerebral injury in ischemic stroke involve complex interactions. Ischemic tissue-derived inflammatory mediators directly damage neural cells while recruiting and activating immune cells. These activated immune cells not only inflict direct neuronal injury but also generate additional inflammatory mediators, establishing a self-perpetuating inflammatory cascade that amplifies cerebral tissue (Zietz et al., 2024).

4 Natural plant compounds alleviates neuroinflammation in ischemic stroke

The exploration of natural plant compounds has become a prominent area of interest in the ongoing search for innovative stroke treatments, offering considerable potential. Natural plant compounds exhibit a diverse array of bioactive properties in

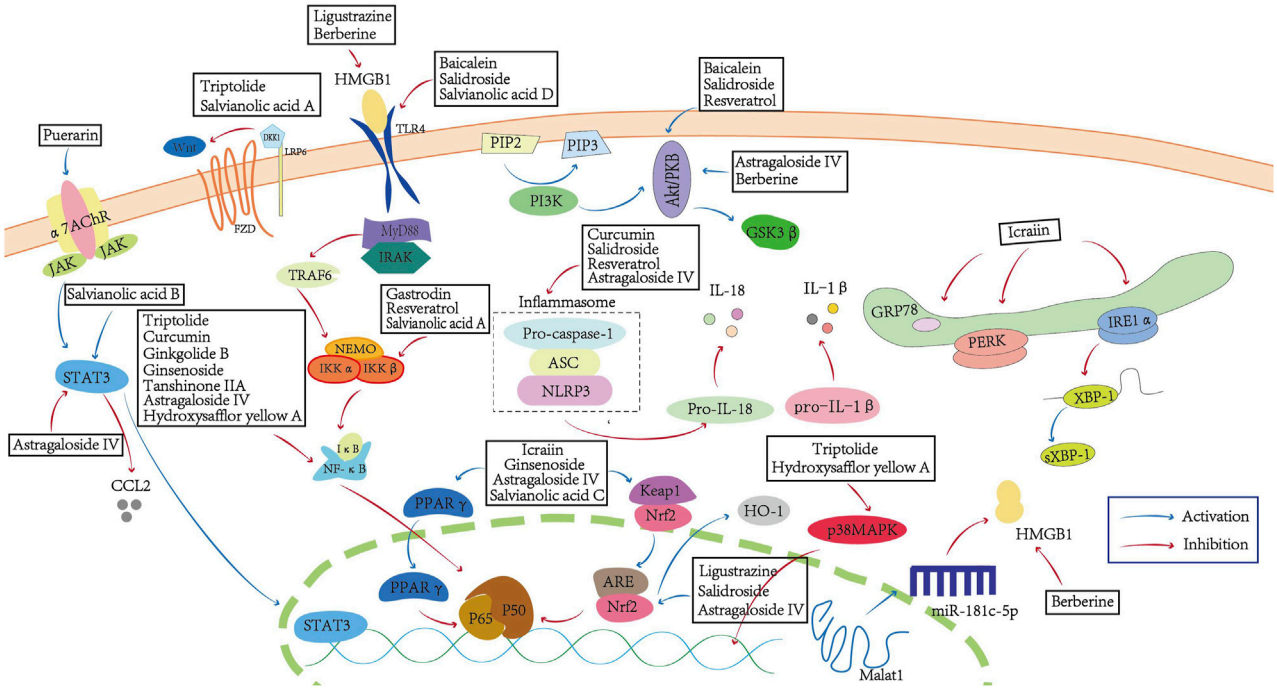


FIGURE 1
Natural plant compounds inhibit neuroinflammation in ischemic stroke. Natural plant compounds inhibit the release of inflammatory mediators and the activation of pro-inflammatory cells through multiple pathways such as the NF-κB signaling pathway, Wnt/β-catenin pathway, STAT3/VEGF signaling pathway, PI3K/AKT signaling pathway, and JAK/STAT signaling pathway.

ischemic stroke therapy, encompassing autophagy regulation, ferroptosis inhibition, anti-inflammatory and immunomodulatory effects, oxidative stress mitigation, anti-apoptotic activity on neurons, blood-brain barrier preservation, and facilitation of neurovascular remodeling (Tao et al., 2020). Given the inherent complexity of traditional Chinese medicinal metabolites that exert multi-target mechanisms through diverse pathways, our review focuses on bioactive natural plant compounds relevant to the pathophysiological mechanisms of ischemic stroke, with particular emphasis on identifying potential therapeutic targets within neuroinflammatory responses. In this review, we collected and summarized the relevant research reports on five categories with anti-inflammatory activities after ischemic stroke. These natural plant compounds can exert anti-inflammatory effects through multiple mechanisms (Figure 1), including inhibiting the production of inflammatory mediators, the activation of glial cells, the activation of proinflammatory signals, and the infiltration of peripheral immune cells.

4.1 Polyphenols

Polyphenols with anti-inflammatory activity in ischemic stroke include salvianolic acid, gastrodin, curcumin, and resveratrol (Table 1).

Salvianolic acid, derived from *Salvia miltiorrhiza* Bunge [Lamiaceae], is obtained from the dried roots and rhizomes of this medicinal plant. The phytochemical metabolites of *Salvia miltiorrhiza* Bunge are primarily divided into two groups: hydrophilic phenolic acids and lipophilic diterpenoids (Wang X. et al., 2020). Among these, salvianolic acid represents the predominant water-soluble fraction, comprising four bioactive metabolites: salvianolic acid A (SAA), salvianolic acid B (SAB), salvianolic acid C (SAC), and salvianolic acid D (SAD) (Zhao et al., 2024). SAA, SAB, SAC and SAD all show anti-inflammatory activity in ischemic stroke.

4.1.1 Salvianolic acid A

Although SAA exhibits poor bioavailability in animal models, its concentration significantly increases in post-ischemic brain tissue, likely due to ischemia-induced disruption of the blood-brain barrier (Feng et al., 2017). Furthermore, the anti-inflammatory properties of SAA in post-ischemic stroke have been well established (Yang H. et al., 2024). Experimental evidence demonstrates that in ischemic stroke models, SAA administration not only significantly reduces the expression of pro-inflammatory cytokines IL-1β and TNF-α, but also effectively suppresses the activation of key inflammatory signaling pathways including TLR2/TLR4, MyD88, and NF-κB (Ling et al., 2021; Chien et al., 2016; Zhang et al., 2018). Furthermore, SAA treatment exhibits protective effects on blood-brain barrier (BBB) integrity, which may contribute to reducing peripheral immune cell infiltration (Chien et al., 2016). However, a notable limitation lies in the insufficient mechanistic depth of current investigations. Although Yun et al. observed attenuated SAA efficacy following TLR2/TLR4 intervention, these findings lack validation through *in vivo* experimental approaches. Moreover, critical rescue experiments remain necessary to conclusively establish TLR2/TLR4 as the pivotal molecular targets mediating SAA's anti-inflammatory actions.

TABLE 1 Polyphenols inhibit neuroinflammation in ischemic stroke.

Natural plant compound	PubChem CID	Sources	Model	Dose	Mechanisms	References
Salvianolic acid A	10364	*Salvia miltiorrhiza* Bunge [Lamiaceae]	OGD/R Rats MCAO model	62.5, 125, 250 μg/mL 5, 10 mg/kg	TLR2/4 signaling pathway	(Ling et al., 2021)
			MCAO model	50, 100 μg/kg	NF-κB signaling pathway	(Chien et al., 2016)
			Rats MCAO	5, 10, 20 mg/kg	MMP-9↓,NF-κB p65 activation↓	(Zhang et al., 2018)
Salvianolic acid B	6451084		Rats tMCAO	3, 6, 12 mg/kg	TLR4/MyD88/ TRAF6 signaling pathway	(Wang et al., 2016)
			Rats MCAO model	10, 20, 40, 60 mg/kg	SIRT1 signaling	(Lv et al., 2015)
Salvianolic acid C	13991590		Rats tMCAO model	25 mg/kg	TLR4-TREM1-NF-κB pathway	(Guo et al., 2024)
			Rats tMCAO	20, 40 mg/kg	The M1 polarization of BV2 cell↓ Glycolysis↓ VEGFR2 and Notch1↑	(Shen et al., 2022)
Salvianolic acid D	75412558		PC12 cell OGD/R Rats MCAO/R model	1, 3, 15 mg/kg 0.2, 1,5 μM	HMGB1/NF-κB signaling pathway	(Zhang et al., 2020)
Gastrodin	115067	Gastrodia elata Blume [Orchidaceae]	OGD/R Rats MCAO/R mode	25, 50, 100 mg/kg 0.5, 5, 20 μM	JAK2/STAT3 signaling pathway	(Zhang et al., 2024b)
			Rats MCAO	100 mg/kg	CRP, IL-1β↓ Bcl-2, VEGF↑	(Wang et al., 2018a)
Curcumin	969516	Zingiberaceae Araceae	Rats MCAO	10 μmol/L	MAPK and NF-κB signaling pathways	(Lan et al., 2024)
			Rats dMCAO model	150 mg/kg 12.5.25 mmol/L	CD16, TNF-α, IL-12p70, IL-6↓ CD20↑	(Lei et al., 2023)
			Rats MCAO model	100 mg/kg	Ca^{2+} activation channel↓ P2X7R↓	(Liu et al., 2017)
Resveratrol	445154	*Vitis vinifera* L. [Vitaceae], Reynoutria japonica Houtt [Polygonaceae]	Rats MCAO/R	200 mg/kg	Th1/Th2 balance and Treg/Th17 balance	(Chiang et al., 2022)

OGD/R, Oxygen-glucose deprivation/reoxygenation; MCAO, Middle cerebral artery occlusion; I/R, ischemia reperfusion; MCAO/R, middle cerebral artery occlusion/reperfusion; OGD, oxygen-glucose deprivation; dMCAO, distal middle cerebral artery occlusion; BMECs, brain microvascular endothelial cells; ↓, downregulation or inhibition; ↑, upregulation or activation.

4.1.2 Salvianolic acid B

SAB demonstrates significant anti-inflammatory properties in both cellular and animal models of ischemic stroke. *In vivo* studies revealed that SAB administration dose-dependently suppressed the expression of pro-inflammatory mediators (IL-1β, IL-6, IL-8, and MCP-1) within the ischemic penumbra. Moreover, it effectively attenuated the pathological overactivation of astrocytes and microglia following cerebral ischemia. *In vitro*, excessive activation of astrocytes and microglia after ischemia (Xu et al., 2017; Wang et al., 2016; Fan et al., 2018). *In vitro*, SAB can not only reduce OGD/R-induced neuronal cell damage, but also inhibit TLR4, MyD88, and NF-kB signals in neurons, and reduce the expression of proinflammatory factors IL-1β, IL-6, and TNF-α (Wang et al., 2016). Although the precise molecular targets of SAB remain to be fully elucidated, recent investigations by Hongdi Lv et al. have identified silent information regulator 1 (SIRT1) as a crucial mediator in SAB's neuroprotective and anti-inflammatory mechanisms (Lv et al., 2015). However, the poor brain availability of SAB, evidenced by its minimal penetration into brain tissue following systemic absorption, remains a significant challenge (Zhang et al., 2021). Studies have demonstrated that SAB co-administered with borneol synergistically enhances its brain delivery (Zhang et al., 2021), thus offering a potential strategy to overcome its limited brain bioavailability.

4.1.3 Salvianolic acid C

Although the therapeutic potential of SAC in ischemic stroke remains relatively under-investigated, emerging evidence highlights its anti-inflammatory properties. Following ischemic stroke, SAC exerts dual regulatory effects by suppressing both the activation of nuclear factor-κB (NF-κB) through inhibition of Toll-like receptor 4 (TLR4)/triggering receptor expressed on myeloid cells-1 (TREM1)

signaling pathways in myeloid cells and microglial overactivation, thereby reducing the expression of proinflammatory mediators (Guo et al., 2024; Shen et al., 2022). Transcriptomic profiling further corroborates these findings, revealing SAC's predominant modulation of neuroinflammatory pathways, acute-phase response signaling, and granulocyte adhesion processes, collectively underscoring its anti-inflammatory role in cerebral ischemia (Guo et al., 2024). Notably, molecular docking analyses by Yang et al. identified nine potential SAC-binding targets, among which peroxisome proliferator-activated receptor gamma (PPARγ) emerges as a critical therapeutic target (Yang et al., 2021). As a nuclear transcription factor abundantly expressed in macrophages and microglia, PPARγ orchestrates multifaceted neuroprotective mechanisms, including cerebral injury repair, anti-inflammatory response potentiation, and mitochondrial biogenesis. Intriguingly, SAC treatment upregulated PPARγ expression, suggesting its potential involvement in SAC-mediated anti-inflammatory effects (Yang et al., 2021; Titus et al., 2024). However, low bioavailability remains a challenge to be addressed for SAC (Song et al., 2016), and its pharmacokinetic profile in brain tissue also requires further elucidation.

4.1.4 Salvianolic acid D

A study by Wen Zhang et al. investigated the therapeutic potential of salvianolic acid D (SAD) in ischemic stroke. Experimental evidence from both middle cerebral artery occlusion (MCAO) rat models and oxygen-glucose deprivation/reoxygenation (OGD/R)-induced cellular models demonstrated that SAD exhibited potent anti-inflammatory properties, as evidenced by its significant suppression of proinflammatory cytokines including TNF-α, IL-1β, and IL-6. Mechanistically, the anti-neuroinflammatory effects of SAD in ischemic stroke may be attributed to its inhibitory action on high mobility group box 1 (HMGB1) nuclear-to-cytoplasmic translocation and subsequent blockade of the TLR4/MyD88/NF-κB signaling cascade activation (Zhang et al., 2020). However, this hypothesis requires further experimental validation through comprehensive mechanistic studies. Besides, the direct molecular targets of SAD and its precise mode of action in modulating these pathways remain to be fully elucidated. Additionally, the pharmacokinetic characteristics of SAD in brain tissue also need to be explored.

4.1.5 Gastrodin

Gastrodin is derived from the dried tuber of the orchid plant Gastrodia elata Blume [Orchidaceae], which has the effect of dispelling wind and unblocking circulation tracts. It has been used to treat central nervous system diseases such as dizziness, headache and epilepsy for hundreds of years (Xiao et al., 2023). Gastrodin can be rapidly absorbed in the intestine and widely distributed in the body, and can pass through the BBB into the brain tissue (Dai et al., 2024). Gastrodin exhibits potent anti-inflammatory properties in various disease models by modulating oxidative stress and the TLR4/NF-κB signaling pathway. In ischemic tissue, endogenous DNA damage mediated by TLR9 exacerbates post-ischemic inflammatory responses, which can be effectively suppressed by gastrodin through downregulation of TLR9 expression. Furthermore, gastrodin may directly bind to JAK2 and reduce the production of inflammatory cytokines after cerebral ischemia by inhibiting JAK2/STAT3 signaling. In addition, as oxidative stress represents a critical factor in exacerbating neuroinflammation, gastrodin also demonstrates regulatory effects on post-ischemic oxidative stress (Zhang M. et al., 2024; Wang S. et al., 2018; Peng et al., 2015). Notably, a clinical study demonstrated that Gastrodin effectively prevented postoperative delirium following cardiac surgery, with no drug-related adverse events reported, highlighting its favorable safety profile in clinical settings (Bai et al., 2025). These findings provide critical support for advancing future clinical trials investigating the therapeutic potential of Gastrodin in ischemic stroke management.

4.1.6 Curcumin

Curcumin is a natural plant compound extracted from the roots and stems of some plants of Zingiberaceae and Araceae, which has anti-inflammatory and antioxidant effects. NOD-like receptor protein 3 (NLRP3) inflammasome mediated microglial pyroptosis is a pro-inflammatory programmed cell death, which plays a crucial role in neuroinflammation and functional recovery after stroke (Han et al., 2023). NF-κB signal transduction is the main pathway to promote inflammatory response, and plays a central role in the aggregation of NLRP3 metabolites and the activation of NLRP3 inflammasome (Lei et al., 2023). Curcumin can downregulate MAPK and NF-κB signal transduction pathway, inhibit the activation of NLPR3 inflammasome, reduce the expression of ICAM-1, MMP-9 and IL-1β exerting anti-inflammatory effect (Yang X. et al., 2024; Wang et al., 2024). Curcumin can also act on the activation stage of microglia, inhibit M1 polarization, promote M2 polarization, inhibit Ca^{2+} activation channel and P2X7 receptor (P2X7R) to inhibit inflammatory reaction (Bissacotti et al., 2023; Lan et al., 2024; Liu et al., 2017; Wang Z. et al., 2020). Notably, poor water solubility, low oral bioavailability, and poor BBB permeability have limited the application of curcumin in the past, but the application of nanotechnology has effectively improved this situation. Currently, nanoparticles loaded with curcumin have been used in the study of ischemic stroke and have shown excellent anti-inflammatory effects (Yang Q. et al., 2024; Li F. et al., 2021). Curcumin is a natural plant compound with great potential for the treatment of ischemic stroke. However, curcumin has been identified as a pan-assay interference compound (Baker, 2017), which requires researchers to focus on in related studies.

4.1.7 Resveratrol

Resveratrol is a stilbene metabolite famously found in *Vitis vinifera* L. [Vitaceae] and Reynoutria japonica Houtt [Polygonaceae] (Fletes-Vargas et al., 2023). It has been widely studied for its cardioprotective and neuroprotective effects. In a cerebral ischemia model, resveratrol exhibits anti-inflammatory and antioxidant effects. Specifically, the anti-inflammatory mechanisms of resveratrol include inhibiting the activation of NF-κB and NLRP3 inflammasomes to reduce the production of inflammatory factors, downregulating the cluster of differentiation 147 (CD147)/MMP-9 axis to inhibit microglial activation and the release of inflammatory factors, activating the PI3K/AKT signaling pathway to participate in the anti-inflammatory process, and regulating the changes in Th17/Tregs and Th1/Th2 polarity mediated by the intestinal lamina propria to

TABLE 2 Flavonoids inhibit neuroinflammation in ischemic stroke.

Natural plant compound	PubChem CID	Sources	Model	Dose	Mechanisms	References
Baicalein	64982	*Scutellaria baicalensis* Georgi [Lamiaceae]	Neuron–astrocyte cocultures OGD/R model	137.5, 68.75, 34.38, 17.19, 8.59, 4.30, 2.15, 1.08 μg/mL	BDNF-TrkB/PI3K/Akt and MAPK/Erk1/2 signaling axes	(Baell and Walters, 2014)
			BV2 and Raw 264.7 cell	0.5, 1, 5, 10, 50, 150, 200 μg/mL	PI3K/AKT/mTOR pathway	(Sowndhararajan et al., 2017)
			BMECs OGD/R model	5, 10, 25 μg/mL	INOS, IL6↓	(Yu et al., 2023)
Icariin	5318997	Epimedium brevicornu Maxim [Berberidaceae]	Microglia OGD/R model	0.37, 0.74, 1.48 μmol/L	IRE1/XBP1s pathway	(Seyedi et al., 2023)
			Rat MCAO model	60 mg/kg	PPARs/Nrf2/NF-κB and JAK2/STAT3/NF-κB pathways	(Mo et al., 2021)
Puerarin	5281807	*Pueraria montana* var. Lobata [Fabaceae]	Rats MCAO model	25, 50, 100 mg/kg	SIRT1/HIF-1α/VEGF signaling pathway	(Dai et al., 2021)
			Rats MCAOmodel	36, 54 mg/kg	α7AChR/JAK2/STAT3 signaling pathway NF-κBp65 protein, IL-1β, IL-6, TNF-α↓	(An et al., 2021)
Hydroxysafflor yellow A	6443665	*Carthamus tinctorius* L. [Asteraceae]	BV2 cell OGD/R model	20, 40, 80, 160, 320, 640, 1,280 μM	NF-κB and p38 MAPK pathway↓	(Yi et al., 2017)

OGD/R, Oxygen-glucose deprivation/reoxygenation; OGD, oxygen-glucose deprivation; I/R, ischemia reperfusion; MCAO, middle cerebral artery occlusion; TBI, Traumatic brain injuries; ↓, downregulation or inhibition; ↑, upregulation or activation.

inhibit systemic inflammation and neurological symptoms after stroke (Tsai et al., 2022; Chiang et al., 2022; Dou et al., 2019). Furthermore, A systematic review evaluated the research on resveratrol in animal models of ischemic stroke and pointed out that resveratrol has beneficial effects on rodent stroke models (López-Morales et al., 2023), suggesting that resveratrol has great potential for the treatment of ischemic stroke. Resveratrol exhibits a short biological half-life, poor water solubility at physiological pH, and low absorption rate, which limits its clinical application. However, nanostructure-based drug delivery systems have shown significant potential to enhance the chemical stability, water dispersibility, bioavailability, and blood-brain barrier (BBB) permeability of resveratrol (Ramalingam and Ko, 2016). Besides, as a pan-assay interference compound (Baell and Walters, 2014), Resveratrol also needs to be paid special attention in research.

4.2 Flavonoids

Flavonoids with anti-inflammatory activity in ischemic stroke include Baicalein, Icraiin, Puerarin, and Hydroxysafflor yellow A (Table 2).

4.2.1 Baicalein

Baicalein, a flavonoid compound extracted from the roots of *Scutellaria baicalensis* Georgi [Lamiaceae], can cross the BBB and penetrate brain tissue within 20–30 min after administration (Sowndhararajan et al., 2017). It has anti-inflammatory and anti-thrombotic effects, and is commonly used in clinical treatment of stroke. It can reduce the increase of NO and MDA after oxygen-glucose deprivation/reoxygenation (OGD/R), reduce TNF-α, IL-1β and IL-6 expression, increase the expression of SOD, which can be reversed by tropomyosin receptor kinase B (TrkB) antagonist ANA-12, indicating that brain-derived neurotrophic factor (BDNF)-TrkB signal transduction is essential for its neuroprotective effect (Li C. et al., 2021). In addition to BDNF-TrkB signal transduction, baicalin also participates in the activation of PI3k/AKT signaling pathway and the inhibition of NF-κB signaling pathway (Long et al., 2025). PI3K/AKT signaling pathway is an important downstream cascade of BDNF-TrkB signaling transduction, and inhibition of inflammatory reaction is the direct consequence of PI3K/AKT signal transduction (Yu et al., 2023). The inhibition of NF-κB signaling pathway is targeted on the TLR upstream. By down-regulating the TLR, suppress the activation of NF-κB and block the signal from being sent to the downstream factor TNF-α, in order to resist immune cell infiltration and relieve ischemic stroke (Lu et al., 2025; Hao et al., 2023). Baicalin exerts anti-inflammatory effects in ischemic stroke through multiple pathways. Furthermore, baicalin-loaded nanoparticles delivered via the nose-to-brain route demonstrate significant efficacy in suppressing oxidative stress and neuroinflammation in rat models of ischemic stroke, indicating promising therapeutic potential (Li X. et al., 2022).

4.2.2 Icariin

Icariin, one of the main active metabolites of Epimedium brevicornu Maxim [Berberidaceae] has many effects, such as enhancing blood flow, regulating immune function, and enhancing bone growth (Seyedi et al., 2023). It was found that icariin could directly regulate glucose-regulated protein 78 (GRP78), inositol requiring enzyme-1α (IRE1α) and protein kinase RNA-like ER kinase (PERK) to inhibit endoplasmic reticulum (ER) stress and repress the inositol-requiring enzyme 1 (IRE1)/X-box binding protein 1 (XBP1s) pathway, reduce microglial activation in the ischemic penumbra, decrease the expression and release of

proteins such as IL-1β and TNF-α to exert anti-inflammatory effects to attenuate ischemic stroke injury (Zheng et al., 2022; Mo et al., 2021; Tao et al., 2024). PPARs are endogenous neuroprotective factors and have three different PPAR isoforms, PPARα, PPARβ and PPARγ. Activation of PPAR subtypes, especially PPARγ, was shown to protect against post-ischemic inflammation and neuronal damage (Titus et al., 2024). Cytokines and oxidative stress can stimulate the JAK phosphorylation, which in turn leads to STAT phosphorylation and is involved in the pathogenesis of inflammatory and autoimmune diseases. Icariin could regulate PPARs and repress JAK2/STAT3 pathway, which in turn inhibited NF-κB activation mediated inflammation to exert neuroprotective effects (Dai et al., 2021). Furthermore, studies demonstrate that icariin is extensively absorbed in the bloodstream and rapidly distributes to the brain within 15–20 min. Thus, icariin exhibits considerable potential for clinical treatment of ischemic stroke (An et al., 2021).

4.2.3 Puerarin

Puerarin is a single metabolite flavonoid glycoside extracted from the dried roots of legume plant *Pueraria montana* var. Lobata [Fabaceae] that has effects of activating blood circulation and eliminating stasis, improving microcirculation, and dilating blood vessels. The neuroprotective effect of Puerarin against cerebral ischemic injury was manifested by attenuating hypoxia-inducible factor-1α (HIF-1α) and TNF-α expression, inhibiting inflammatory responses, apoptosis, and neutrophil activation (Liu et al., 2023). Puerarin pretreatment can inhibit NF-κB expression and by stimulating α7 nicotinic acetylcholine receptor (α7AChR) activates the JAK3/STAT3 signaling pathway to regulate inflammatory responses (Liu et al., 2013). However, the clinical application of puerarin is significantly limited by its suboptimal physicochemical properties and BBB permeability, evidenced by a low brain-to-serum area under the curve (AUC) ratio of only 2.92% following intravenous administration. Notably, research by Tao Yi et al. demonstrates that co-administration of borneol with a self-microemulsifying drug delivery system (SMEDDS) enhances the oral absorption and brain penetration of puerarin in mice (Yi et al., 2017), suggesting a promising strategy for developing oral puerarin formulations for ischemic stroke.

4.2.4 Hydroxysafflor yellow A (HSYA)

Carthamus tinctorius L. [Asteraceae] has the efficacy of activating blood circulation, eliminating stasis and relieving pain, and HSYA is the most effective water-soluble site for the pharmacological efficacy of Carthamus tinctorius L. *In vitro*, cell experiments found that HSYA could reduce the levels of pro-inflammatory factors after OGD, inhibit the OGD-induced degradation of IκB and nuclear translocation of p65 and the phosphorylation of p38 MAPK, exerting anti-inflammatory properties, enhances cell viability, and inhibits cell death (Zhao et al., 2020; Li et al., 2013). *In vivo*, HSYA decreased the levels of inflammatory marker TNF-α and reduced neuroinflammation, which could be reversed by an opener of the mitochondrial permeability transition pore (mPTP), indicating that HSYA might exert neuroprotective effects by inhibiting the opening of the mPTP (Yi et al., 2022). Furthermore, despite its slow rate of absorption from blood into cerebrospinal fluid, HSYA can penetrate brain tissue and exert neuroprotective effects following ischemic stroke (Du et al., 2022; Sheng et al., 2020).

4.3 Saponins

Saponins with anti-inflammatory activity in ischemic stroke include Ginsenoside Rd, Astragaloside IV, and Salidroside (Table 3).

4.3.1 Ginsenoside Rd

Ginsenosides are regarded as the main active metabolites of ginseng, which can be extracted from Araliaceae such as *Panax ginseng* C.A.Mey. [Araliaceae], *Panax quinquefolius* L. [Araliaceae] and *Panax notoginseng* (Burkill) F.H.Chen [Araliaceae], contain various monomeric saponin metabolites such as Rd, Rg1 and Rb1, are widely studied. The mechanism underlying the neuroprotective effect of Rd is the inhibition of microglial proteasome activity, thereby inhibiting microglial activation and reducing inflammatory factors, iNOS, and COX-2 expression, followed by the inhibition of inhibitory subunit of IκBα phosphorylation and NF-κB nuclear translocation mediated inflammation, and its anti-inflammatory inhibition had significantly less adverse effects than glucocorticoids (Zhou et al., 2024); Rg1 reversed cerebral I/R-induced increases in IL-1β, caspase-1, ASC, and NLPR1. Alleviates neuroinflammation in ischemic stroke by inhibiting NADPH oxidase 2 (NOX2) mediated ROS accumulation and Ca^{2+} mediated calcineurin-nuclear factor of activated t-cells 1(CN-NFAT1) activation (Mohebbati, 2023). Its anti-inflammatory mechanisms also include decreased pro-inflammatory factor expression, inhibition of IκBα phosphorylation and NF-κB nuclear translocation, decreases proteasome activity and protein aggregate accumulation in the brain (Wang L. et al., 2018; Zheng et al., 2019). RB1 can activate PPARγ, decrease phosphorylated NF-κB p65 levels, which inhibit the production of proinflammatory cytokines, as well as neuroinflammation by inhibiting MMP-9 and NADPH oxidase 4 (NOX4) derived free radicals, thereby maintaining the integrity of the BBB ischemic stroke and ameliorating brain injury (Su et al., 2022; Chen et al., 2015; Yu et al., 2024). Although ginsenosides exhibit the lowest distribution to the brain compared to other organs, their brain-to-serum ratio was significantly higher than that of albumin, indicating their ability to cross the BBB (Ye et al., 2013).

4.3.2 Astragaloside IV

Astragaloside IV, the most major active metabolite of *Astragalus mongholicus* (Fisch.) Bunge [Fabaceae] membranaceus, has shown positive therapeutic effects on ischemic stroke in both animal and cellular experiments. Inflammation is one of the essential pathological processes in ischemic stroke, and the anti-inflammatory mechanisms of Astragaloside IV include activation of Nrf2 to inhibit NLRP3/caspase-1/Gasdermin-D (GSDMD) pathway mediated pyroptosis, inhibition of NLRP3 inflammasome activation and NF-κB phosphorylation, increased PPARγ expression, promotes the polarization of microglia/macrophages from M1 to M2 phenotype, enhances the secretion of neurotrophic and growth factors by M2 microglia/macrophages in the ischemic area, and promotes neurogenesis and angiogenesis (Li L. et al., 2021; Chen et al., 2022; Xiao et al., 2021; Tang et al., 2019; Hou et al., 2024). Inhibition of STAT2 activation to suppress CCL3 mediated brain infiltration of NK cells, reduced NK cell transcription of Natural Killer Group 2D and IFN-γ release,

TABLE 3 Saponins inhibit neuroinflammation in ischemic stroke.

Natural plant compound	PubChem CID	Sources	Model	Dose	Mechanisms	References
Ginsenoide Rg1	441923	*Panax ginseng* C.A.Mey. [Araliaceae], *Panax quinquefolius* L. [Araliaceae], *Panax notoginseng* (Burkill) F.H.Chen [Araliaceae]	Rats MCAO model	20, 40 mg/kg	BDNF↑ IL-1β, IL-6, TNF-α, Glu,Asp↓	(Zhou et al., 2024)
			Rats I/R model	10, 20, 40 mg/kg	IκBα phosphorylation and NF-κB nuclear translocatin↓ Proteasomal activity and protein aggregate accumulation↓	(Mohebbati, 2023)
Ginsenoside Rb1	9898279		Rats MCAO/R model	50 mg/kg	PPARγ signaling pathway	(Wang et al., 2018b)
			Rats MCAO model	5, 20, 40 mg/kg	Nitric oxide synthase, IL-1β, MMP9, NOX↓ Arginase 1, IL-10↑ Tight junction proteins↑	(Zheng et al., 2019)
Astragaloside IV	13943297	*Astragalus mongholicus* (Fisch.) Bunge [Fabaceae]	OGD/R Rats MCAO/R model	10 mL/kg 5, 10, 20, 40, 80 μg/mL	NLRP3/Caspase-1/GSDMD pathway	(Li et al., 2021c)
			Rats I/R model	28, 56 mg/kg	NLRP3, NF-κB, Caspase-1, IL-1β, IL-18↓	(Chen et al., 2022)
			Rats MCAO	20.40 mg/kg	STAT3↓ NK cell infiltration and activation↓	(Tang et al., 2019)
			Photochemical brain ischemia model, IL-17 knock out mice	200 mg/kg	Akt/GSK-3β and Wnt/β-catenin pathway	(Hou et al., 2024)
Salidroside	159278	*Rhodiola rosea* L. [Crassulaceae]	BV2 cell OGD/R model, Rats MCAO/R model	6.25, 12.5, 25, 50, 100 μM 50 mg/kg	TLR4/NF-κB signaling pathway	(Sun et al., 2020)
			Rats MCAO/R model	50 mg/kg	PI3K/Akt/HIF Signaling pathway	(Stępnik and Kukula-Koch, 2020)
			Rats pMCAO model	25, 50, 100 mg/kg	PI3K/PKB/Nrf2/NFκB Signaling Pathway	(Liu et al., 2021)

OGD, Oxygen-glucose deprivation; I/R, ischemia reperfusion; MCAO, middle cerebral artery occlusion; OGD/R, oxygen-glucose deprivation/reoxygenation; MCAO/R, middle cerebral artery occlusion/reperfusion; NSCs, neural stem cells; tMCAO, transient middle cerebral artery occlusion; pMCAO, permanent middle cerebral artery occlusion; ↓, downregulation or inhibition; ↑, upregulation or activation.

attenuated NK cell-mediated cytolytic killing of neurons and brain injury (Li S. et al., 2021). Downregulation of IL-7 protein expression, followed by upregulation of AKT/glycogen synthase kinase-3β (GSK-3β) and Wnt/β-catenin pathway, which activates the stemness of neural stem cells, inhibits neuronal apoptosis, promotes neurogenesis, and ultimately alleviates cognitive deficits after stroke (Sun et al., 2020). Although pharmacokinetic studies indicate that astragaloside IV exhibits poor oral bioavailability and blood-brain barrier (BBB) permeability, it can be detected in brain tissue following ischemic stroke (Du et al., 2022; Stępnik and Kukula-Koch, 2020). This phenomenon is likely attributable to BBB disruption after ischemic stroke.

4.3.3 Salidroside

Salidroside, the main active metabolite of *Rhodiola rosea* L. [Crassulaceae], has anti-inflammatory, antioxidant, immunomodulatory, free radical scavenging, and inhibitory effects on neural apoptosis. The anti-inflammatory effects of salidroside involve multiple signaling pathways, including inhibition of TLR3/NF-κB signaling pathway that inhibits cerebral I/R-induced NLRP4 inflammasome activation and apoptosis in microglia. Activation of the PI3K/AKT/HIF signaling pathway reduces the expression of integrin alpha-M (CD11b) and inflammatory mediators. Regulation of PI3K/PKB/Nrf2/NF-κB signaling pathways to reduce neuroinflammation and nerve injury (Liu et al., 2021; Wei et al., 2017; Zhang et al., 2019). Moreover, salidroside can also promote microglial M2 polarization, enhance microglial phagocytosis and inhibit microglial derived inflammatory cytokine release (Ji et al., 2025). Despite demonstrating significant anti-inflammatory efficacy following ischemic stroke, salidroside exhibits extremely low bioavailability in the brain based on pharmacokinetic studies—a critical challenge requiring resolution (Fan et al., 2020).

TABLE 4 Diterpenoids inhibits neuroinflammation in ischemic stroke.

Natural plant compound	PubChem CID	Sources	Model	Dose	Mechanisms	References
Tanshinne IIA	164676	*Salvia miltiorrhiza* Bunge [Lamiaceae], *Saxifraga stolonifera* Curtis [Saxifragaceae]	Rats MCAO model	8 mg/kg	GFAP, caspase-3, caspase-8↓	(Arefnezhad et al., 2024)
			Rats I/R model	4, 8 mg/kg	NeuN, Protein disulfide isomerase, Na, K-ATPase↑ Microglial activation↓	(Carpi et al., 2023)
			Rats pMCAO model	5, 10, 20 mg/kg	SOD↑ MDA, NO, iNOS, NF-κB nuclear translocatin↓	(Zhou et al., 2015)
			Rats I/R model	25 mg/kg	MIF, MPO, IL-6, TNF-α, NF-κB↓	(Wen et al., 2016)
Triptolide	107985	*Tripterygium wilfordii* Hook.f. [Celastraceae]	PBMCs and NK Cells	0.4, 2, 5 ng/mL	p38MAPK signaling pathways	(Long et al., 2020)
			Rats I/R model	12.5, 25, 50 mg/kg	Wnt/β-catenin signaling pathway SOD↑ MDA, ROS↓	(Gao et al., 2021)
Ginkgolide B	11973122	*Ginkgo biloba* L. [Ginkgoaceae]	BV2 microglia, macrophages, Rats tMCAO model	5 μg/mL 5 mg/kg	Microglia/macrophage M2 polarization↑	(Pan and Xu, 2020)
			Rats tMCAO model	10, 20, 40 mg/kg	NF-κB signaling pathway	(Yang et al., 2024e)

MCAO, Middle cerebral artery occlusion; I/R, ischemia reperfusion; pMCAO, permanent middle cerebral artery occlusion; OGD, oxygen-glucose deprivation; tMCAO, transient middle cerebral artery occlusion; BBB, blood-brain barrier; ↓, downregulation or inhibition; ↑, upregulation or activation.

4.4 Diterpenoids

Diterpenoids with anti-inflammatory activity in ischemic stroke include Tanshinone IIA, Triptolide, and Ginkgolide B (Table 4).

4.4.1 Tanshinone IIA

Tanshinone IIA can be extracted from *Salvia miltiorrhiza* Bunge, *Saxifraga stolonifera* Curtis [Saxifragaceae] and other traditional Chinese medicines, it has many effects such as improving coronary circulation, anti-atherosclerosis, and anti-inflammation. Cerebral I/R injury is closely related to the inflammatory response, and Tanshinone IIA can relieve the pro-inflammatory response induced by I/R. The mechanisms include reducing the expression of GFAP, inhibiting the excessive activation of astrocytes, inhibiting the activation of microglia, downregulating the expression of macrophage migration inhibitory factor in neurons, decreasing NF-κB activation and the release of various inflammatory factors, inhibiting oxidative stress and free radical mediated inflammatory damage (Xie et al., 2023; Arefnezhad et al., 2024; Carpi et al., 2023; Zhou et al., 2015; Wen et al., 2016; Dong et al., 2009; Chen et al., 2012). Studies indicate that Tanshinone IIA monomer exhibits poor BBB permeability; however, borneol significantly enhances its delivery to brain tissue (Zhang et al., 2021). Furthermore, intranasal administration demonstrates considerable potential in facilitating Tanshinone IIA transport to the brain (Long et al., 2020).

4.4.2 Triptolide

Triptolide is an important diterpene active compound in *Tripterygium wilfordii* Hook.f. [Celastraceae] Since Kupchan et al. first isolated triptolide and found its significant anti-leukaemic effects, it has also been proven to have significant anti-inflammatory, immunosuppressive, anticancer and other important biological activities (Gao et al., 2021). Triptolide is a strong anti-inflammatory agent, reduces astrocyte activation, downregulates NF-κB and p38 MAPK signaling pathway, and inhibits the release of IL-1β, iNOS, COX-2, TNF-α and other proinflammatory factors, alleviates BBB damage (Wang N. et al., 2023). In addition to the classical NF-κB pathway the mechanism of Triptolide treatment in ischemic stroke also involves inhibition of inflammatory responses mediated by activation of the Wnt/β-catenin signaling pathway (Pan and Xu, 2020). Furthermore, its favorable BBB permeability and comparable pharmacokinetic profiles simultaneously observed in cerebral and systemic compartments highlight triptolide's promise as a clinically translatable natural plant compound (Zhu et al., 2020).

4.4.3 Ginkgolide B

Ginkgolides, a class of active metabolites extracted from *Ginkgo biloba* L. [Ginkgoaceae], of which Ginkgolide B is the most physiologically active and the strongest antagonist of platelet activating factor discovered to date, are commonly used in the clinic to treat ischemic stroke and also have protective effects on damaged neurons. The protective function exhibited by Ginkgolide B in ischemic stroke, such as reducing infarct volume, ameliorating neurological deficits, and alleviating the increase in BBB permeability, is associated with its anti-inflammatory properties. Both *in vivo* and *in vitro* experiments have confirmed its ability to promote the conversion of microglia/macrophages from an inflammatory M1 phenotype to an anti-inflammatory

TABLE 5 Alkaloids inhibit neuroinflammation in ischemic stroke.

Natural plant compound	PubChem CID	Sources	Model	Dose	Mechanisms	References
Ligustrazine (Tetramethylpyrazine)	14296	*Conioselinum anthriscoides* 'Chuanxiong' [Apiaceae]	Rats I/R model	40 mg/kg	JAK/STAT signaling pathway	(Qi et al., 2024)
			Rats MCAO model	25, 50 mg/kg	MCPIP1↑ IL-1β, IL-6, TNF-α, MMP-9↓	(Gu et al., 2012)
			Rats MCAO model	20 mg/kg	Circ_0008146-miR-709-Cx3cr1 axis	(Jin et al., 2021)
			Permanent cerebral ischemia rat model	20 mg/kg	Akt and ERK phosphorylation, HMGB1, TLR4, iNOS, NO↓ Nrf2, HO-1↑	(Gong et al., 2019)
Berberine	2353	*Coptis chinensis* Franch. [Ranunculaceae], *Phellodendron amurense* Rupr. [Rutaceae]	OGD/R Rats tMCAO model	10 μM	AMPK signaling pathways	(Mu et al., 2023)
			Rats tMCAO model	25, 50 mg/kg	HMGB1/TLR4/NF-κB signaling	(Ding et al., 2023)
			Rats MCAO/R models	50 mg/kg	IL-1β,TNF-α,IL-6↓ IL-10↑	(Zhu et al., 2019)
			Rats MCAO model	50 mg/kg	Malat1/miR-181c-5p/HMGB1 axis	(Zhu et al., 2018)

I/R, Ischemia reperfusion; MCAO, Middle cerebral artery occlusion; tMCAO, transient middle cerebral artery occlusion; pMCAO, permanent middle cerebral artery occlusion; BBB, blood-brain barrier; ↓, downregulation or inhibition; ↑, upregulation or activation.

M2 phenotype (Li et al., 2023; Yang Y. et al., 2024); Ginkgolide B anti-inflammatory mechanism mainly involves NF-κB signaling pathway, which plays a neuroprotective role by inhibiting the activation of NF-κB and subsequent sequence responses (Huang et al., 2021; Gu et al., 2012). The poor hydrophilicity and lipophilicity of ginkgolide B significantly limit its ability to cross the BBB. However, research by Yang Li et al. demonstrated that conjugating ginkgolide B with highly lipophilic docosahexaenoic acid (DHA) to form the covalent complex ginkgolide B-DHA not only enhances the pharmacological effects of ginkgolide B, but also facilitates its stable encapsulation within liposomes. This approach improves solubility and consequently enhances therapeutic efficacy against ischemic stroke (Li et al., 2023).

4.5 Alkaloids

Alkaloids with anti-inflammatory activity in ischemic stroke include Ligustrazine and Berberine (Table 5).

4.5.1 Ligustrazine

The traditional Chinese medicine *Conioselinum anthriscoides* 'Chuanxiong' [Apiaceae] has the effects of invigorating blood flow and relieving pain, Ligustrazine also known as tetramethylpyrazine, is a potent monomer isolated from the alkaloids of *Conioselinum anthriscoides* because its anti-inflammatory properties are widely used in ischemic stroke (Qi et al., 2024). Mechanistically, tetramethylpyrazine can suppress inflammation and protect BBB integrity by blocking the JAK/STAT signaling pathway as well as upregulating monocyte chemotactic protein-induced protein 1 (MCPIP1) expression (Jin et al., 2021; Gong et al., 2019). The most recent study found that tetramethylpyrazine significantly increased mir-709 expression levels, decreased C-X3-C Motif Chemokine Receptor 1 (Cx3cr1) expression levels, and decreased the concentrations of IL-2 and TNF-α that significantly inhibited cell death, improved neurological scores, reduced infarct volumes. Knocked down circ_0008146 also presented the same effect, suggesting that tetramethylpyrazine targeted circ_0008146/miR-709/Cx3cr1 axis exerts neuroprotective and anti-inflammatory effects (Li L. et al., 2022). Furthermore, tetramethylpyrazine exerts neuroprotective effects in cerebral ischemia by elevating Nrf2/HO-1 expression and inhibiting HMGB1/TLR4, AKT, and Extracellular Signal-Regulated Kinase (ERK) signaling to suppress neutrophil activation, promote endogenous anti-inflammatory defenses, and attenuate proinflammatory responses (Chang et al., 2015). Ligustrazine exhibits low bioavailability and poor BBB permeability; however, nanoparticle and liposome encapsulation strategies demonstrate considerable potential in addressing these limitations (Wen et al., 2022; Mu et al., 2023).

4.5.2 Berberine

Berberine, an isoquinoline derivative alkaloid extracted from medicinal herbs such as *Coptis chinensis* Franch. [Ranunculaceae] and *Phellodendron amurense* Rupr. [Rutaceae], exhibits diverse pharmacological activities (Ding et al., 2023). In ischemic stroke models, berberine demonstrates significant anti-inflammatory effects through multiple molecular mechanisms. The underlying anti-inflammatory mechanisms may involve: (1) modulation of microglial polarization via AMPK signaling pathway regulation (Zhu et al., 2019); (2) suppression of TLR4/NF-κB signaling activation by inhibiting HMGB1 nucleocytoplasmic translocation (Zhu et al., 2018; Duan et al., 2023); and (3) enhancement of butyrate production through gut microbiota

modulation to reduce inflammatory cytokine expression. Notably, recent studies have revealed that berberine's regulatory effect on HMGB1 is associated with lncRNA Malat1, where lncRNA Malat1 competitively binds miR-181c-5p - a direct HMGB1 target - to regulate HMGB1 expression following ischemic stroke (Cao et al., 2020). However, the precise molecular targets directly mediating berberine's anti-inflammatory actions remain to be fully elucidated. Furthermore, berberine is a substrate of P-glycoprotein and cytochrome P450 enzymes. Its low oral bioavailability and poor BBB penetration significantly limit its clinical application. However, promisingly, numerous studies have demonstrated the potential of novel delivery strategies or encapsulation materials to improve berberine's bioavailability and BBB permeability (Murakami et al., 2023; Abo El-Enin et al., 2022).

5 Discussion and perspectives

Inflammatory storm mediated by activated immune cells and inflammatory mediators constitutes a critical factor exacerbating cerebral tissue damage following ischemic stroke (DeLong et al., 2022). Targeting neuroinflammation has emerged as a pivotal strategy for improving clinical outcomes in stroke patients. Natural plant compounds with diverse bioactivities have been extensively investigated in various disease models, including ischemic stroke. These phytochemicals can attenuate post-ischemic neuroinflammation through multiple mechanisms: suppressing immune cell activation, inhibiting inflammatory mediator production, and blocking peripheral immune cell infiltration. The remarkable anti-inflammatory properties of natural plant compounds position them as promising therapeutic candidates for ischemic stroke treatment (Shang et al., 2022).

Nevertheless, several unresolved challenges persist in advancing natural plant compounds from preclinical research to clinical applications for stroke therapy. The primary limitation lies in their suboptimal bioavailability. Many phytochemicals exhibit poor water solubility, directly compromising their absorption and distribution. Furthermore, the selective permeability of the BBB substantially restricts compound delivery to cerebral ischemic regions (Huang et al., 2024). A second critical issue involves ambiguous pharmacological targets and mechanisms of action. While numerous natural products demonstrate anti-inflammatory properties in experimental stroke models, their precise molecular targets and downstream signaling pathways remain incompletely characterized. This knowledge gap hinders rational drug optimization and target validation. Thirdly, methodological limitations in preclinical studies warrant attention: Translational relevance is compromised by predominant use of young animals in modeling a predominantly geriatric disease (Finger et al., 2022); Temporal dynamics of therapeutic evaluation require refinement, as most investigations focus on acute phase outcomes despite the chronic nature of post-stroke inflammation (Ahsan et al., 2021); Standardization issues persist regarding dosage regimens (administration routes and therapeutic windows), introducing variability that undermines inter-study comparability. Finally, clinical translation faces dual barriers: The translational gap between rodent models and human pathophysiology, particularly regarding comorbidities and aging-related biological changes; Significant inter-patient heterogeneity in stroke manifestations and treatment responses, necessitating large-scale clinical trials to ensure robust conclusions. Addressing these challenges through integrated pharmacokinetic optimization, mechanism-driven research, standardized preclinical protocols, and rigorous clinical validation will be crucial for harnessing the therapeutic potential of natural plant compounds in stroke management (Tao et al., 2020; Gong et al., 2023).

Recent advancements in pharmacology, molecular biology, nanotechnology, and artificial intelligence have unveiled novel therapeutic avenues for natural plant compounds in stroke management (Tao et al., 2020). Notably, nanoparticle-based delivery systems have demonstrated significant potential in overcoming solubility limitations and enhancing BBB penetration. For instance, curcumin-loaded nanoparticles effectively improve bioavailability and facilitate anti-inflammatory effects in cerebral ischemia models (Li F. et al., 2021). Additionally, baicalin nanoparticles delivered via the intranasal route successfully target brain tissues in experimental stroke models (Yu et al., 2023). The integration of omics technologies, including transcriptomics, metabolomics, and proteomics, has substantially accelerated mechanistic investigations of various natural plant compounds in ischemic stroke. Concurrently, artificial intelligence-assisted molecular docking techniques have been widely adopted for natural target screening, proving crucial for identifying direct molecular targets and elucidating pharmacological mechanisms of natural plant compounds. Moreover, while clinical trials directly investigating natural plant compounds in ischemic stroke remain limited, their safety profiles have been extensively documented through clinical studies in other disease contexts (Bai et al., 2025; Conforti et al., 2024). This existing evidence provides a foundation for conducting well-designed clinical trials under strict ethical guidelines to evaluate natural product-based interventions for ischemic stroke. Such systematic clinical investigations would significantly advance the clinical translation of natural therapeutics for cerebrovascular protection, potentially offering novel treatment strategies to improve neurological outcomes in stroke patients.

Author contributions

PN: Data curation, Formal Analysis, Writing – original draft, Writing – review and editing, Conceptualization, Software. YZ: Project administration, Visualization, Conceptualization, Writing – original draft, Writing – review and editing, Data curation. BW: Conceptualization, Software, Supervision, Writing – review and editing. SQ: Conceptualization, Formal Analysis, Validation, Visualization, Writing – review and editing. QD: Conceptualization, Formal Analysis, Investigation, Project administration, Resources, Writing – review and editing. LL: Data curation, Formal Analysis, Resources, Writing – review and editing. YL: Conceptualization, Data curation, Methodology, Writing – review and editing.

Funding

The author(s) declare that financial support was received for the research and/or publication of this article. This work was supported by grants from the Huzhou Public Welfare Application Research Project (2022GYB03) and Zhejiang University of Traditional Chinese Medicine 2023 Affiliated Hospital Research Specialization (2023FSYYZY18).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declare that no Generative AI was used in the creation of this manuscript.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

Abo El-Enin, H. A., Elkomy, M. H., Naguib, I. A., Ahmed, M. F., Alsaidan, O. A., Alsalahat, I., et al. (2022). Lipid nanocarriers overlaid with chitosan for brain delivery of berberine via the nasal route. *Pharmaceuticals (Basel)* 15, 281. doi:10.3390/ph15030281

Ahsan, A., Liu, M., Zheng, Y., Yan, W., Pan, L., Li, Y., et al. (2021). Natural compounds modulate the autophagy with potential implication of stroke. *Acta Pharm. Sin. B* 11, 1708–1720. doi:10.1016/j.apsb.2020.10.018

An, H.-M., Li, M.-N., Yang, H., Pang, H.-Q., Qu, C., Xu, Y., et al. (2021). A validated UHPLC-MS/MS method for pharmacokinetic and brain distribution studies of twenty constituents in rat after oral administration of Jia-Wei-Qi-Fu-Yin. *J. Pharm. Biomed. Anal.* 202, 114140. doi:10.1016/j.jpba.2021.114140

Arefnezhad, R., Nejabat, A., Behjati, F., Torkamanche, M., Zarei, H., Yekkehbash, M., et al. (2024). Tanshinone IIA against cerebral ischemic stroke and Ischemia- reperfusion injury: a review of the current documents. *Mini Rev. Med. Chem.* 24, 1701–1709. doi:10.2174/0113895575299721240227070032

Baell, J., and Walters, M. A. (2014). Chemistry: chemical con artists foil drug discovery. *Nature* 513, 481–483. doi:10.1038/513481a

Bai, Y.-X., Wu, H.-L., Xie, W.-L., Li, X., Han, J.-J., Liu, J., et al. (2025). Efficacy and safety of gastrodin in preventing postoperative delirium following cardiac surgery: a randomized placebo controlled clinical trial. *Crit. Care* 29, 108. doi:10.1186/s13054-025-05331-9

Baker, M. (2017). Deceptive curcumin offers cautionary tale for chemists. *Nature* 541, 144–145. doi:10.1038/541144a

Bissacotti, B. F., Copetti, P. M., Bottari, N. B., Palma, T. V., Pillat, M. M., de Andrade, C. M., et al. (2023). Curcumin modulates neurogliogenesis and purinergic receptor expression in neural precursor cells infected with Toxoplasma gondii. *Parasitol. Res.* 122, 77–84. doi:10.1007/s00436-022-07698-y

Cao, D.-W., Liu, M.-M., Duan, R., Tao, Y.-F., Zhou, J.-S., Fang, W.-R., et al. (2020). The lncRNA Malat1 functions as a ceRNA to contribute to berberine-mediated inhibition of HMGB1 by sponging miR-181c-5p in poststroke inflammation. *Acta Pharmacol. Sin.* 41, 22–33. doi:10.1038/s41401-019-0284-y

Carpi, S., Quarta, S., Doccini, S., Saviano, A., Marigliano, N., Polini, B., et al. (2023). Tanshinone IIA and cryptotanshinone counteract inflammation by regulating gene and miRNA expression in human SGBS adipocytes. *Biomolecules* 13, 1029. doi:10.3390/biom13071029

Chang, C. Y., Kao, T. K., Chen, W. Y., Ou, Y. C., Li, J. R., Liao, S. L., et al. (2015). Tetramethylpyrazine inhibits neutrophil activation following permanent cerebral ischemia in rats. *Biochem. Biophys. Res. Commun.* 463, 421–427. doi:10.1016/j.bbrc.2015.05.088

Chen, R., Zhang, X., Gu, L., Zhu, H., Zhong, Y., Ye, Y., et al. (2021). New insight into neutrophils: a potential therapeutic target for cerebral ischemia. *Front. Immunol.* 12, 692061. doi:10.3389/fimmu.2021.692061

Chen, T., Li, Z., Li, S., Zou, Y., Gao, X., Shu, S., et al. (2022). Cycloastragenol suppresses M1 and promotes M2 polarization in LPS-Stimulated BV-2 cells and ischemic stroke mice. *Int. Immunopharmacol.* 113, 109290. doi:10.1016/j.intimp.2022.109290

Chen, W., Guo, Y., Yang, W., Zheng, P., Zeng, J., and Tong, W. (2015). Protective effect of ginsenoside Rb1 on integrity of blood-brain barrier following cerebral ischemia. *Exp. Brain Res.* 233, 2823–2831. doi:10.1007/s00221-015-4352-3

Chen, Y., Wu, X., Yu, S., Lin, X., Wu, J., Li, L., et al. (2012). Neuroprotection of tanshinone IIA against cerebral ischemia/reperfusion injury through inhibition of macrophage migration inhibitory factor in rats. *PLoS One* 7, e40165. doi:10.1371/journal.pone.0040165

Chiang, M.-C., Nicol, C. J. B., Lo, S.-S., Hung, S.-W., Wang, C.-J., and Lin, C.-H. (2022). Resveratrol mitigates oxygen and glucose deprivation-induced inflammation, NLRP3 inflammasome, and oxidative stress in 3D neuronal culture. *Int. J. Mol. Sci.* 23, 11678. doi:10.3390/ijms231911678

Chien, M.-Y., Chuang, C.-H., Chern, C.-M., Liou, K.-T., Liu, D.-Z., Hou, Y.-C., et al. (2016). Salvianolic acid A alleviates ischemic brain injury through the inhibition of inflammation and apoptosis and the promotion of neurogenesis in mice. *Free Radic. Biol. Med.* 99, 508–519. doi:10.1016/j.freeradbiomed.2016.09.006

Conforti, A., Iorio, G. G., Di Girolamo, R., Rovetto, M. Y., Picarelli, S., Cariati, F., et al. (2024). The impact of resveratrol on the outcome of the *in vitro* fertilization: an exploratory randomized placebo-controlled trial. *J. Ovarian Res.* 17, 81. doi:10.1186/s13048-024-01391-7

Dai, M., Chen, B., Wang, X., Gao, C., and Yu, H. (2021). Icariin enhance mild hypothermia-induced neuroprotection via inhibiting the activation of NF-κB in experimental ischemic stroke. *Metab. Brain Dis.* 36, 1779–1790. doi:10.1007/s11011-021-00731-6

Dai, Y., Ban, W., and Yang, Z. (2024). Gastrodin, a promising natural small molecule for the treatment of central nervous system disorders, and its recent progress in synthesis, pharmacology and pharmacokinetics. *Int. J. Mol. Sci.* 25, 9540. doi:10.3390/ijms25179540

DeLong, J. H., Ohashi, S. N., O'Connor, K. C., and Sansing, L. H. (2022). Inflammatory responses after ischemic stroke. *Semin. Immunopathol.* 44, 625–648. doi:10.1007/s00281-022-00943-7

Ding, W., Gu, Q., Liu, M., Zou, J., Sun, J., and Zhu, J. (2023). Astrocytes-derived exosomes pre-treated by berberine inhibit neuroinflammation after stroke via miR-182-5p/Rac1 pathway. *Int. Immunopharmacol.* 118, 110047. doi:10.1016/j.intimp.2023.110047

Dong, K., Xu, W., Yang, J., Qiao, H., and Wu, L. (2009). Neuroprotective effects of Tanshinone IIA on permanent focal cerebral ischemia in mice. *Phytother. Res.* 23, 608–613. doi:10.1002/ptr.2615

Dou, Z., Rong, X., Zhao, E., Zhang, L., and Lv, Y. (2019). Neuroprotection of resveratrol against focal cerebral ischemia/reperfusion injury in mice through a mechanism targeting gut-brain axis. *Cell Mol. Neurobiol.* 39, 883–898. doi:10.1007/s10571-019-00687-3

Du, Y., Li, C., Xu, S., Yang, J., Wan, H., and He, Y. (2022). LC-MS/MS combined with blood-brain dual channel microdialysis for simultaneous determination of active components of astragali radix-safflower combination and neurotransmitters in rats with cerebral ischemia reperfusion injury: application in pharmacokinetic and pharmacodynamic study. *Phytomedicine* 106, 154432. doi:10.1016/j.phymed.2022.154432

Duan, H., Hu, J., Deng, Y., Zou, J., Ding, W., Peng, Q., et al. (2023). Berberine mediates the production of butyrate to ameliorate cerebral ischemia via the gut microbiota in mice. *Nutrients* 16, 9. doi:10.3390/nu16010009

Fan, F., Yang, L., Li, R., Zou, X., Li, N., Meng, X., et al. (2020). Salidroside as a potential neuroprotective agent for ischemic stroke: a review of sources, pharmacokinetics, mechanism and safety. *Biomed. Pharmacother.* 129, 110458. doi:10.1016/j.biopha.2020.110458

Fan, Y., Luo, Q., Wei, J., Lin, R., Lin, L., Li, Y., et al. (2018). Mechanism of salvianolic acid B neuroprotection against ischemia/reperfusion induced cerebral injury. *Brain Res.* 1679, 125–133. doi:10.1016/j.brainres.2017.11.027

Feigin, V. L., Brainin, M., Norrving, B., Martins, S. O., Pandian, J., Lindsay, P., et al. (2025). World stroke organization: global stroke fact sheet 2025. *Int. J. Stroke* 20, 132–144. doi:10.1177/17474930241308142

Feng, S.-Q., Aa, N., Geng, J.-L., Huang, J.-Q., Sun, R.-B., Ge, C., et al. (2017). Pharmacokinetic and metabolomic analyses of the neuroprotective effects of salvianolic acid A in a rat ischemic stroke model. *Acta Pharmacol. Sin.* 38, 1435–1444. doi:10.1038/aps.2017.114

Feng, Z., Sun, Q., Chen, W., Bai, Y., Hu, D., and Xie, X. (2019). The neuroprotective mechanisms of ginkgolides and bilobalide in cerebral ischemic injury: a literature review. *Mol. Med.* 25, 57. doi:10.1186/s10020-019-0125-y

Finger, C. E., Moreno-Gonzalez, I., Gutierrez, A., Moruno-Manchon, J. F., and McCullough, L. D. (2022). Age-related immune alterations and cerebrovascular inflammation. *Mol. Psychiatry* 27, 803–818. doi:10.1038/s41380-021-01361-1

Fletes-Vargas, G., Rodríguez-Rodríguez, R., Pacheco, N., Pérez-Larios, A., and Espinosa-Andrews, H. (2023). Evaluation of the biological properties of an optimized extract of Polygonum cuspidatum using ultrasonic-assisted extraction. *Molecules* 28, 4079. doi:10.3390/molecules28104079

Gao, J., Zhang, Y., Liu, X., Wu, X., Huang, L., and Gao, W. (2021). Triptolide: pharmacological spectrum, biosynthesis, chemical synthesis and derivatives. *Theranostics* 11, 7199–7221. doi:10.7150/thno.57745

Gong, P., Zhang, Z., Zou, Y., Tian, Q., Han, S., Xu, Z., et al. (2019). Tetramethylpyrazine attenuates blood-brain barrier disruption in ischemia/reperfusion injury through the JAK/STAT signaling pathway. *Eur. J. Pharmacol.* 854, 289–297. doi:10.1016/j.ejphar.2019.04.028

Gong, Z., Guo, J., Liu, B., Guo, Y., Cheng, C., Jiang, Y., et al. (2023). Mechanisms of immune response and cell death in ischemic stroke and their regulation by natural compounds. *Front. Immunol.* 14, 1287857. doi:10.3389/fimmu.2023.1287857

Gu, J. H., Ge, J. B., Li, M., Wu, F., Zhang, W., and Qin, Z. H. (2012). Inhibition of NF-κB activation is associated with anti-inflammatory and anti-apoptotic effects of ginkgolide B in a mouse model of cerebral ischemia/reperfusion injury. *Eur. J. Pharm. Sci.* 47, 652–660. doi:10.1016/j.ejps.2012.07.016

Guo, W., Xu, X., Xiao, Y., Zhang, J., Shen, P., Lu, X., et al. (2024). Salvianolic acid C attenuates cerebral ischemic injury through inhibiting neuroinflammation via the TLR4-TREM1-NF-κB pathway. *Chin. Med.* 19, 46. doi:10.1186/s13020-024-00914-0

Han, P.-P., Han, Y., Shen, X.-Y., Gao, Z.-K., and Bi, X. (2023). NLRP3 inflammasome activation after ischemic stroke. *Behav. Brain Res.* 452, 114578. doi:10.1016/j.bbr.2023.114578

Hao, Z., Zhang, Z., Zhao, Y., and Wang, D. (2023). Baicalin reduces immune cell infiltration by inhibiting inflammation and protecting tight junctions in ischemic stroke injury. *Am. J. Chin. Med.* 51, 355–372. doi:10.1142/S0192415X23500180

Hilkens, N. A., Casolla, B., Leung, T. W., and de Leeuw, F.-E. (2024). Stroke. *Lancet.* 403, 2820–2836. doi:10.1016/S0140-6736(24)00642-1

Hou, Y., Yan, Z., Wan, H., Yang, J., Ding, Z., and He, Y. (2024). A combination of astragaloside IV and hydroxysafflor yellow A attenuates cerebral ischemia-reperfusion injury via NF-κB/NLRP3/Caspase-1/GSDMD pathway. *Brain Sci.* 14, 781. doi:10.3390/brainsci14080781

Huang, L., Huang, X.-H., Yang, X., Hu, J.-Q., Zhu, Y.-Z., Yan, P.-Y., et al. (2024). Novel nano-drug delivery system for natural products and their application. *Pharmacol. Res.* 201, 107100. doi:10.1016/j.phrs.2024.107100

Huang, L., Shi, Y., and Zhao, L. (2021). Ginkgolide B alleviates learning and memory impairment in rats with vascular dementia by reducing neuroinflammation via regulating NF-κB pathway. *Front. Pharmacol.* 12, 676392. doi:10.3389/fphar.2021.676392

Jayaraj, R. L., Azimullah, S., Beiram, R., Jalal, F. Y., and Rosenberg, G. A. (2019). Neuroinflammation: friend and foe for ischemic stroke. *J. Neuroinflammation* 16, 142. doi:10.1186/s12974-019-1516-2

Ji, W., Zhang, Z., Jin, T., Meng, D., Zhou, X., Hu, J., et al. (2025). Salidroside attenuates cognitive deficits induced by chronic cerebral hypoperfusion via modulating microglial phenotypic transformation in mice. *J. Neuroimmunol.* 400, 578544. doi:10.1016/j.jneuroim.2025.578544

Jin, Z., Liang, J., and Kolattukudy, P. E. (2021). Tetramethylpyrazine preserves the integrity of blood-brain barrier associated with upregulation of MCPIP1 in a murine model of focal ischemic stroke. *Front. Pharmacol.* 12, 710358. doi:10.3389/fphar.2021.710358

Lan, Z., Tan, F., He, J., Liu, J., Lu, M., Hu, Z., et al. (2024). Curcumin-primed olfactory mucosa-derived mesenchymal stem cells mitigate cerebral ischemia/reperfusion injury-induced neuronal PANoptosis by modulating microglial polarization. *Phytomedicine* 129, 155635. doi:10.1016/j.phymed.2024.155635

Lei, P., Li, Z., Hua, Q., Song, P., Gao, L., Zhou, L., et al. (2023). Ursolic acid alleviates neuroinflammation after intracerebral hemorrhage by mediating microglial pyroptosis via the NF-κB/NLRP3/GSDMD pathway. *Int. J. Mol. Sci.* 24, 14771. doi:10.3390/ijms241914771

Li, C., Sui, C., Wang, W., Yan, J., Deng, N., Du, X., et al. (2021b). Baicalin attenuates oxygen-glucose Deprivation/reoxygenation-induced injury by modulating the BDNF-TrkB/PI3K/Akt and MAPK/Erk1/2 signaling axes in neuron-astrocyte cocultures. *Front. Pharmacol.* 12, 599543. doi:10.3389/fphar.2021.599543

Li, F., Xu, Y., Li, X., Wang, X., Yang, Z., Li, W., et al. (2021a). Triblock copolymer nanomicelles loaded with curcumin attenuates inflammation via inhibiting the NF-κB pathway in the rat model of cerebral ischemia. *Int. J. Nanomedicine* 16, 3173–3183. doi:10.2147/IJN.S300379

Li, J., Zhang, S., Lu, M., Chen, Z., Chen, C., Han, L., et al. (2013). Hydroxysafflor yellow A suppresses inflammatory responses of BV2 microglia after oxygen-glucose deprivation. *Neurosci. Lett.* 535, 51–56. doi:10.1016/j.neulet.2012.12.056

Li, L., Gan, H., Jin, H., Fang, Y., Yang, Y., Zhang, J., et al. (2021c). Astragaloside IV promotes microglia/macrophages M2 polarization and enhances neurogenesis and angiogenesis through PPARγ pathway after cerebral ischemia/reperfusion injury in rats. *Int. Immunopharmacol.* 92, 107335. doi:10.1016/j.intimp.2020.107335

Li, L., Zhang, D., Yao, W., Wu, Z., Cheng, J., Ji, Y., et al. (2022b). Ligustrazine exerts neuroprotective effects via circ_0008146/miR-709/Cx3cr1 axis to inhibit cell apoptosis and inflammation after cerebral ischemia/reperfusion injury. *Brain Res. Bull.* 190, 244–255. doi:10.1016/j.brainresbull.2022.10.011

Li, S., Dou, B., Shu, S., Wei, L., Zhu, S., Ke, Z., et al. (2021d). Suppressing NK cells by astragaloside IV protects against acute ischemic stroke in mice via inhibiting STAT3. *Front. Pharmacol.* 12, 802047. doi:10.3389/fphar.2021.802047

Li, X., Li, S., Ma, C., Li, T., and Yang, L. (2022a). Preparation of baicalin-loaded ligand-modified nanoparticles for nose-to-brain delivery for neuroprotection in cerebral ischemia. *Drug Deliv.* 29, 1282–1298. doi:10.1080/10717544.2022.2064564

Li, Y., Zhang, M., Li, S., Zhang, L., Kim, J., Qiu, Q., et al. (2023). Selective ischemic-hemisphere targeting ginkgolide B liposomes with improved solubility and therapeutic efficacy for cerebral ischemia-reperfusion injury. *Asian J. Pharm. Sci.* 18, 100783. doi:10.1016/j.ajps.2023.100783

Ling, Y., Jin, L., Ma, Q., Huang, Y., Yang, Q., Chen, M., et al. (2021). Salvianolic acid A alleviated inflammatory response mediated by microglia through inhibiting the activation of TLR2/4 in acute cerebral ischemia-reperfusion. *Phytomedicine* 87, 153569. doi:10.1016/j.phymed.2021.153569

Liu, J., Ma, W., Zang, C. H., Wang, G. D., Zhang, S. J., Wu, H. J., et al. (2021). Salidroside inhibits NLRP3 inflammasome activation and apoptosis in microglia induced by cerebral ischemia/reperfusion injury by inhibiting the TLR4/NF-κB signaling pathway. *Ann. Transl. Med.* 9, 1694. doi:10.21037/atm-21-5752

Liu, X., Mei, Z., Qian, J., Zeng, Y., and Wang, M. (2013). Puerarin partly counteracts the inflammatory response after cerebral ischemia/reperfusion via activating the cholinergic anti-inflammatory pathway. *Neural Regen. Res.* 8, 3203–3215. doi:10.3969/j.issn.1673-5374.2013.34.004

Liu, X., Sui, X., Zhang, Y., Yue, R., and Yin, S. (2023). Efficacy of puerarin in rats with focal cerebral ischemia through modulation of the SIRT1/HIF-1α/VEGF signaling pathway and its effect on synaptic plasticity. *Heliyon* 9, e15872. doi:10.1016/j.heliyon.2023.e15872

Liu, Z., Ran, Y., Huang, S., Wen, S., Zhang, W., Liu, X., et al. (2017). Curcumin protects against ischemic stroke by titrating microglia/macrophage polarization. *Front. Aging Neurosci.* 9, 233. doi:10.3389/fnagi.2017.00233

Long, Y., Li, X., He, X., Wang, Z., Wu, Y., Sun, L., et al. (2025). Baicalin liposomes ameliorate cerebral ischemia-reperfusion-induced acute lung injury by modulating the inflammatory response. *Brain Res.* 1859, 149642. doi:10.1016/j.brainres.2025.149642

Long, Y., Yang, Q., Xiang, Y., Zhang, Y., Wan, J., Liu, S., et al. (2020). Nose to brain drug delivery - a promising strategy for active components from herbal medicine for treating cerebral ischemia reperfusion. *Pharmacol. Res.* 159, 104795. doi:10.1016/j.phrs.2020.104795

López-Morales, M. A., Castelló-Ruiz, M., Burguete, M. C., Hervás, D., Pérez-Pinzón, M. A., and Salom, J. B. (2023). Effect and mechanisms of resveratrol in animal models of ischemic stroke: a systematic review and Bayesian meta-analysis. *J. Cereb. Blood Flow. Metab.* 43, 2013–2028. doi:10.1177/0271678X231206236

Lu, Y., Zhou, R., Zhu, R., Wu, X., Liu, J., Ma, Y., et al. (2025). Baicalin ameliorates neuroinflammation by targeting TLR4/MD2 complex on microglia via PI3K/AKT/NF-κB signaling pathway. *Neuropharmacology* 267, 110296. doi:10.1016/j.neuropharm.2025.110296

Lv, H., Wang, L., Shen, J., Hao, S., Ming, A., Wang, X., et al. (2015). Salvianolic acid B attenuates apoptosis and inflammation via SIRT1 activation in experimental stroke rats. *Brain Res. Bull.* 115, 30–36. doi:10.1016/j.brainresbull.2015.05.002

Mo, Z. T., Zheng, J., and Liao, Y. L. (2021). Icariin inhibits the expression of IL-1β, IL-6 and TNF-α induced by OGD/R through the IRE1/XBP1s pathway in microglia. *Pharm. Biol.* 59, 1473–1479. doi:10.1080/13880209.2021.1991959

Mohebbati, R. (2023). Ginsenoside Rg1 attenuates cerebral ischemia-reperfusion injury through inhibiting the inflammation activation of microglia. *Exp. Cell Res.* 430, 113690. doi:10.1016/j.yexcr.2023.113690

Mu, Q., Yao, K., Syeda, M. Z., Zhang, M., Cheng, Q., Zhang, Y., et al. (2023). Ligustrazine nanoparticle hitchhiking on neutrophils for enhanced therapy of cerebral ischemia-reperfusion injury. *Adv. Sci. (Weinh)* 10, e2301348. doi:10.1002/advs.202301348

Murakami, T., Bodor, E., and Bodor, N. (2023). Approaching strategy to increase the oral bioavailability of berberine, a Quaternary ammonium isoquinoline alkaloid: part 1. Physicochemical and pharmacokinetic properties. *Expert Opin. Drug Metab. Toxicol.* 19, 129–137. doi:10.1080/17425255.2023.2203857

Pan, W., and Xu, Z. (2020). Triptolide mediates Wnt/β-catenin signalling pathway to reduce cerebral ischemia-reperfusion injury in rats. *Folia Neuropathol.* 58, 324–333. doi:10.5114/fn.2020.102435

Peng, Z., Wang, S., Chen, G., Cai, M., Liu, R., Deng, J., et al. (2015). Gastrodin alleviates cerebral ischemic damage in mice by improving anti-oxidant and anti-inflammation activities and inhibiting apoptosis pathway. *Neurochem. Res.* 40, 661–673. doi:10.1007/s11064-015-1513-5

Potter, T. B. H., Tannous, J., and Vahidy, F. S. (2022). A contemporary review of epidemiology, risk factors, etiology, and outcomes of premature stroke. *Curr. Atheroscler. Rep.* 24, 939–948. doi:10.1007/s11883-022-01067-x

Qi, M., Su, X., Li, Z., Huang, H., Wang, J., Lin, N., et al. (2024). Bibliometric analysis of research progress on tetramethylpyrazine and its effects on ischemia-reperfusion injury. *Pharmacol. Ther.* 259, 108656. doi:10.1016/j.pharmthera.2024.108656

Ramalingam, P., and Ko, Y. T. (2016). Validated LC-MS/MS method for simultaneous quantification of resveratrol levels in mouse plasma and brain and its application to pharmacokinetic and brain distribution studies. *J. Pharm. Biomed. Anal.* 119, 71–75. doi:10.1016/j.jpba.2015.11.026

Seyedi, Z., Amiri, M. S., Mohammadzadeh, V., Hashemzadeh, A., Haddad-Mashadrizeh, A., Mashreghi, M., et al. (2023). Icariin: a promising natural product in biomedicine and tissue engineering. *J. Funct. Biomater.* 14, 44. doi:10.3390/jfb14010044

Shang, Y., Zhang, Z., Tian, J., and Li, X. (2022). Anti-inflammatory effects of natural products on cerebral ischemia. *Front. Pharmacol.* 13, 914630. doi:10.3389/fphar.2022.914630

Shen, H., Pei, H., Zhai, L., Guan, Q., and Wang, G. (2022). Salvianolic acid C improves cerebral ischemia reperfusion injury through suppressing microglial cell M1 polarization and promoting cerebral angiogenesis. *Int. Immunopharmacol.* 110, 109021. doi:10.1016/j.intimp.2022.109021

Sheng, C., Peng, W., Xia, Z., and Wang, Y. (2020). Plasma and cerebrospinal fluid pharmacokinetics of hydroxysafflor yellow A in patients with traumatic brain injury after intravenous administration of xuebijing using LC-MS/MS method. *Xenobiotica* 50, 545–551. doi:10.1080/00498254.2019.1668983

Si, W., He, P., Wang, Y., Fu, Y., Li, X., Lin, X., et al. (2019). Complement complex C5b-9 levels are associated with the clinical outcomes of acute ischemic stroke and carotid plaque stability. *Transl. Stroke Res.* 10, 279–286. doi:10.1007/s12975-018-0658-3

Song, J., Zhang, W., Sun, J., Zhang, X., Xu, X., Zhang, L., et al. (2016). Determination of salvianolic acid C in rat plasma using liquid chromatography-mass spectrometry and its application to pharmacokinetic study. *Biomed. Chromatogr.* 30, 376–383. doi:10.1002/bmc.3558

Sowndhararajan, K., Deepa, P., Kim, M., Park, S. J., and Kim, S. (2017). Baicalein as a potent neuroprotective agent: a review. *Biomed. Pharmacother.* 95, 1021–1032. doi:10.1016/j.biopha.2017.08.135

Stępnik, K., and Kukula-Koch, W. (2020). *In silico* studies on triterpenoid saponins permeation through the blood-brain barrier combined with postmortem research on the brain tissues of mice affected by astragaloside IV administration. *Int. J. Mol. Sci.* 21, 2534. doi:10.3390/ijms21072534

Su, L. J., Ren, Y. C., Chen, Z., Ma, H. F., Zheng, F., Li, F., et al. (2022). Ginsenoside Rb1 improves brain, lung, and intestinal barrier damage in middle cerebral artery occlusion/reperfusion (MCAO/R) micevia the PPARγ signaling pathway. *Chin. J. Nat. Med.* 20, 561–571. doi:10.1016/s1875-5364(22)60204-8

Sun, L., Han, R., Guo, F., Chen, H., Wang, W., Chen, Z., et al. (2020). Antagonistic effects of IL-17 and astragaloside IV on cortical neurogenesis and cognitive behavior after stroke in adult mice through Akt/GSK-3β pathway. *Cell Death Discov.* 6, 74. doi:10.1038/s41420-020-00298-8

Széplaki, G., Szegedi, R., Hirschberg, K., Gombos, T., Varga, L., Karádi, I., et al. (2009). Strong complement activation after acute ischemic stroke is associated with unfavorable outcomes. *Atherosclerosis* 204, 315–320. doi:10.1016/j.atherosclerosis.2008.07.044

Tang, B., Tang, W. J., Tang, Y. H., and Deng, C. Q. (2019). Astragaloside IV attenuates cerebral ischemia and reperfusion injury and reduces activation of NLRP3 inflammasome and NF-κB phosphorylation in rats following a transient middle cerebral artery occlusion. *Sheng Li Xue Bao* 71, 424–430.

Tao, B., Pei, J., Li, H., Yang, G., Shi, X., Zhang, Z., et al. (2024). Inhibition of P2X7R alleviates neuroinflammation and brain edema after traumatic brain injury by suppressing the NF-κB/NLRP3 inflammasome pathway. *J. Neurorestoratology* 12, 100106. doi:10.1016/j.jnrt.2024.100106

Tao, T., Liu, M., Chen, M., Luo, Y., Wang, C., Xu, T., et al. (2020). Natural medicine in neuroprotection for ischemic stroke: challenges and prospective. *Pharmacol. Ther.* 216, 107695. doi:10.1016/j.pharmthera.2020.107695

Titus, C., Hoque, M. T., and Bendayan, R. (2024). PPAR agonists for the treatment of neuroinflammatory diseases. *Trends Pharmacol. Sci.* 45, 9–23. doi:10.1016/j.tips.2023.11.004

Tsai, M.-M., Chen, J.-L., Lee, T.-H., Liu, H., Shanmugam, V., and Hsieh, H.-L. (2022). Brain protective effect of resveratrol via ameliorating Interleukin-1β-Induced MMP-9-Mediated disruption of ZO-1 arranged integrity. *Biomedicines* 10, 1270. doi:10.3390/biomedicines10061270

Wang, H., Zhang, S., Xie, L., Zhong, Z., and Yan, F. (2023a). Neuroinflammation and peripheral immunity: focus on ischemic stroke. *Int. Immunopharmacol.* 120, 110332. doi:10.1016/j.intimp.2023.110332

Wang, J., Bian, L., Du, Y., Wang, D., Jiang, R., Lu, J., et al. (2022). The roles of chemokines following intracerebral hemorrhage in animal models and humans. *Front. Mol. Neurosci.* 15, 1091498. doi:10.3389/fnmol.2022.1091498

Wang, L., Zhao, H., Zhai, Z. Z., and Qu, L. X. (2018b). Protective effect and mechanism of ginsenoside Rg1 in cerebral ischaemia-reperfusion injury in mice. *Biomed. Pharmacother.* 99, 876–882. doi:10.1016/j.biopha.2018.01.136

Wang, N., Min, X., Ma, N., Zhu, Z., Cao, B., Wang, Y., et al. (2023b). The negative impact of triptolide on the immune function of human natural killer cells. *Pharmaceuticals (Basel)* 16, 458. doi:10.3390/ph16030458

Wang, S., Nan, Y., Zhu, W., Yang, T., Tong, Y., and Fan, Y. (2018a). Gastrodin improves the neurological score in MCAO rats by inhibiting inflammation and apoptosis, promoting revascularization. *Int. J. Clin. Exp. Pathol.* 11, 5343–5350.

Wang, X., Yang, Y., Liu, X., and Gao, X. (2020a). Pharmacological properties of tanshinones, the natural products from *Salvia miltiorrhiza*. *Adv. Pharmacol.* 87, 43–70. doi:10.1016/bs.apha.2019.10.001

Wang, X., Yu, H., Zhang, Y., Chang, X., Liu, C., Wen, X., et al. (2024). Curcumin alleviates osteoarthritis through the p38MAPK pathway: network pharmacological prediction and experimental confirmation. *J. Inflamm. Res.* 17, 5039–5056. doi:10.2147/JIR.S459867

Wang, Y., Chen, G., Yu, X., Li, Y., Zhang, L., He, Z., et al. (2016). Salvianolic acid B ameliorates cerebral ischemia/reperfusion injury through inhibiting TLR4/MyD88 signaling pathway. *Inflammation* 39, 1503–1513. doi:10.1007/s10753-016-0384-5

Wang, Z., Ren, W., Zhao, F., Han, Y., Liu, C., and Jia, K. (2020b). Curcumin amends Ca(2+) dysregulation in microglia by suppressing the activation of P2X7 receptor. *Mol. Cell Biochem.* 465, 65–73. doi:10.1007/s11010-019-03668-8

Wei, Y., Hong, H., Zhang, X., Lai, W., Wang, Y., Chu, K., et al. (2017). Salidroside inhibits inflammation through PI3K/Akt/HIF signaling after focal cerebral ischemia in rats. *Inflammation* 40, 1297–1309. doi:10.1007/s10753-017-0573-x

Wen, P. Y., Li, J., Lu, B. L., Liu, J., Yang, F. Z., Zhou, L., et al. (2016). Tanshinone IIA increases levels of NeuN, protein disulfide isomerase, and Na+/K+-ATPase and decreases evidence of microglial activation after cerebral ischemic injury. *Neuroreport* 27, 435–444. doi:10.1097/wnr.0000000000000559

Wen, Y., Zhang, Z., Cai, Z., Liu, B., Wu, Z., and Liu, Y. (2022). Ligustrazine-loaded borneol liposome alleviates cerebral ischemia-reperfusion injury in rats. *ACS Biomater. Sci. Eng.* 8, 4930–4941. doi:10.1021/acsbiomaterials.2c00847

Xiao, G., Tang, R., Yang, N., and Chen, Y. (2023). Review on pharmacological effects of gastrodin. *Arch. Pharm. Res.* 46, 744–770. doi:10.1007/s12272-023-01463-0

Xiao, L., Dai, Z., Tang, W., Liu, C., and Tang, B. (2021). Astragaloside IV alleviates cerebral ischemia-reperfusion injury through NLRP3 inflammasome-mediated pyroptosis inhibition via activating Nrf2. *Oxid. Med. Cell Longev.* 2021, 9925561. doi:10.1155/2021/9925561

Xie, X., Xu, Y., Zhou, X., Su, P., Jiang, X., and Jin, Z. (2023). The protective effect of an extract of *Salvia miltiorrhiza* bunge (danshen) on cerebral ischemic injury in animal models: a systematic review and meta-analysis. *J. Ethnopharmacol.* 317, 116772. doi:10.1016/j.jep.2023.116772

Xu, G., Dong, F., Su, L., Tan, Z.-X., Lei, M., Li, L., et al. (2024). The role and therapeutic potential of nuclear factor κB (NF-κB) in ischemic stroke. *Biomed. Pharmacother.* 171, 116140. doi:10.1016/j.biopha.2024.116140

Xu, S., Zhong, A., Ma, H., Li, D., Hu, Y., Xu, Y., et al. (2017). Neuroprotective effect of salvianolic acid B against cerebral ischemic injury in rats via the CD40/NF-κB pathway associated with suppression of platelets activation and neuroinflammation. *Brain Res.* 1661, 37–48. doi:10.1016/j.brainres.2017.02.011

Yang, H., Ibrahim, M. M., Zhang, S., Sun, Y., Chang, J., Qi, H., et al. (2024b). Targeting post-stroke neuroinflammation with salvianolic acid A: molecular mechanisms and preclinical evidence. *Front. Immunol.* 15, 1433590. doi:10.3389/fimmu.2024.1433590

Yang, K., Zeng, L., He, Q., Wang, S., Xu, H., and Ge, J. (2024a). Advancements in research on the immune-inflammatory mechanisms mediated by NLRP3 inflammasome in ischemic stroke and the regulatory role of natural plant products. *Front. Pharmacol.* 15, 1250918. doi:10.3389/fphar.2024.1250918

Yang, Q., Li, R., Hong, Y., Liu, H., Jian, C., and Zhao, S. (2024d). Curcumin-loaded gelatin nanoparticles cross the blood-brain barrier to treat ischemic stroke by attenuating oxidative stress and neuroinflammation. *Int. J. Nanomedicine* 19, 11633–11649. doi:10.2147/IJN.S487628

Yang, X., Xia, L., Shen, C., Li, J., Dong, X., and Liu, J. (2024c). Curcumin alleviates heatstroke-induced liver injury in dry-heat environments by inhibiting the expression of NF-κB, iNOS, and ICAM-1 in rats. *PLoS One* 19, e0309598. doi:10.1371/journal.pone.0309598

Yang, Y., He, Y., Wei, X., Wan, H., Ding, Z., Yang, J., et al. (2021). Network pharmacology and molecular docking-based mechanism study to reveal the protective effect of salvianolic acid C in a rat model of ischemic stroke. *Front. Pharmacol.* 12, 799448. doi:10.3389/fphar.2021.799448

Yang, Y., Wu, Q., Shan, X., Zhou, H., Wang, J., Hu, Y., et al. (2024e). Ginkgolide B attenuates cerebral ischemia-reperfusion injury via inhibition of ferroptosis through disrupting NCOA4-FTH1 interaction. *J. Ethnopharmacol.* 318, 116982. doi:10.1016/j.jep.2023.116982

Ye, R., Zhao, G., and Liu, X. (2013). Ginsenoside Rd for acute ischemic stroke: translating from bench to bedside. *Expert Rev. Neurother.* 13, 603–613. doi:10.1586/ern.13.51

Yi, T., Tang, D., Wang, F., Zhang, J., Zhang, J., Wang, J., et al. (2017). Enhancing both oral bioavailability and brain penetration of puerarin using borneol in combination with preparation technologies. *Drug Deliv.* 24, 422–429. doi:10.1080/10717544.2016.1259372

Yi, X., Wang, J., Lin, W., Chen, L., Li, H., Lan, T., et al. (2022). Hydroxysafflower yellow A alleviates HK-2 cells injury in cold hypoxia/reoxygenation via mitochondrial apoptosis. *Transpl. Immunol.* 74, 101610. doi:10.1016/j.trim.2022.101610

Yu, C., Zhang, Y., Guo, Y., Shen, Z., Li, K., Chen, W., et al. (2024). Ginsenoside Rb1 attenuates mouse cerebral ischemia/reperfusion induced neurological impairments through modulation of microglial polarization. *Folia Neuropathol.* 62, 215–222. doi:10.5114/fn.2024.134300

Yu, S., Li, D., Shi, A., Long, Y., Deng, J., Ma, Y., et al. (2023). Multidrug-loaded liposomes prevent ischemic stroke through intranasal administration. *Biomed. Pharmacother.* 162, 114542. doi:10.1016/j.biopha.2023.114542

Zhang, J., Liu, S.-L., Wang, H., Shi, L.-Y., Li, J.-P., Jia, L.-J., et al. (2021). The effects of borneol on the pharmacokinetics and brain distribution of tanshinone IIA, salvianolic acid B and ginsenoside Rg1 in fufang danshen preparation in rats. *Chin. J. Nat. Med.* 19, 153–160. doi:10.1016/S1875-5364(21)60016-X

Zhang, M., Zhang, Y., Peng, J., Huang, Y., Gong, Z., Lu, H., et al. (2024b). Gastrodin against oxidative stress-inflammation crosstalk via inhibiting mtDNA/TLR9 and JAK2/STAT3 signaling to ameliorate ischemic stroke injury. *Int. Immunopharmacol.* 141, 113012. doi:10.1016/j.intimp.2024.113012

Zhang, W., Song, J., Li, W., Kong, D., Liang, Y., Zhao, X., et al. (2020). Salvianolic acid D alleviates cerebral ischemia-reperfusion injury by suppressing the cytoplasmic translocation and release of HMGB1-Triggered NF-κB activation to inhibit inflammatory response. *Mediat. Inflamm.* 2020, 9049614. doi:10.1155/2020/9049614

Zhang, W., Song, J.-K., Zhang, X., Zhou, Q.-M., He, G.-R., Xu, X.-N., et al. (2018). Salvianolic acid A attenuates ischemia reperfusion induced rat brain damage by protecting the blood brain barrier through MMP-9 inhibition and anti-inflammation. *Chin. J. Nat. Med.* 16, 184–193. doi:10.1016/S1875-5364(18)30046-3

Zhang, X., Lai, W., Ying, X., Xu, L., Chu, K., Brown, J., et al. (2019). Salidroside reduces inflammation and brain injury after permanent middle cerebral artery occlusion in rats by regulating PI3K/PKB/Nrf2/NFκB signaling rather than complement C3 activity. *Inflammation* 42, 1830–1842. doi:10.1007/s10753-019-01045-7

Zhang, Y., Ye, P., Zhu, H., Gu, L., Li, Y., Feng, S., et al. (2024a). Neutral polysaccharide from Gastrodia elata alleviates cerebral ischemia-reperfusion injury by inhibiting ferroptosis-mediated neuroinflammation via the NRF2/HO-1 signaling pathway. *CNS Neurosci. Ther.* 30, e14456. doi:10.1111/cns.14456

Zhao, C., Bai, X., Wen, A., Wang, J., and Ding, Y. (2024). The therapeutic effects of salvianolic acids on ischemic stroke: from molecular mechanisms to clinical applications. *Pharmacol. Res.* 210, 107527. doi:10.1016/j.phrs.2024.107527

Zhao, F., Wang, P., Jiao, Y., Zhang, X., Chen, D., and Xu, H. (2020). Hydroxysafflor yellow A: a systematical review on botanical resources, physicochemical properties, drug delivery system, pharmacokinetics, and pharmacological effects. *Front. Pharmacol.* 11, 579332. doi:10.3389/fphar.2020.579332

Zhao, Y., Zhang, X., Chen, X., and Wei, Y. (2022). Neuronal injuries in cerebral infarction and ischemic stroke: from mechanisms to treatment (review). *Int. J. Mol. Med.* 49, 15. doi:10.3892/ijmm.2021.5070

Zheng, J., Liao, Y., Xu, Y., and Mo, Z. (2022). Icariin attenuates ischaemic stroke through suppressing inflammation mediated by endoplasmic reticulum stress signalling pathway in rats. *Clin. Exp. Pharmacol. Physiol.* 49, 719–730. doi:10.1111/1440-1681.13645

Zheng, T., Jiang, H., Jin, R., Zhao, Y., Bai, Y., Xu, H., et al. (2019). Ginsenoside Rg1 attenuates protein aggregation and inflammatory response following cerebral ischemia and reperfusion injury. *Eur. J. Pharmacol.* 853, 65–73. doi:10.1016/j.ejphar.2019.02.018

Zhou, L., Bondy, S. C., Jian, L., Wen, P., Yang, F., Luo, H., et al. (2015). Tanshinone IIA attenuates the cerebral ischemic injury-induced increase in levels of GFAP and of caspases-3 and -8. *Neuroscience* 288, 105–111. doi:10.1016/j.neuroscience.2014.12.028

Zhou, L., Tan, F., Zhang, X., Li, Y., and Yin, W. (2024). Neuroprotection and mechanisms of ginsenosides in nervous system diseases: progress and perspectives. *IUBMB Life* 76, 862–882. doi:10.1002/iub.2862

Zhu, J., Cao, D., Guo, C., Liu, M., Tao, Y., Zhou, J., et al. (2019). Berberine facilitates angiogenesis against ischemic stroke through modulating microglial polarization via AMPK signaling. *Cell Mol. Neurobiol.* 39, 751–768. doi:10.1007/s10571-019-00675-7

Zhu, J.-R., Lu, H.-D., Guo, C., Fang, W.-R., Zhao, H.-D., Zhou, J.-S., et al. (2018). Berberine attenuates ischemia-reperfusion injury through inhibiting HMGB1 release and NF-κB nuclear translocation. *Acta Pharmacol. Sin.* 39, 1706–1715. doi:10.1038/s41401-018-0160-1

Zhu, S., Wang, X., Zheng, Z., Zhao, X.-E., Bai, Y., and Liu, H. (2020). Synchronous measuring of triptolide changes in rat brain and blood and its application to a comparative pharmacokinetic study in normal and alzheimer's disease rats. *J. Pharm. Biomed. Anal.* 185, 113263. doi:10.1016/j.jpba.2020.113263

Zietz, A., Gorey, S., Kelly, P. J., Katan, M., and McCabe, J. J. (2024). Targeting inflammation to reduce recurrent stroke. *Int. J. Stroke* 19, 379–387. doi:10.1177/17474930231207777

frontiers | Frontiers in Pharmacology

TYPE Systematic Review
PUBLISHED 21 July 2025
DOI 10.3389/fphar.2025.1573069

Check for updates

OPEN ACCESS

EDITED BY
Huazheng Liang,
Monash University - Southeast University Joint Research Institute, China

REVIEWED BY
Yuxiang Fei,
China Pharmaceutical University, China
Yin Huang,
Sichuan University, China

*CORRESPONDENCE
Yabin Zhou,
zhouyabin@hotmail.com

RECEIVED 08 February 2025
ACCEPTED 03 July 2025
PUBLISHED 21 July 2025

CITATION
Wu Z, Fu J, Zhou Y and Zhou Y (2025) Efficacy and safety of Tongxinluo in the treatment of stroke: a systematic review and meta-analysis of randomized controlled trials.
Front. Pharmacol. 16:1573069.
doi: 10.3389/fphar.2025.1573069

COPYRIGHT
© 2025 Wu, Fu, Zhou and Zhou. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

Efficacy and safety of Tongxinluo in the treatment of stroke: a systematic review and meta-analysis of randomized controlled trials

Zhixin Wu[1], Jiamei Fu[1], Yumeng Zhou[1] and Yabin Zhou[2]*

[1]Heilongjiang University of Chinese Medicine, Harbin, Heilongjiang, China, [2]The First Affiliated Hospital of Heilongjiang University of Chinese Medicine, Harbin, Heilongjiang, China

Objective: To evaluates the efficacy and safety of Tongxinluo in treating stroke.

Methods: PubMed, Web of Science, Cochrane Library, Embase, China National Knowledge Infrastructure and Wanfang databases were performed to search literature from 2000 to 2024. Randomized controlled trials evaluating Tongxinluo for stroke were included. The primary outcomes were efficacy and safety. Sensitivity and subgroup analyses were conducted to assess result stability and identify sources of heterogeneity. All analyses were conducted using Review Manager 5.4 and STATA 15.1.

Results: Fifty-one RCTs including 9,577 participants for analysis. Tongxinluo significantly outperformed the control group in efficacy [RR = 1.20, 95% CI (1.16, 1.25)]. Adverse event incidence between groups showed no significant difference [RR = 1.01, 95% CI (0.90, 1.12)]. Additionally, Tongxinluo significantly improved NIHSS, total cholesterol (TC), and serum hypersensitive C-reactive protein (hs-CRP) levels in stroke individuals.

Conclusion: Tongxinluo, as an adjunctive treatment for stroke, offers superior clinical efficacy compared to conventional treatments without increasing adverse event risk. However, due to study limitations, further multicenter, large-sample RCTs are required to confirm.

Systematic Review Registration: https://www.crd.york.ac.uk/PROSPERO/.

KEYWORDS

tongxinluo, adjunctive treatment, traditional Chinese medicine, stroke, meta-analysis

1 Introduction

Stroke is an acute neurological disorder caused by cerebrovascular disease, leading to impaired cerebral blood circulation. It can be ischemic (cerebral infarction) or hemorrhagic (including intracerebral hemorrhage, subarachnoid hemorrhage, and others). Stroke has a high incidence, recurrence, disability, mortality, and economic burden. It is the second leading cause of death worldwide and the primary cause of adult disability. In developing countries, stroke-related deaths account for about two-thirds of global deaths (Tu et al., 2023a). The 2013–2020 Stroke High-Risk Population Screening and Intervention Program in China found that the average age of first stroke onset for individuals aged 40 and older

ranged from 60.9 to 63.4 years, with over 66.6% of patients aged 40–64 years (Tu et al., 2023b). Although enrollment data standardization methods may vary, the findings suggest a younger age of stroke onset in China. Stroke incidence is higher in men than women, with the male incidence rate increasing annually from 2013 to 2020, while the female rate remained stable (Guzik and Bushnell, 2017). Primary stroke treatments, including thrombolysis, surgery, and pharmacological therapy, are associated with adverse effects. Efforts to improve patients' post-treatment quality of life are needed. Traditional Chinese medicine (TCM), with its long history in stroke treatment, is referred to as "Zhongfeng." This term, first mentioned in the *Jinkui Yaolue* (Essential Prescriptions of the Golden Cabinet), describes symptoms such as hemiplegia, numbness, facial drooping, speech difficulties, and, in severe cases, collapse and loss of consciousness. The *Jinkui Yaolue* also recorded the use of Chinese herbal decoctions for stroke treatment (Zhong et al., 2020).

Tongxinluo is an antioxidant that protects the blood-brain barrier, promotes axonal plasticity, stabilizes vulnerable plaques, inhibits delayed neuronal death, and improves stroke prognosis. Approved by the China Food and Drug Administration in 1996, it is widely used in China to treat acute unstable angina (Wu et al., 2006). Tongxinluo contains 12 traditional Chinese herbs: *Boswellia sacra Flück.,Dalbergia odorifera T.C.Chen, Panax ginseng C.A.Mey.,Paeonia lactiflora Pall.,Ziziphus jujuba Mill.,Santalum album L.,Blumea balsamifera (L.) DC.,Cucumis melo L.,Salvia miltiorrhiza Bunge, Girardinia diversifolia subsp. diversifolia, Eupolyphaga sinensis Walker, Scolopendra subspinipes mutilans* L. Koch (Chen et al., 2024). Pharmacological studies show that Tongxinluo has vasodilatory, antiplatelet, anticoagulant, thrombolytic, and lipid-lowering effects, protecting against cerebral ischemic injury (Bu et al., 2008).

A 2013 study by Jianjun Wen demonstrated that Tongxinluo effectively treats acute cerebral infarction. Involving 278 patients, it showed significant improvement in NIHSS scores after 4 weeks of treatment (Wen et al., 2013). Similarly, a 2008 study by Junke Cui, involving 112 patients, found significant improvements in acute cerebral infarction outcomes after 30 days of treatment (Cui et al., 2008).

However, Zhou et al. (2016) reported no significant improvement in NIHSS scores among stroke patients treated with Lan et al. (2013) likewise found no substantial benefit in overall therapeutic outcomes. These findings indicate that existing evidence remains inadequate to substantiate the clinical value of Tongxinluo in stroke management, with limited support from evidence-based studies. To address this gap, we conducted the first systematic review and meta-analysis to assess its efficacy, safety, and potential moderating factors in the treatment of stroke.

2 Methods and materials

2.1 Protocol and registration

Following the PRISMA guidelines and registering the study in PROSPERO (registration number CRD42024621065).

2.2 Search method

Systematic searches were conducted up to October 2024 in PubMed ((("tongxinluo" [Supplementary Concept]) OR (Tongxinluo)) AND (("Stroke, Lacunar" [Mesh]) OR ((((((((((((((((Strokes) OR (Cerebrovascular Accident)) OR (Cerebrovascular Accidents)) OR (Cerebral Stroke)) OR (Cerebral Strokes)) OR (Cerebrovascular Apoplexy)) OR (Brain Vascular Accident)) OR (Brain Vascular Accidents)) OR (Cerebrovascular Stroke)) OR (Cerebrovascular Stroke)) OR (Apoplexy)) OR (CVA)) OR (CVAs)) OR (Acute Stroke)) OR (Acute Strokes)) OR (Acute Cerebrovascular Accident)) OR (Acute Cerebrovascular Accidents)))) AND (random*), Embase, Cochrane, Web of Science, VIP Database, Chinese Biomedical Literature Database, China National Knowledge Infrastructure (CNKI), and Wanfang databases to explore the use of Tongxinluo in stroke treatment. Search terms included "Tongxinluo," "Stroke," "Cerebrovascular Accident," "Brain Vascular Accident," and "randomized controlled trials," with no linguistic or geographical restrictions. Additionally, we manually reviewed references from identified articles meeting the inclusion criteria to supplement the search. The search details were depicted in Supplementary Table S1.

2.3 Study selection

Eligible RCTs were included following a screening and evaluation of the literature through the title, abstract, and full text.

Inclusion criteria:

(a) Participants: Adults (≥18 years) with clinically diagnosed stroke, with no gender or racial restrictions.
(b) Intervention and comparison: Conventional with Tongxinluo capsules and conventional treatments.
(c) More than one outcome **exists**, including primary outcomes such as the clinical response rate and adverse event rate. Secondary outcomes-NIHSS, total cholesterol (TC), triglyceride (TG), HDL, LDL, TNF-α, hs-CRP, IL-6, IL-18, nitric oxide (NO), Barthel Index, whole blood viscosity (high and low cut), plasma viscosity, erythrocyte ratio, plasma fibrinogen, quality of life score, Fugl-Meyer (FM), MMSE, inflammatory myofibroblastic tumor (IMT), plaque area, platelet aggregation function.

The main metabolites of Tongxinluo are shown in Supplementary Table S2.

Exclusion criteria:

(a) Non-RCTs, retrospective studies, animal studies, and review literature.
(b) Patients with other cardiovascular conditions. For example, atrial fibrillation, coronary heart disease, heart failure, cardiomyopathy, valvular heart disease, and hypertension.
(c) Studies where the intervention group received alternative traditional Chinese medicine treatments, including Chinese patent medicines, pills, injections, acupuncture, massage, or auricular acupuncture.

(d) Studies with inaccurate data, incomplete outcome measurements, or lack of access to original data.
(e) Duplicate publications.

2.4 Composition and preparation methods of tongxinluo

Tongxinluo contains 12 traditional Chinese medicinal ingredients: *Boswellia sacra Flück.*, *Dalbergia odorifera T.C. Chen*, *Panax ginseng C.A. Mey.*, *Paeonia lactiflora Pall.*, *Ziziphus jujuba Mill.*, *Santalum album L.*, *Blumea balsamifera (L.) DC.*, *Cucumis melo L.*, *Salvia miltiorrhiza Bunge*, *Girardinia diversifolia subsp. diversifolia*, *E. sinensis Walker*, and *S. subspinipes mutilans L. Koch*.

The extraction of each herbal component in the Tongxinluo capsules used in this study strictly followed national pharmacopeial standards and modern Chinese medicine preparation protocols. Methods were determined based on the characteristics of each herb and the nature of its active constituents, as outlined below:

Frankincense (*Boswellia sacra Flück.*), dalbergia odorifera (*Dalbergia odorifera T.C.Chen*), and sandalwood (*Santalum album L.*): These herbs were processed using ethanol reflux extraction combined with steam distillation. After pulverization, the materials were extracted twice with six times the volume of 85% ethanol for 1.5 h each. The combined ethanol extracts were recovered and concentrated to obtain a resinous extract rich in lipophilic constituents. The residues were then subjected to steam distillation to collect volatile oils, mainly containing monoterpenes and sesquiterpenes.

Ginseng (*Panax ginseng C.A.Mey.*): Extracted by water decoction. Ginseng slices were soaked in ten times the volume of water for 0.5 h and decocted three times (2 h, 1.5 h, 1.5 h). The combined decoctions were filtered and concentrated into a clear extract with a relative density of 1.10–1.15 at 60°C, or further processed by spray drying to obtain ginseng extract powder containing ginsenosides and polysaccharides.

White peony root (*Paeonia lactiflora Pall.*) and sour jujube seed (*Ziziphus jujuba Mill.*): Extracted by water decoction. The herbs were decocted three times with eight times the volume of water (1.5 h each time). The combined extracts were filtered and concentrated. Ethanol precipitation was used to remove impurities (adjusted to 60%–70% ethanol), followed by standing and collection of the supernatant. Ethanol was recovered, and the remaining solution was dried to yield purified extracts rich in paeoniflorin, saponins, and flavonoids.

Borneo (*Blumea balsamifera (L.) DC.*) (natural borneol): As a sublimable crystalline substance, it was directly ground to a fine powder (passed through a No. 9 sieve, 200 mesh) without extraction.

Medicinal leech (*Cucumis melo L.*), cicada molt (*Salvia miltiorrhiza Bunge*), Chinese scorpion (*Girardinia diversifolia subsp. diversifolia*), Chinese cockroach (*E. sinensis* Walker), and centipede (*S. subspinipes mutilans* L. Koch): These animal-derived ingredients are rich in proteins, enzymes, and bioactive peptides and are heat-sensitive. A low-temperature ultrafine grinding technique was used. After selection and cleaning, the materials were dried below 60°C, sterilized using either cobalt-60 irradiation or steam flash sterilization, and ground into ultrafine powder (≤45 μm, passed through a 325-mesh sieve) to preserve biological activity.

The processed products were mixed according to the prescription ratio: refined water-ethanol extracts (or dried powder) of ginseng, white peony root, and sour jujube seed; ethanol extracts and volatile oils (often cyclodextrin-encapsulated) of frankincense, dalbergia odorifera, and sandalwood; fine powder of borneo; and ultrafine powders of centipede, medicinal leech, cicada molt, Chinese scorpion, and Chinese cockroach. Appropriate excipients were added, and the blend was processed through granulation, drying, sizing, and capsule filling. All procedures complied with Good Manufacturing Practice (GMP) standards.

2.5 Data extraction

Two investigators collected data independently, and the information including:

(a) Publication details;
(b) Study characteristics;
(c) Participant characteristics;
(d) Intervention details;
(e) The mean and standard deviation were recorded about continuous data, while the events' number and participants' number were noted about categorical data. Disagreements were resolved by another researcher.

2.6 Quality assessment

Two researchers assessed the literature quality using the Cochrane Risk of Bias tool. Seven components including random sequence generation, allocation concealment, blinding, completeness of outcome data, reporting bias, and other biases. Studies were classified as "high risk," "low risk," or "unclear risk." Disagreements were resolved by another researcher.

2.7 Statistical analysis

EndNote 9.0, Excel, RevMan 5.4, and Stata 15.1 were used for literature management, data organization, and analysis. For binary outcomes, the risk ratio (RR) was used; for continuous outcomes, the standardized mean difference (SMD) was applied. Cochrane's Q test and the I^2 test were used for heterogeneity assessment, with significant heterogeneity defined by $p \geq 0.1$ and $I^2 < 50\%$. A random-effects model was applied with $\alpha = 0.05$. Sensitivity analysis was performed using the one-by-one elimination method, and funnel plots along with Egger's test were used to assess publication bias. Subgroup analysis, based on population, intervention duration, and age, was performed to assess result stability and identify potential sources of heterogeneity.

3 Results

3.1 Study searching and selection

1904 articles with 623 remaining after excluding 471 duplicates included. 373 literatures were excluded according to the title and

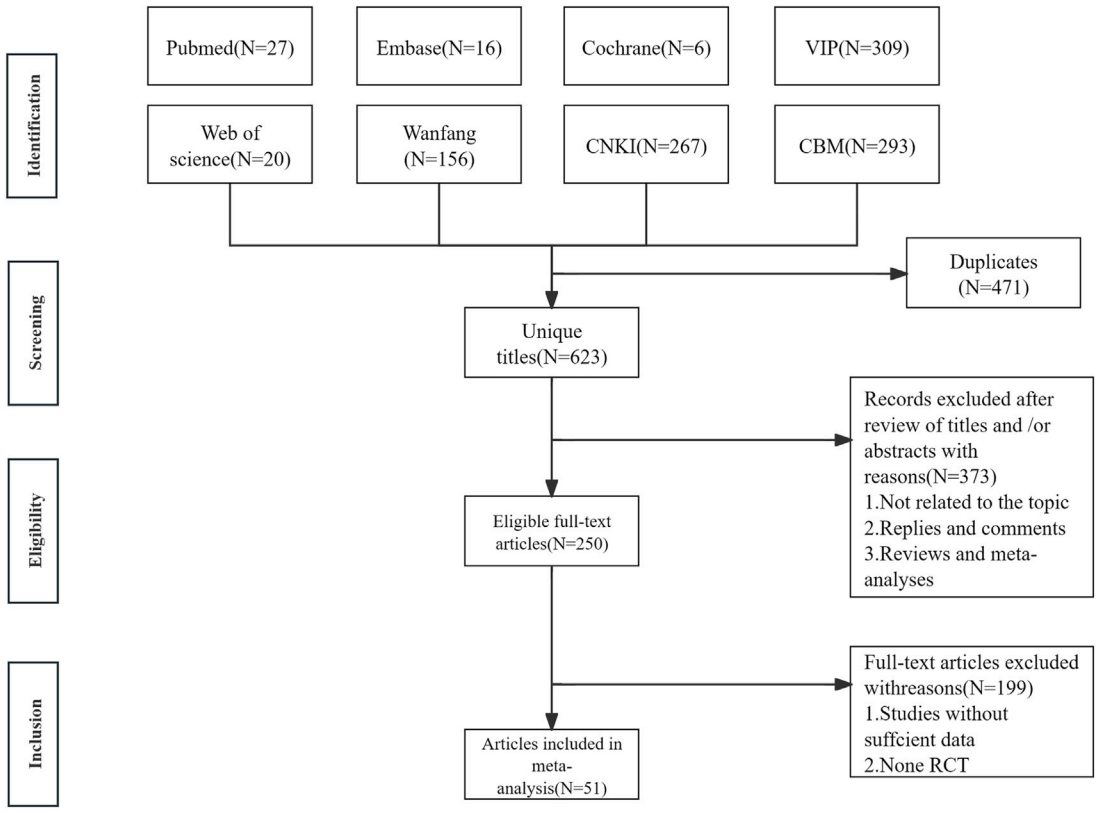


FIGURE 1
The literature searching and screening flowchart.

abstract. After reviewing the full texts, 199 articles were excluded, leaving 51 articles, as shown in Figure 1.

3.2 Study characteristics

Fifty-one RCTs' studies (Wen et al., 2013; Cui et al., 2008; Sun et al., 2000; Luo et al., 2002; Liu et al., 2003; Wang et al., 2004; Wang et al., 2005; Duan, 2006; Wang et al., 2006; Wang; Huang et al., 2007; Lui et al., 2007; Lv, 2008; Ma, 2008; Wang, 2008; Yan et al., 2008; Yang, 2008; Sun et al., 2009; Yang et al., 2009; Chu, 2010; Kong et al., 2010; Wei et al., 2010; Wu et al., 2010; Yu et al., 2010; Su et al., 2011; Liu and LI, 2012; Lan et al., 2013; Cao et al., 2014; Guo et al., 2014; Ruan and Ruan, 2014; Wang, 2014; Wang and Gai, 2014; Xue et al., 2014; Di, 2015; Huang, 2015; Tang and Yu, 2015; Wang, 2015; Wang et al., 2015; Wu et al., 2010; Wang et al., 2016; Zhou et al., 2016; Bai et al., 2017; Mi et al., 2017; Xiao, 2017; Qin et al., 2018; Chen et al., 2021; Li P.-X. et al., 2021; Wu et al., 2021; Deng and Jia, 2024; Dong et al., 2024) with 9,577 patients were included, 5,019 in the intervention group and 4,558 in the control group, both with sample sizes exceeding 100. The average age ranged from 51.20 to 72.67 years in the intervention group and from 51.20 to 71.83 years in the control group. Treatment duration varied from 2 to 48 weeks. The control group received conventional treatments such as simvastatin, aspirin, liraglutide, edaravone, and acupuncture. The intervention group received Tongxinluo capsules in addition to the control treatments (Table 1).

3.3 Bias risk assessment results

The Cochrane risk assessment tool was performed to assess literature quality. Nineteen articles were classified as low risk based on the standard allocation method. Thirty-two studies did not specify the allocation method and were classified as having an unclear risk. Three studies employed a double-blind method for allocation concealment, classified as low risk, while the others did not provide this information and were classified as having an unclear risk. Two studies reported blinding, classified as low risk, while the others lacked details and were classified as having an unclear risk. All studies had complete outcome data, classified as low risk. Seventeen studies were classified as low risk for selective reporting, while the others were classified as unclear risk. No literature identified additional sources of bias, and these were classified as low risk (Figure 2).

3.4 Results of meta-analysis

3.4.1 Overall efficacy

The overall efficacy was assessed in 35 studies (Wen et al., 2013; Cui et al., 2008; Luo et al., 2002; Liu et al., 2003; Wang et al., 2004; Wang; Huang et al., 2007; Lui et al., 2007; Lv, 2008; Ma, 2008; Yang, 2008; Sun et al., 2009; Yang et al., 2009; Chu, 2010; Kong et al., 2010; Yu et al., 2010; Liu and LI, 2012; Lan et al., 2013; Cao et al., 2014; Ruan and Ruan, 2014; Wang, 2014; Wang and Gai, 2014; Xue et al.,

TABLE 1 The basic characteristics of literature.

Study	study period	region	study design	Population	Intervention	Intervention time	Control
Liu et al. (2003)	2002–2003	China	RCT	Cerebral infarction	Tongxinluo	30 days	Buyang also five soup + acupuncture
Xiao (2017)	2013–2015	China	RCT	Acute cerebral infarction	Tongxinluo + Conventional treatment + acupuncture	—	Conventional treatment
Deng and Jia (2024)	2021–2023	China	RCT	Cerebral infarction + Type 2 diabetes	Tongxinluo + Conventional treatment	12 weeks	Conventional treatment + liraglutide
Deng and Jia (2024)	2021–2024	China	RCT	Cerebral infarction + Type 2 diabetes	Tongxinluo + Conventional treatment + liraglutide	12 weeks	Conventional treatment + liraglutide
Cao et al. (2014)	—	China	RCT	Cerebral infarction	Tongxinluo	28 days	Naoxintong capsule
Su et al. (2011)	2003–2004	China	RCT	Cerebral infarction	Tongxinluo	28 days	Naoxintong capsule
Wu et al. (2010)	2008–2009	China	RCT	Acute cerebral infarction	Tongxinluo + Conventional treatment	—	Simvastatin + Conventional treatment
Wang et al. (2004)	—	China	RCT	Acute cerebral infarction	Tongxinluo + Conventional treatment	3 weeks	Cerebral thrombosis tablet + Conventional treatment
Yan et al. (2008)	2003–2004	China	RCT	Acute cerebral infarction	Tongxinluo + Conventional treatment	12 months	Conventional treatment
Yao et al. (2006)	—	China	RCT	Cerebral infarction	Tongxinluo + aspirin	15 days	Dihydroergosine mesylate + aspirin
Wu and Li (2015)	2009–2013	China	RCT	Cerebral infarction + Type 2 diabetes	Tongxinluo	4 weeks	Simvastatin + Conventional treatment
Wu and Li (2015)	2,209–2013	China	RCT	Cerebral infarction + Type 2 diabetes	Tongxinluo + Simvastatin	4 weeks	Simvastatin + Conventional treatment
Huang (2015)	2008–2011	China	RCT	Cerebral infarction + Type 2 diabetes	Tongxinluo	4 weeks	Simvastatin + Conventional treatment
Huang (2015)	2008–2011	China	RCT	Cerebral infarction + Type 2 diabetes	Tongxinluo + Simvastatin	4 weeks	Simvastatin + Conventional treatment
Kong et al. (2010)	2004–2009	China	RCT	Acute cerebral infarction	Tongxinluo + Conventional treatment	3 weeks	Conventional treatment
Lui et al. (2007)	2002–2005	China	RCT	Cerebral infarction convalescence period	Tongxinluo + Conventional treatment	30 days	Conventional treatment
Wang (2006)	2002–2005	China	RCT	Cerebral infarction convalescence period	Tongxinluo + Conventional treatment	24 weeks	Conventional treatment
Lan et al. (2013)	2011–2013	China	RCT	Acute cerebral infarction	Tongxinluo + Conventional treatment + Acupuncture and moxibustion	30 days	Conventional treatment + acupuncture
Guo et al. (2014)	2012–2013	China	RCT	Cerebral infarction	Tongxinluo	8 weeks	Simvastatin
Lv (2008)	2005–2006	China	RCT	Acute cerebral infarction	Tongxinluo	4 weeks	Conventional treatment

(Continued on following page)

10.3389/fphar.2025.1573069

TABLE 1 (*Continued*) The basic characteristics of literature.

Study	study period	region	study design	Population	Intervention	Intervention time	Control
Xue et al. (2014)	—	China	RCT	Cerebral infarction	Tongxinluo + Atorvastatin + aspirin	3 months	atorvastatin + aspirin
Wang and Gai (2014)	2010–2012	China	RCT	Acute cerebral infarction	Tongxinluo + Clopidogrel + aspirin	14 days	aspirin
Li et al. (2021a)	2015–2020	China	RCT	Acute cerebral infarction	Tongxinluo + Clopidogrel	1 month	clopidogrel
Wang et al. (2016)	2014–2015	China	RCT	Cerebral infarction	Tongxinluo + Conventional treatment + Probucol	12 months	probucol + Conventional treatment
Zhou et al. (2016)	2014–2015	China	RCT	hypertensive intracerebral hemorrhage	Tongxinluo + Conventional treatment + Nimodipine	4 weeks	nimodipine + Conventional treatment
Chen et al. (2021)	2017–2019	China	RCT	Acute cerebral infarction	Tongxinluo + Conventional treatment + Edaravone	—	Edaravone + Conventional treatment
Wu et al. (2021)	2017–2019	China	RCT	Acute cerebral infarction	Tongxinluo + Conventional treatment + Edaravone	14 days	Edaravone + Conventional treatment
Ma (2008)	—	China	RCT	Cerebral infarction	Tongxinluo + Conventional treatment	1 month	Weinolutong + Conventional treatment
Wang and Gai (2014)	2011–2013	China	RCT	hypertensive cerebral hemorrhage	Tongxinluo + Conventional treatment	—	Conventional treatment
Wang (2006)	2002–2005	China	RCT	Acute cerebral infarction	Tongxinluo + Conventional treatment	2 weeks	Conventional treatment
Ruan and Ruan (2014)	2009–2013	China	RCT	Acute cerebral infarction	Tongxinluo + Salvia ligustrazine injection + biaspirin	15 days	Salvia ligustrazine injection + biaspirin
Wen et al. (2013)	2010–2013	China	RCT	Acute cerebral infarction	Tongxinluo + Conventional treatment	4 weeks	Conventional treatment
Wei et al. (2010)	2006–2006	China	RCT	Acute cerebral infarction	Tongxinluo + Conventional treatment	3 months	Conventional treatment
Liu and Li (2012)	2009–2011	China	RCT	Cerebral infarction	Tongxinluo + Conventional treatment	3 months	Conventional treatment
Di (2015)	—	China	RCT	Acute cerebral infarction	Tongxinluo + Conventional treatment	30 days	Conventional treatment
Chu (2010)	2006–2008	China	RCT	Cerebral infarction	Tongxinluo + aspirin	28 days	aspirin
Luo et al. (2002)	2001–2002	China	RCT	Acute cerebral infarction	Tongxinluo + Conventional treatment	4 weeks	Danshen injection + Conventional treatment
Cui et al. (2008)	2006–2007	China	RCT	Acute cerebral infarction	Tongxinluo + Conventional treatment + puerarin	30 days	Conventional treatment + puerarin
Tang and Wu (2015)	2011–2013	China	RCT	Cerebral infarction	Tongxinluo + Atorvastatin	6 eeksw	atorvastatin
Sun et al. (2000)	—	China	RCT	Cerebral infarction	Tongxinluo + Danshen injection	4 weeks	Danshen injection
Wang et al. (2005)	2003–2004	China	RCT	Cerebral infarction	Tongxinluo + Conventional treatment	14 days	Conventional treatment

(Continued on following page)

10.3389/fphar.2025.1573069

TABLE 1 (*Continued*) The basic characteristics of literature.

Study	study period	region	study design	Population	Intervention	Intervention time	Control
Yu et al. (2010)	2007–2008	China	RCT	Cerebral infarction complicated with hyperlipidemia	Tongxinluo + Cytidiphosphate choline, aspirin, A-Lo	1 month	Cytidiphosphate choline, aspirin, A-Lo
Yang et al. (2008)	2007–2008	China	RCT	Acute cerebral infarction	Tongxinluo + Cytidiphosphate choline, aspirin, Salvia miltiorrhiza	4 weeks	Cytidiphosphate choline, aspirin, Salvia miltiorrhiza
Wang (2015)	2010–2013	China	RCT	Acute cerebral infarction	Tongxinluo + butylphenylpeptide	2.5 months	butylphenylpeptide
Yang et al. (2009)	2007–2009	China	RCT	Acute cerebral infarction	Tongxinluo + Cytidiphosphate choline, aspirin, Salvia miltiorrhiza, piracetam	4 weeks	Cytidiphosphate choline, aspirin, Salvia miltiorrhiza, piracetam
Mi et al. (2017)	2014–2015	China	RCT	Cerebral infarction	Tongxinluo + Conventional treatment	6 months	Conventional treatment
Duan (2006)	2004–2005	China	RCT	Acute cerebral infarction	Tongxinluo + Conventional treatment	4 weeks	metabolite Xueshuantong capsule + Conventional treatment
Huang et al. (2007)	2004–2005	China	RCT	Acute cerebral infarction	Tongxinluo + Conventional treatment	2 weeks	Conventional treatment
Wang et al. (2008)	—	China	RCT	Cerebral infarction	Tongxinluo + Cytidiphosphate choline	4 weeks	Cytidiphosphate choline + piracetam
Qin et al. (2018)	2016–2017	China	RCT	Cerebral infarction	Tongxinluo	—	acupuncture
Wang et al. (2015)	2012–2013	China	RCT	Cerebral infarction	Tongxinluo	14 days	acupuncture
Bai et al. (2017)	2012–2014	China	RCT	Cerebral infarction	Tongxinluo + Conventional treatment	12 months	Conventional treatment
Sun et al. (2009)	2007–2008	China	RCT	Acute cerebral infarction	Tongxinluo + Conventional treatment	8 weeks	Conventional treatment
Dong et al. (2024)	2014–2016	United States of America	RCT	Acute cerebral infarction	Tongxinluo	90 days	placebo

Study	Patients		Mean/median age		Male		Mean/median course of disease		Research findings
	Intervention	Control	Intervention	Control	Intervention	Control	Intervention	Control	
Liu et al. (2003)	60	60	57.26 ± 11.49	58.74 ± 11.74	91		17.22 ± 4.45 d	19.76 ± 6.02 d	Tongxinluo demonstrated an overall efficacy rate of 81.67% and a marked improvement rate of 48.33% in stroke patients. No significant adverse reactions were observed during or after the treatment period.
Xiao (2017)	63	63	65.1 ± 4.7	64.7 ± 4.1	35	33	—		**Tongxinluo combined with acupuncture significantly improves the efficacy rate and NIHSS scores in stroke patients compared to conventional treatment.**
Deng and Jia (2024)	50	50	62.59 ± 6.20		91		—		Compared to liraglutide alone, Tongxinluo combined with liraglutide significantly improves patients' IMT, TC, TG, LDL-C, TNF-α, IL-6, hs-

(Continued on following page)

10.3389/fphar.2025.1573069

TABLE 1 (*Continued*) The basic characteristics of literature.

Study	Patients		Mean/median age		Male		Mean/median course of disease		Research findings
	Intervention	Control	Intervention	Control	Intervention	Control	Intervention	Control	
Deng and Jia (2024)	50	50							CRP, and HDL-C levels, with no serious adverse reactions observed during the treatment period.
Cao et al. (2014)	198	65	63.1 ± 9.4	63.3 ± 9.2	111	45	2.6 ± 2.3 m	2.3 ± 1.4 m	Compared to Naoxintong, Tongxinluo treatment significantly improves the efficacy rate and traditional Chinese medicine (TCM) syndrome scores in stroke patients, with no noticeable adverse reactions observed during the treatment period.
Su et al. (2011)	63	61	—	—	31	33	—	—	Tongxinluo treatment significantly improves the efficacy rate in stroke patients compared to Naoxintong.
Wu et al. (2010)	67	67	—	—	—	—	—	—	Compared to conventional treatment, Tongxinluo significantly improves TNF-α, hs-CRP, LDL-C, and TC levels in stroke patients.
Wang et al. (2004)	52	50	68.2 ± 3.3	67.4 ± 3.5	29	28	—	—	Compared to conventional treatment, Tongxinluo significantly improves clinical symptoms, neurological deficit scores, and plasma levels of endothelin (ET), calcitonin gene-related peptide (CGRP), and nitric oxide (NO) in stroke patients.
Yan et al. (2008)	182	178	—	—	—	—	—	—	**Compared to conventional treatment, Tongxinluo significantly improves Barthel index scores and neurological deficit scores in stroke patients, with no significant adverse reactions observed during the treatment period.**
Yao(2006)	228	38	—	—	108	17	—	—	Compared to conventional treatment, Tongxinluo significantly improves the efficacy rate in stroke patients.
Wu and Li (2015)	51	51	66.1 ± 10.5	65.7 ± 11.4	30	31	—	—	Compared to conventional treatment, the Tongxinluo group showed significant improvements in TC, TG, LDL-C, HDL-C, TNF-α, and hs-CRP levels in stroke patients.
Wu and Li (2015)	52	51	63.6 ± 10.7		35		—		
Huang (2015)	50	50	51.2 ± 5.1	51.2 ± 5.2	27	28	9.1 ± 1.1 h	9.3 ± 1.2 h	**Compared to simvastatin alone, Tongxinluo combined with simvastatin significantly improves TC, TG, LDL-C, HDL-C, and hs-CRP levels, as well as the overall efficacy rate in stroke patients.**
Huang (2015)	50		51.4 ± 5.3		27		9.4 ± 1.3 h		
Kong et al. (2010)	186	189	61.4 ± 11.3	61.3 ± 11.2	110	108	—	—	Compared to conventional treatment, Tongxinluo significantly improves clinical efficacy and hemorheological parameters in stroke patients.
Lui et al. (2007)	51	51	61.35 ± 12.61	62.93 ± 7.62	30	29	6.6 ± 0.78 m	6.5 ± 0.69 m	Compared to conventional treatment, Tongxinluo significantly improves neurological function, enhances activities of daily living, and increases overall clinical efficacy in stroke patients, with no serious adverse reactions observed during the treatment period.
Wang (2006)	65	59	—	—	—	—	—	—	Compared to conventional treatment, Tongxinluo significantly improves motor function as measured by the Fugl-Meyer (FM) assessment and cognitive function as assessed by the Mini-Mental State Examination

(Continued on following page)

10.3389/fphar.2025.1573069

TABLE 1 (*Continued*) The basic characteristics of literature.

Study	Patients		Mean/median age		Male		Mean/median course of disease		Research findings
	Intervention	Control	Intervention	Control	Intervention	Control	Intervention	Control	
									(MMSE), enhances limb function recovery, and to some extent improves hemorheological parameters in stroke patients.
Lan et al. (2013)	60	60	57.0 ± 2.3	56.0 ± 3.6	36	38	29.2 ± 9.6 h	28.0 ± 12.6 h	**Compared to conventional treatment, Tongxinluo significantly improves the efficacy rate, neurological deficit scores, Barthel index scores, levels of hs-CRP, TNF-α, IL-6, blood lipids, and hemorheological parameters in stroke patients, with no serious adverse reactions observed during the treatment period.**
Guo et al. (2014)	84	84	66.4±7.8	65.9 ± 8.7	45	47	—	—	Compared to simvastatin alone, Tongxinluo combined with simvastatin significantly improves total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), apolipoprotein A1 (ApoA1), apolipoprotein B (ApoB), and the ApoA1/ApoB ratio in stroke patients, indicating an improvement in dyslipidemia. No noticeable adverse reactions were observed during the treatment period.
Lv (2008)	60	60	60 ± 10	61 ± 10	39	33	—	—	Compared to conventional treatment, Tongxinluo significantly improves the efficacy rate, degree of neurological deficits, quality of life scores, and lipid profiles in stroke patients.
Xue et al. (2014)	74	74	66.1 ± 7.2	65.8 ± 6.3	42	43	—	—	Compared to conventional treatment alone, Tongxinluo combined with conventional therapy significantly improves total cholesterol, triglycerides, low-density lipoprotein cholesterol, C-reactive protein, interleukin-6, vascular stenosis rate, platelet aggregation rate, and high-density lipoprotein cholesterol in stroke patients, with no noticeable adverse reactions observed during the treatment period.
Wang (2014)	120	80	51.3 ± 10.1	53.0 ± 9.8	80	51	—	—	Compared to conventional treatment, Tongxinluo combined with clopidogrel and aspirin significantly improves neurological deficit scores in stroke patients, with no serious adverse reactions observed during the treatment period.
Li et al. (2021b)	62	61	53.7 ± 8.5	54.6 ± 8.4	29	30	24.6 ± 6.0 h	23.9 ± 5.8 h	Compared to conventional treatment combined with clopidogrel, Tongxinluo combined with clopidogrel significantly improves the overall clinical efficacy, cognitive function during the recovery phase, and quality of life in stroke patients.
Wang et al. (2016)	53	53	62.5 ± 9.8	63.8 ± 9.4	33	31	—	—	**Compared to probucol alone, Tongxinluo combined with probucol significantly reduces serum levels of ox-LDL, MMP-7, and IL-18 in stroke patients, suggesting a potential role in delaying or reversing the progression of carotid atherosclerotic plaques. No significant adverse reactions were observed during the treatment period.**
Zhou et al. (2016)	51	51	56.9 ± 6.6	55.6 ± 7.6	32	28	9.8 ± 3.5 y	10.1 ± 4.2 y	Compared to nimodipine alone, Tongxinluo combined with nimodipine significantly reduces serum levels of hs-CRP and Tau protein, increases IGF-1 levels, and improves neurological deficit symptoms in stroke

(Continued on following page)

10.3389/fphar.2025.1573069

TABLE 1 (*Continued*) The basic characteristics of literature.

Study	Patients		Mean/median age		Male		Mean/median course of disease		Research findings
	Intervention	Control	Intervention	Control	Intervention	Control	Intervention	Control	
									patients, with no serious adverse reactions observed during the treatment period.
Chen et al. (2021)	57	57	72.67 ± 7.43	71.83 ± 7.51	32	31	9.85 ± 2.69 h	9.67 ± 2.84 h	Compared to edaravone alone, Tongxinluo combined with edaravone significantly improves neurological deficit scores (NIHSS), serum total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), high-sensitivity C-reactive protein (hs-CRP), nitric oxide (NO) levels, plaque area, and intima-media thickness (IMT) in stroke patients.
Wu et al. (2021)	53	53	61.41 ± 8.12	60.94 ± 8.21	29	32	—	—	Compared to edaravone alone, Tongxinluo combined with edaravone significantly improves lipid profiles and inflammatory markers, enhances the overall treatment efficacy, and shows no significant adverse reactions during the treatment period in stroke patients.
Ma (2008)	56	56	—	—	35	36	—	—	**Compared to Vinpocetine Capsules combined with conventional treatment, Tongxinluo combined with conventional treatment significantly improves overall efficacy, limb function recovery, and cognitive function scores in stroke patients.**
Wang (2014)	56	54	—	—	—	—	—	—	Compared to conventional treatment alone, Tongxinluo combined with conventional therapy significantly improves neurological function and blood viscosity in stroke patients.
Wang (2006)	56	56	62.31 ± 10.05	61.64 ± 9.87	34	32	—	—	Compared to conventional treatment alone, Tongxinluo combined with conventional therapy significantly improves the overall efficacy rate and neurological function in stroke patients, with no noticeable adverse reactions observed during the treatment period.
Ruan et al. (2014)	63	63	61.5 ± 7.2	61.1 ± 6.9	39	38	—	—	**Compared to conventional treatment alone, Tongxinluo combined with conventional therapy significantly improves the overall efficacy rate, neurological function, and hemorheological parameters in stroke patients.**
Wen et al. (2013)	136	142	64.9	63.8	64	70	—	—	**Compared to conventional treatment alone, Tongxinluo combined with conventional therapy significantly improves the overall efficacy rate, neurological deficit scores, and Barthel index in stroke patients, with no noticeable adverse reactions observed during the treatment period.**
Wei et al. (2010)	50	50	68.64 ± 10.52	69.05 ± 10.53	23	25	—	—	Compared to conventional treatment alone, Tongxinluo combined with conventional therapy significantly improves total cholesterol (TC), triglycerides (TG), blood glucose levels, activities of daily living (ADL), and modified Rankin Scale (mRS) scores in stroke patients.
Liu et al. (2012)	50	56	—	—	—	—	—	—	**Compared to conventional treatment alone, Tongxinluo combined with conventional therapy significantly improves neurological deficit scores, lipid profiles, carotid plaque burden, and lumen stenosis, thereby enhancing overall clinical efficacy in stroke patients.**

(Continued on following page)

10.3389/fphar.2025.1573069

TABLE 1 (*Continued*) The basic characteristics of literature.

Study	Patients		Mean/median age		Male		Mean/median course of disease		Research findings
	Intervention	Control	Intervention	Control	Intervention	Control	Intervention	Control	
Di (2015)	72	72	—	—	—	—	—	—	Compared to conventional treatment alone, Tongxinluo combined with conventional therapy significantly improves neurological deficit scores and blood viscosity in stroke patients, with no noticeable adverse reactions observed during the treatment period.
Chu (2010)	50	50	68.7	69.2	28	30	—	—	Compared to conventional treatment alone, Tongxinluo combined with conventional therapy significantly improves neurological deficit scores and increases the overall efficacy rate in stroke patients.
Luo et al. (2002)	88	72	62.76	61.48	58	48	—	—	Compared to Salvia miltiorrhiza injection combined with conventional treatment, Tongxinluo combined with conventional therapy significantly improves lipid metabolism, blood viscosity, hemorheological parameters, and platelet aggregation function in stroke patients. It effectively enhances cerebral blood circulation, reduces delayed neuronal injury, and improves neurological deficits.
Cui et al. (2008)	72	40	63.2 ± 12.1	59.6 ± 15.9	43	23	—	—	Compared to conventional treatment alone, Tongxinluo combined with conventional therapy significantly improves neurological deficit scores and increases the overall efficacy rate in stroke patients.
Tang et al. (2015)	60	60	55.6 ± 2.5	54.9 ± 3.0	27	28	—	—	**Compared to conventional treatment alone, Tongxinluo combined with conventional therapy significantly improves serum cytokine levels and enhances the overall clinical efficacy in stroke patients.**
Sun et al. (2000)	50	50	—	—	28	30	—	—	Compared to Salvia miltiorrhiza injection, Tongxinluo treatment significantly improves neurological deficits and enhances clinical efficacy in stroke patients.
Wang et al. (2005)	105	102	61	60	58	57	11 h	10 h	Compared to conventional treatment alone, Tongxinluo combined with conventional therapy significantly improves vertebrobasilar insufficiency and enhances clinical efficacy in stroke patients, with no serious adverse reactions observed during the treatment period.
Yu et al. (2010)	82	80	—	—	42	39	—	—	**Compared to conventional treatment alone, Tongxinluo combined with conventional therapy significantly improves lipid profiles in stroke patients.**
Yang (2008)	67	67	62 ± 7.35	61 ± 6.64	40	38	—	—	**Compared to conventional treatment alone, Tongxinluo combined with low-molecular-weight heparin and conventional therapy significantly improves overall therapeutic efficacy and neurological deficits in stroke patients.**
Wang (2015)	60	60	64.46 ± 7.24	63.24 ± 7.36	37	35	—	—	**Compared to butylphthalide alone, Tongxinluo combined with butylphthalide significantly improves overall therapeutic efficacy, blood viscosity, and neurological deficits in stroke patients, with no noticeable adverse reactions observed during the treatment period.**

(Continued on following page)

10.3389/fphar.2025.1573069

TABLE 1 (*Continued*) The basic characteristics of literature.

Study	Patients		Mean/median age		Male		Mean/median course of disease		Research findings
	Intervention	Control	Intervention	Control	Intervention	Control	Intervention	Control	
Yang et al. (2009)	128	128	63 ± 6.41	62.8	70	75	—	—	**Compared to conventional treatment alone, Tongxinluo combined with low-molecular-weight heparin significantly improves overall therapeutic efficacy and neurological deficits in stroke patients.**
Mi et al. (2017)	84	84	58.6 ± 3.2	59.1 ± 2.7	56	55	7.4 ± 1.5 d	7.9 ± 1.1 d	Compared to conventional treatment alone, Tongxinluo combined with conventional therapy significantly improves carotid intima-media thickness, atherosclerotic plaque area, carotid stenosis rate, and reduces levels of hs-CRP and D-dimer in stroke patients.
Duan (2006)	66	64	60.1	60	34	34	—	—	Compared to conventional treatment alone, Tongxinluo combined with conventional therapy significantly improves overall therapeutic efficacy and hemorheological parameters in stroke patients, with no noticeable adverse reactions observed during the treatment period.
Huang et al. (2007)	95	97	59.3	57.6	53	57	—	—	Compared to conventional treatment alone, Tongxinluo combined with conventional therapy significantly improves overall therapeutic efficacy in stroke patients, with marked improvements in arrhythmia, ST-T changes, and myocardial enzyme levels. No noticeable adverse reactions were observed during the treatment period.
Wang (2008)	60	60	—	—	—	—	—	—	Compared to Naofukang combined with conventional treatment, Tongxinluo combined with conventional therapy significantly improves the efficacy rate, limb function recovery, and cognitive function scores in stroke patients.
Qin et al. (2018)	41	41	69.03 ± 5.17	68.14 ± 4.63	23	24	3.21 ± 0.92 m	3.19 ± 0.87 m	Compared to conventional treatment, Tongxinluo combined with acupuncture effectively promotes angiogenesis and neural repair, inhibits apoptosis, and enhances functional recovery in stroke patients.
Wang et al. (2015)	34	35	68 ± 10	67 ± 10	16	18	3.08 ± 1.52 m	3.28 ± 1.64 m	**Compared to conventional treatment, Tongxinluo combined with acupuncture improves bone marrow stem cell mobilization and neurological deficits in stroke patients.**
Bai et al. (2017)	68	77	66.88 ± 10.60	66.99 ± 10.24	32	33	—	—	Compared to conventional treatment alone, Tongxinluo combined with conventional therapy reduces the recurrence rate of stroke, promotes neurological recovery, and contributes to better blood pressure and lipid control, with no serious adverse reactions observed during the treatment period.
Sun et al. (2009)	105	103	62 ± 12	63 ± 13	56	53	—	—	Compared to conventional treatment alone, Tongxinluo combined with conventional therapy improves overall efficacy, neurological deficits, activities of daily living, and motor function in stroke patients.
Dong et al. (2024)	973	973	61.3	60.9	677	665	—	—	Compared to conventional treatment alone, Tongxinluo combined with conventional therapy improves the overall efficacy rate in stroke patients, with no serious adverse reactions observed during the treatment period.

10.3389/fphar.2025.1573069

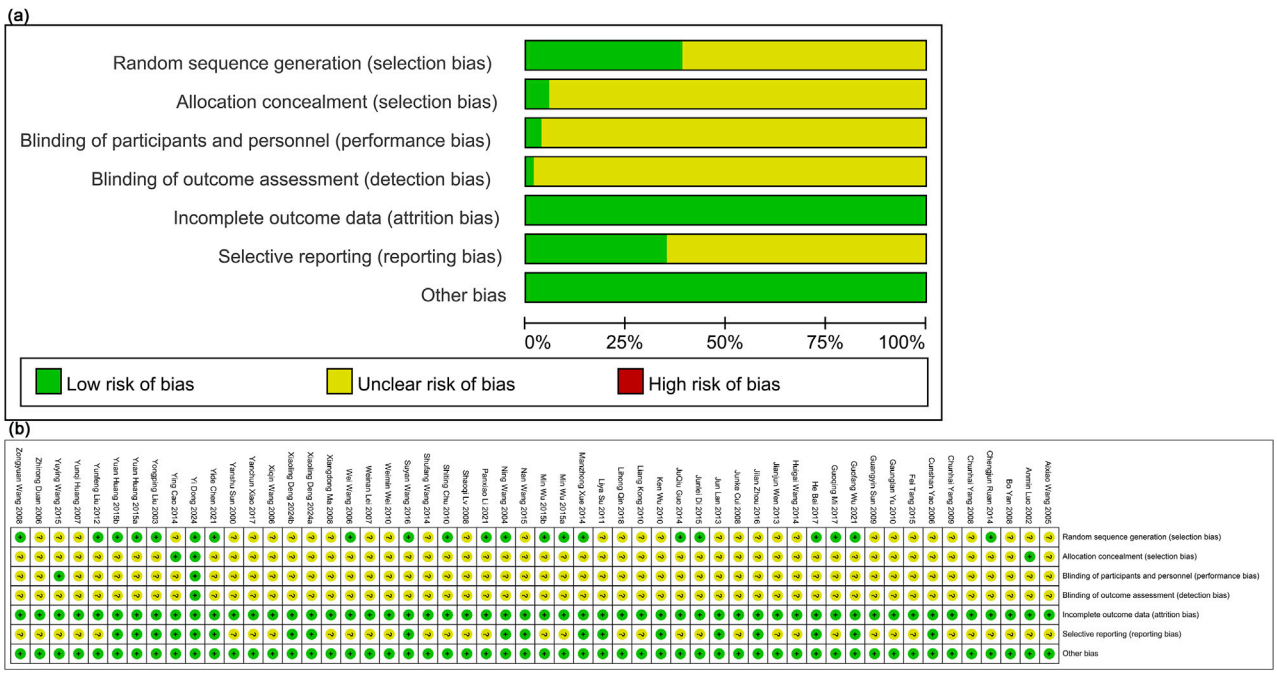


FIGURE 2
Literature quality evaluation. **(a)** Risk of bias graph; **(B)** Risk of bias summary.

2014; Di, 2015; Huang, 2015; Tang and Yu, 2015; Wang, 2015; Zhou et al., 2016; Xiao, 2017; Chen et al., 2021; Li P.-X. et al., 2021; Wu et al., 2021). Results showed that Tongxinluo treatment for stroke was significantly more effective [RR = 1.20, 95% CI (1.16, 1.25)], with considerable heterogeneity (I^2 = 49%) (Figure 3a). Funnel plot analysis (Figure 3b) and Egger's test (P = 0.001) indicated a significant publication bias.

3.4.2 Adverse events

Adverse events were depicted in 14 studies (Liu et al., 2003; Lui et al., 2007; Cao et al., 2014; Wang and Gai, 2014; Xue et al., 2014; Huang, 2015; Wang, 2015; Bai et al., 2017; Chen et al., 2021; Li P.-X. et al., 2021; Deng and Jia, 2024; Dong et al., 2024). Results showed a higher adverse reaction rate in the Tongxinluo group (RR = 1.01), but the 95% CI (0.90, 1.12) indicated no significant difference, suggesting similar rates. Heterogeneity was 0% (I^2), showing no variability between groups (Figure 4a). Funnel plot analysis (Figure 4b) and Egger's test (P = 0.988) revealed no publication bias.

3.4.3 NIHSS score

The NIHSS score was reported in 21 studies (Wen et al., 2013; Cui et al., 2008; Wang et al., 2004; Wang; Lui et al., 2007; Lv, 2008; Yan et al., 2008; Yang, 2008; Sun et al., 2009; Yang et al., 2009; Lan et al., 2013; Ruan and Ruan, 2014; Wang and Gai, 2014; Huang, 2015; Wang, 2015; Wang et al., 2015; Zhou et al., 2016; Xiao, 2017; Qin et al., 2018; Chen et al., 2021). Results showed a significantly greater improvement in the Tongxinluo group [SMD = −0.42, 95% CI (−0.77 to −0.07)], with no significant heterogeneity (I^2 = 95%) (Figure 5a). The funnel plot (Figure 5b) and Egger's test (P = 0.845) indicated no publication bias.

3.4.4 TC

TC data were obtained from 17 studies (Luo et al., 2002; Yao et al.; Wu et al., 2010; Yu et al., 2010; Liu and LI, 2012; Lan et al., 2013; Guo et al., 2014; Ruan and Ruan, 2014; Xue et al., 2014; Huang, 2015; Wu and Li, 2015; Chen et al., 2021; Wu et al., 2021; Deng and Jia, 2024). The Tongxinluo group showed significantly better TC levels [SMD = −0.55, 95% CI (−0.92 to −0.17)], with no significant heterogeneity (I^2 = 94%) (Figure 6a). The funnel plot (Figure 6b) and Egger's test (P = 0.075) indicated no publication bias.

3.4.5 TG

TG data were obtained from 17 studies (Luo et al., 2002; Yao et al.; Wu et al., 2010; Yu et al., 2010; Liu and LI, 2012; Lan et al., 2013; Guo et al., 2014; Ruan and Ruan, 2014; Xue et al., 2014; Huang, 2015; Wu and Li, 2015; Chen et al., 2021; Wu et al., 2021; Deng and Jia, 2024). No significant difference in TG level improvement was observed [SMD = −0.22, 95% CI (−0.61 to −0.16)], with no significant heterogeneity (I^2 = 94%) (Supplementary Figure S1a). The funnel plot (Supplementary Figure S1b) and Egger's test (P = 0.055) indicated no publication bias.

3.4.6 hs-CRP

hs-CRP data were obtained from 14 studies (Wu et al., 2010; Lan et al., 2013; Xue et al., 2014; Huang, 2015; Tang and Yu, 2015; Wu and Li, 2015; Zhou et al., 2016; Mi et al., 2017; Chen et al., 2021; Wu et al., 2021; Deng and Jia, 2024). Results showed significantly greater hs-CRP improvement in the Tongxinluo group (SMD = −0.40, 95% CI [−0.68 to −0.12]), with no significant heterogeneity (I^2 = 87%) (Figure 7a). The funnel plot (Figure 7b) and Egger's test (P = 0.816) indicated no publication bias.

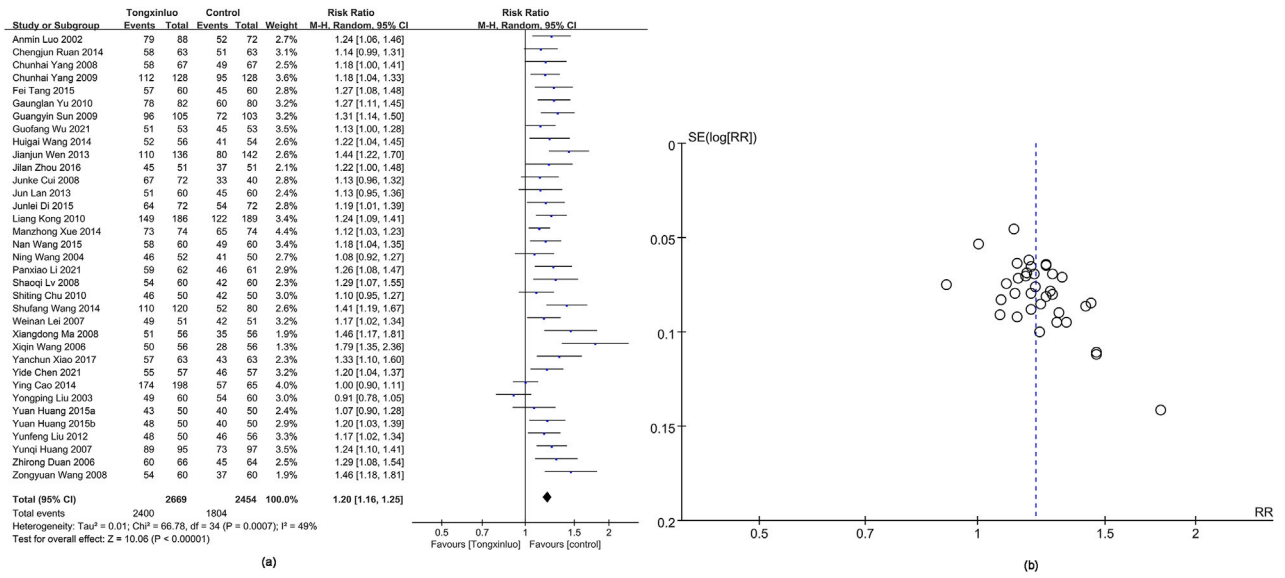


FIGURE 3
Forest and funnel map of overall efficacy. **(a)** Forest map; **(b)** Funnel map.

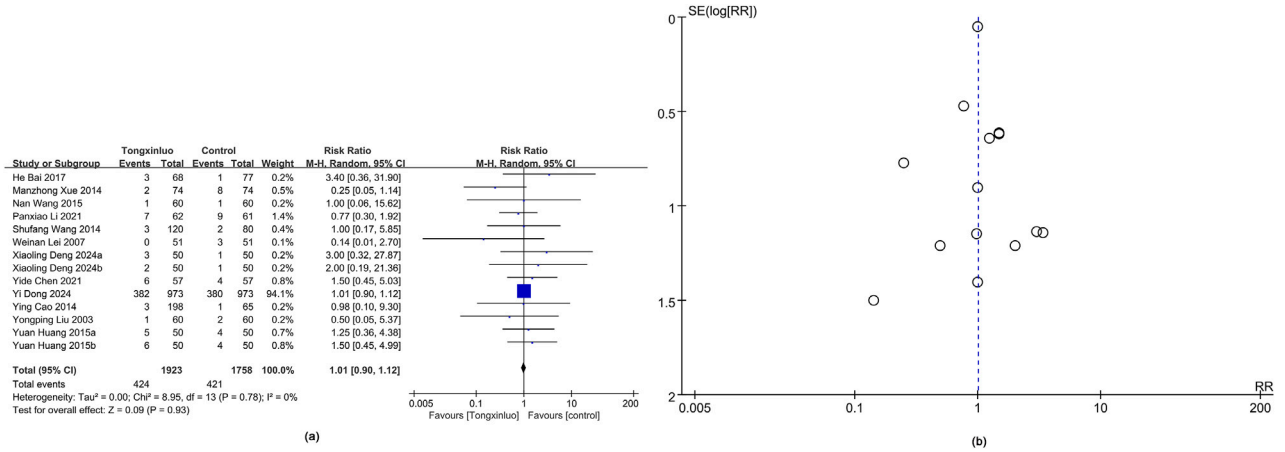


FIGURE 4
Forest and funnel map of adverse events. **(a)** Forest map; **(b)** Funnel map.

3.4.7 LDL

LDL data were obtained from 14 studies (Yao et al.; Wu et al., 2010; Yu et al., 2010; Liu and LI, 2012; Lan et al., 2013; Guo et al., 2014; Xue et al., 2014; Huang, 2015; Wu and Li, 2015; Chen et al., 2021; Deng and Jia, 2024). Results showed significantly greater LDL improvement in the Tongxinluo group (SMD = −0.84, 95% CI [−1.37 to −0.30]), with no significant heterogeneity (I^2 = 96%) (Supplementary Figure S2a). The funnel plot (Supplementary Figure S2b) and Egger's test (P = 0.001) indicated publication bias.

3.4.8 HDL

HDL data were obtained from 13 studies (Luo et al., 2002; Yao et al.; Wu et al., 2010; Yu et al., 2010; Lan et al., 2013; Guo et al., 2014; Xue et al., 2014; Huang, 2015; Wu and Li, 2015; Deng and Jia, 2024). Results showed significantly greater HDL improvement in the Tongxinluo group (SMD = 0.79, 95% CI [0.31, 1.28]), with no significant heterogeneity (I^2 = 95%) (Supplementary Figure S3a). The funnel plot (Supplementary Figure S3b) and Egger's test (P = 0.017) indicated publication bias.

3.4.9 TNF-α

TNF-α data were obtained from nine studies (Wu et al., 2010; Lan et al., 2013; Huang, 2015; Wu and Li, 2015; Wu et al., 2021; Deng and Jia, 2024). Results showed significantly greater TNF-α improvement in the Tongxinluo group (SMD = −0.60, 95% CI [−1.15 to −0.04]), with no significant heterogeneity (I^2 = 94%)

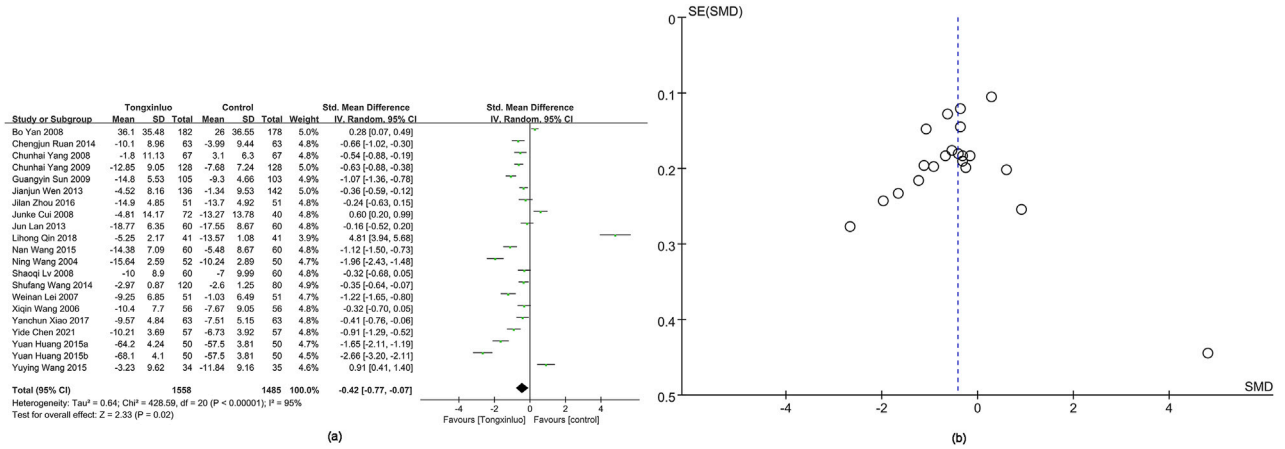


FIGURE 5
Forest and funnel map of NIHSS score. **(a)** Forest map; **(b)** Funnel map.

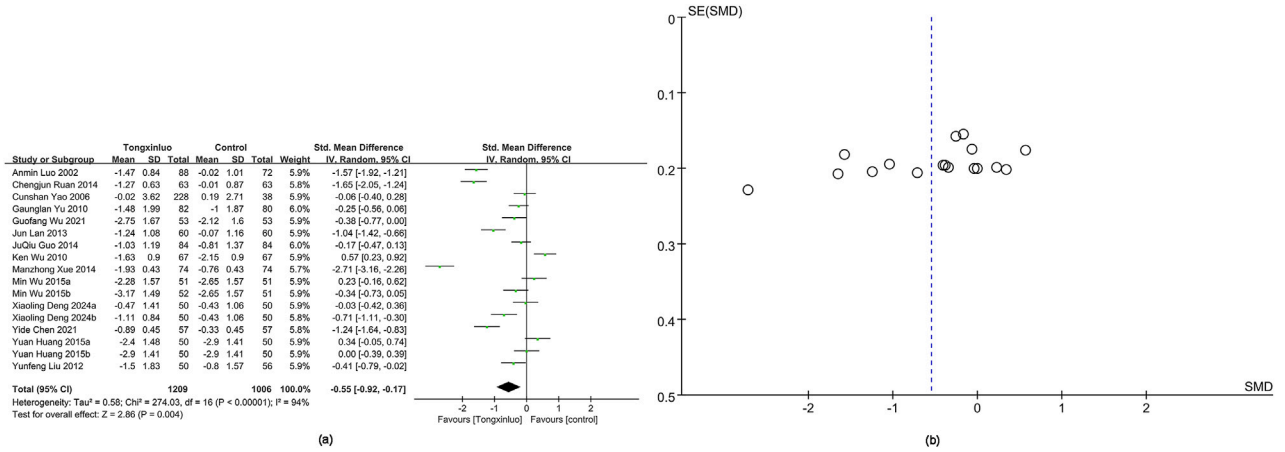


FIGURE 6
Forest and funnel map of TC. **(a)** Forest map; **(B)** Funnel map.

(Supplementary Figure S4b). The funnel plot (Supplementary Figure S4b) and Egger's test (P = 0.014) indicated publication bias.

3.4.10 IL-6

IL-6 data were obtained from 10 studies (Lan et al., 2013; Xue et al., 2014; Huang, 2015; Tang and Yu, 2015; Wu and Li, 2015; Wu et al., 2021; Deng and Jia, 2024). Results showed significantly greater IL-6 improvement in the Tongxinluo group (SMD = −0.49, 95% CI [−0.80 to −0.18]), with no significant heterogeneity (I^2 = 85%) (Supplementary Figure S5a). The funnel plot (Supplementary Figure S5b) and Egger's test (P = 0.986) indicated no publication bias.

3.4.11 High-shear whole blood viscosity

High-shear whole blood viscosity data were obtained from five studies (Luo et al., 2002; Kong et al., 2010; Lan et al., 2013; Di, 2015; Wang, 2015). Results showed significantly greater improvement in the Tongxinluo group (SMD = −1.88, 95% CI [−2.87 to −0.88]), with no significant heterogeneity (I^2 = 97%) (Supplementary Figure S6a). The funnel plot (Supplementary Figure S6b) and Egger's test (P = 0.092) indicated no publication bias.

3.4.12 Low-shear whole blood viscosity

Low-shear whole blood viscosity data were obtained from five studies (Luo et al., 2002; Kong et al., 2010; Lan et al., 2013; Di, 2015; Wang, 2015). Results showed significantly greater improvement in the Tongxinluo group (SMD = −0.61, 95% CI [−0.94 to −0.28]), with no significant heterogeneity (I^2 = 82%) (Supplementary Figure S7a). The funnel plot (Supplementary Figure S7b) and Egger's test (P = 0.391) indicated no publication bias.

3.4.13 Barthel index score

Barthel Index score data were obtained from four studies (Wen et al., 2013; Lui et al., 2007; Yan et al., 2008; Sun et al., 2009). Results showed significantly greater improvement in the Tongxinluo group

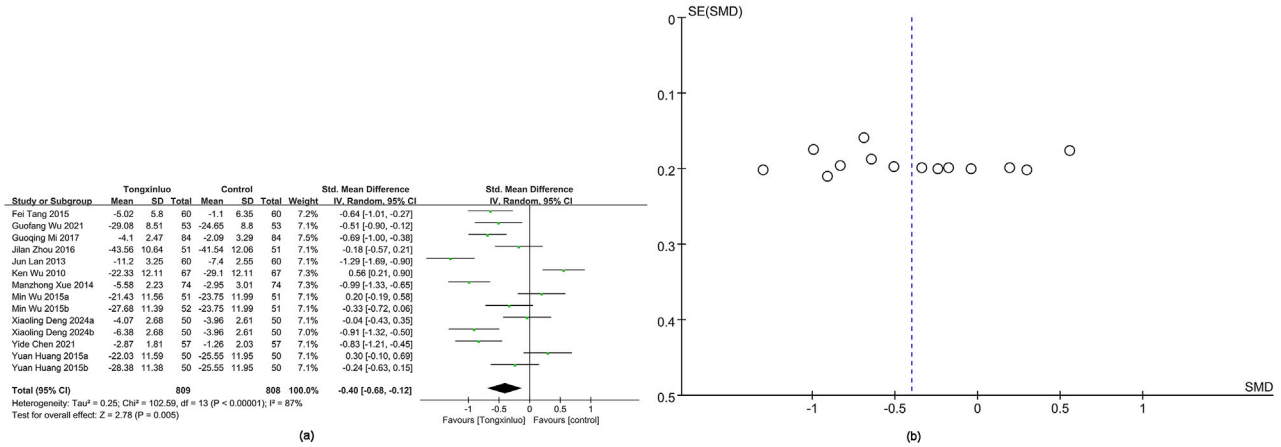


FIGURE 7
Forest and funnel map of hs-CRP. **(a)** Forest map; **(b)** Funnel map.

(SMD = 0.73, 95% CI [0.25, 1.20]), with no significant heterogeneity (I^2 = 92%) (Supplementary Figure S8a). The funnel plot (Supplementary Figure S8b) and Egger's test (P = 0.501) indicated no publication bias.

3.4.14 Plaque area

Plaque area data were obtained from four studies (Liu and LI, 2012; Wang et al., 2016; Mi et al., 2017; Chen et al., 2021). Results showed significantly greater improvement in the Tongxinluo group (SMD = −1.28, 95% CI [−1.84 to −0.71]), with no significant heterogeneity (I^2 = 88%) (Supplementary Figure S9a). The funnel plot (Supplementary Figure S9b) and Egger's test (P = 0.616) indicated no publication bias.

3.4.15 MMSE score

MMSE score data were obtained from four studies (Wang, 2006; Ma, 2008; Wang, 2008; Li P.-X. et al., 2021). Results showed significantly greater improvement in MMSE scores in the Tongxinluo group (SMD = 0.93, 95% CI [0.26, 1.61]), with no significant heterogeneity (I^2 = 92%) (Supplementary Figure S10a). The funnel plot (Supplementary Figure S10b) and Egger's test (P = 0.049) indicated publication bias.

3.4.16 FM score

FM score data were derived from three studies (Wang, 2006; Ma, 2008; Wang, 2008). Results showed significantly greater improvement in FM scores in the Tongxinluo group (SMD = 0.42, 95% CI [0.13, 0.71]), with significant heterogeneity (I^2 = 47%) (Supplementary Figure S11a). The funnel plot (Supplementary Figure S11b) and Egger's test (P = 0.370) indicated no publication bias.

3.4.17 Plasma viscosity

Plasma viscosity data were derived from seven studies (Luo et al., 2002; Duan, 2006; Kong et al., 2010; Lan et al., 2013; Ruan and Ruan, 2014; Wang, 2014; Di, 2015). Results showed significantly greater improvement in plasma viscosity in the Tongxinluo group (SMD = −1.28, 95% CI [−2.01 to −0.55]), with no significant heterogeneity (I^2 = 97%) (Supplementary Figure S12a). The funnel plot (Supplementary Figure S12b) and Egger's test (P = 0.738) indicated no publication bias.

3.4.18 Plasma fibrinogen

Plasma fibrinogen data were derived from six studies (Luo et al., 2002; Duan, 2006; Kong et al., 2010; Lan et al., 2013; Ruan and Ruan, 2014; Wang, 2015). Results showed significantly greater improvement in plasma fibrinogen in the Tongxinluo group (SMD = −0.47, 95% CI [−0.68 to −0.27]), with no significant heterogeneity (I^2 = 60%) (Supplementary Figure S13a). The funnel plot (Supplementary Figure S13b) and Egger's test (P = 0.527) indicated no publication bias.

3.4.19 Hematocrit

Hematocrit data were derived from two studies (Luo et al., 2002; Duan, 2006). Results showed significantly greater improvement in hematocrit in the Tongxinluo group (SMD = −1.26, 95% CI [−2.10 to −0.42]), with no significant heterogeneity (I^2 = 91%) (Supplementary Figure S14a), as shown in the funnel plot (Supplementary Figure S14b).

3.4.20 NO

NO data were derived from two studies (Wang et al., 2004; Chen et al., 2021). Results showed significantly greater improvement in NO levels in the Tongxinluo group (SMD = 1.05, 95% CI [0.24, 1.86]), with no significant heterogeneity (I^2 = 87%) (Supplementary Figure S15a), as shown in the funnel plot (Supplementary Figure S15b).

3.4.21 IL-18

IL-18 data were derived from two studies (Tang and Yu, 2015; Wang et al., 2016). Results showed no significant difference in IL-18 level improvement (SMD = −2.81, 95% CI [−6.25, 0.63]), with no significant heterogeneity (I^2 = 99%) (Supplementary Figure S16a), as indicated by the funnel plot (Supplementary Figure S16b).

TABLE 2 The subgroup results.

Subgroup	Overall efficacy				Change in NIHSSS			
	Study	RR [95% CI]	*P* Value	I^2	Study	SMD [95% CI]	*P* Value	I^2
Total	35	1.20 [1.16–1.25]	<0.00001	49%	21	−0.42 [−0.77 to −0.07]	0.02	95%
Patients								
Simple acute stroke	21	1.22 [1.18–1.27]	<0.00001	22%	15	−0.62 [−0.87 to −0.37]	<0.00001	88%
Stroke with diabetes	2	1.15 [1.02–1.29]	0.02	0%	2	−2.14 [−3.13 to −1.15]	<0.0001	87%
Intervention time								
>4 weeks	13	1.18 [1.12–1.25]	<0.00001	48%	6	−0.44 [−1.08 to 0.19]	0.17	96%
≤4 weeks	19	1.21 [1.15–1.28]	<0.00001	57%	12	−0.72 [−1.10 to −0.33]	0.0003	93%
Mean/median age								
>65 years	4	1.13 [1.06–1.20]	0.0002	0%	4	0.68 [−1.47 to 2.83]	0.53	99%
≤65 years	25	1.20 [1.15–1.26]	<0.00001	55%	16	−0.68 [−0.96 to −0.40]	<0.00001	91%

3.4.22 Platelet aggregation function

Platelet aggregation function data were derived from two studies (Luo et al., 2002; Xue et al., 2014). Results showed no significant difference in improvement (SMD = −0.74, 95% CI [−1.49, 0.01]), with no significant heterogeneity (I^2 = 86%) (Supplementary Figure S17a), as indicated by the funnel plot (Supplementary Figure S17b).

3.4.23 Quality of life scores

Quality of life score data were derived from two studies (Lv, 2008; Li P.-X. et al., 2021). Results showed significantly greater improvement in the Tongxinluo group (SMD = 0.65, 95% CI [0.27, 1.03]), with no significant heterogeneity (I^2 = 54%) (Supplementary Figure S18a), as shown by the funnel plot (Supplementary Figure S18b).

3.5 Sensitivity analysis

A univariate sensitivity analysis was conducted to assess the total efficacy (Supplementary Figure S19a), adverse events (Supplementary Figure S19b), NIHSS scores (Supplementary Figure S19c), TC (Supplementary Figure S19d), TG (Supplementary Figure S19e), HDL (Supplementary Figure S19f), LDL (Supplementary Figure S19g), hs-CRP (Supplementary Figure S19h), TNF-α (Supplementary Figure S19i), IL-6 (Supplementary Figure S19j), high-shear whole blood viscosity (Supplementary Figure S19k), low-shear whole blood viscosity (Supplementary Figure S19l), Barthel scores (Supplementary Figure S19m), plaque area (Supplementary Figure S19n), FM scores (Supplementary Figure S19o), plasma viscosity (Supplementary Figure S19p), plasma fibrinogen (Supplementary Figure S19q), MMSE scores (Supplementary Figure S19r), red blood cell hematocrit, NO, IL-18, platelet aggregation function, and quality of life scores by sequentially excluding individual studies. This analysis assessed each study's impact on the overall effect. Results showed significant changes in NIHSS scores, FM scores, MMSE scores, TG, and TNF-α after excluding certain studies, indicating instability in these outcomes. Caution is needed when interpreting results for these indicators. Excluding studies had minimal impact on the pooled effect size for other outcomes, indicating good stability.

3.6 Subgroup analysis

Subgroup analysis was conducted based on population, intervention duration, and disease duration to assess total efficacy and NIHSS scores. The total efficacy analysis indicated that Tongxinluo was effective across all subgroups. The NIHSS scores analysis showed that Tongxinluo was ineffective in studies with intervention durations longer than 4 weeks and in studies involving participants older than 65 years. However, it was effective in other subgroups. Detailed results are provided in Table 2.

4 Discussion

Tongxinluo capsules are a traditional and safe Chinese medicine used effectively for the secondary prevention of coronary artery diseases. Approved by the China Food and Drug Administration over 20 years ago, it is indicated for the treatment of coronary heart disease, angina, ischemic stroke, and related conditions (Zhuo et al., 2008; Wang and Liu, 2024). A 2015 study by Junlei Di, which included 144 patients, found that Tongxinluo showed significant efficacy in treating acute cerebral infarction (Di, 2015). Tongxinluo is commonly used as an adjuvant in stroke treatment, co-administered with agents such as aspirin, clopidogrel, edaravone, butylphthalide, and statins to achieve synergistic therapeutic effects.

Our study found that Tongxinluo significantly improved the overall therapeutic effect, NIHSS score, TC, TG, hs-CRP, LDL, HDL, TNF-α, IL-6, high-shear and low-shear whole blood viscosity, Barthel index, plaque area, MMSE score, FM score, plasma viscosity, plasma fibrinogen, hematocrit, NO, and quality of life index. However, no significant effects were observed in TG, IL-18, and platelet aggregation function. Sensitivity analysis revealed instability in FM score, MMSE score, NIHSS score, TG, and

TNF-α. Egger's test for overall efficacy, LDL, HDL, TNF-α, and MMSE score indicated publication bias. A 2024 study by Yi Dong (Dong et al., 2024), which included 1,946 patients, demonstrated that after 90 days of treatment, Tongxinluo significantly treated acute ischemic stroke. Our findings align with Yi Dong's results and further validate the efficacy and safety of Tongxinluo in adjunctive stroke treatment, providing evidence to support its clinical application.

Subgroup analysis revealed that the effect was not significant when the intervention duration exceeded 4 weeks, likely due to patient adherence issues. Long-term use of Tongxinluo may cause adverse reactions, including gastrointestinal discomfort, nausea, vomiting, bloating, and diarrhea, potentially resulting from gastrointestinal irritation. Allergic reactions such as rashes and itching, as well as headaches and dizziness, may be linked to its impact on the vascular and nervous systems (Li P. et al., 2021). Prolonged use can decrease adherence, leading to irregular medication usage and affecting treatment outcomes (Lv et al., 2022). Therefore, based on this study's findings, it is recommended that Tongxinluo be used for no more than 4 weeks in clinical adjunctive stroke treatment. For patients over 65, Tongxinluo showed no significant effect on NIHSS scores, likely due to the presence of multiple underlying health conditions that may reduce its efficacy (Dong et al., 2024).

Tongxinluo enhances myocardial reperfusion, reduces no-reflow incidence, and decreases infarct size, thereby improving heart function. It also exhibits anti-atherosclerotic effects by stabilizing plaques and preventing their progression. Its mechanisms of action include regulating cytophysical functions, hormone secretion, protein binding, immune responses, inflammation, and improving lipid metabolism (Liu et al., 2023). The diverse therapeutic effects of Tongxinluo contribute to its potential in stroke treatment and support its clinical application in cerebrovascular disease management. Studies indicate that Tongxinluo capsules effectively regulate blood lipids, prevent coagulation, and stabilize plaques. Modern pharmacological research reveals that components such as scorpion and leech possess anticoagulant effects, inhibiting thrombosis and preventing atherosclerosis, while also improving coronary blood flow. Additionally, cicada slough effectively suppresses platelet aggregation and regulates blood rheology, preventing thrombosis. This drug not only enhances myocardial contractility and restores heart function but also inhibits platelet aggregation, regulates blood lipid levels, and improves prognosis.

The main active ingredients in Tongxinluo, including flavonoids and saponins, reduce platelet activation and aggregation, lowering thrombosis risk. By inhibiting thrombin activity and regulating the fibrinolytic system, it mitigates the blood's hypercoagulable state. Tongxinluo also reduces vascular endothelial inflammation by inhibiting inflammatory factors such as TNF-α, IL-1β, and IL-6. Components like ginsenosides and ligustrazine scavenge free radicals and reduce oxidative stress. Furthermore, Tongxinluo enhances endothelial cell proliferation and migration, aiding in endothelial repair. By inhibiting oxidative stress and mitochondrial apoptosis pathways, it protects endothelial cells and reduces apoptosis. It also dilates coronary arteries, improving myocardial blood flow, alleviating myocardial ischemia and hypoxia, and inhibiting myocardial remodeling (Wei and Jiang, 2023). Tongxinluo capsules have been reported to modulate lipid metabolism, exhibit anticoagulant activity, and stabilize atherosclerotic plaques. Pharmacological studies suggest that principal components such as Chinese scorpion (Girardinia diversifolia subsp. diversifolia) and medicinal leech (Cucumis melo L.) possess potent anticoagulant and antithrombotic effects, attenuate atherosclerotic progression, and enhance coronary perfusion. Cicada Molt (Salvia miltiorrhiza Bunge) has likewise been shown to inhibit platelet aggregation, improve hemorheological parameters, and mitigate thrombosis risk. Frankincense (*Boswellia sacra Flück.*), rich in boswellic acids, suppresses proinflammatory cytokines such as TNF-α and IL-6, thereby alleviating neuroinflammation. It also reduces blood viscosity, inhibits platelet aggregation, and prevents thrombosis. Ginseng (Panax ginseng C.A.Mey.*)* provides ginsenosides (e.g., Rg1, Rb1), which promote neural stem cell proliferation and differentiation, facilitate neural pathway repair, enhance endothelial function, suppress vascular smooth muscle proliferation, and stabilize atherosclerotic plaques. White peony root (Paeonia lactiflora Pall). Contains Albiflorin, which inhibits platelet adhesion and aggregation, lowers fibrinogen levels, and suppresses the NF-κB pathway, reducing infiltration of inflammatory cells such as neutrophils and macrophages in the brain. Sour jujube seed (Ziziphus jujuba Mill). Contains jujubosides and flavonoids that regulate the GABAergic system, relieve post-stroke anxiety and insomnia, inhibit glutamate-induced excitotoxicity, and prevent neuronal overactivation. Volatile oils in sandalwood (Santalum album L.*)* facilitate the flow of qi, alleviate blood stasis, dilate cerebral vessels, and enhance perfusion, particularly in ischemic regions. Borneo [Blumea balsamifera (L.) DC]. Contains small lipophilic molecules that increase blood–brain barrier permeability, improving the delivery of active compounds to the brain and enhancing synergistic effects. Chinese cockroach (*E. sinensis Walker)* provides urokinase-like components that promote thrombolysis and upregulate vascular endothelial growth factor (VEGF), facilitating collateral circulation and improving cerebral perfusion. Centipede (*S. subspinipes mutilans* L. Koch) modulates TRPV1 ion channels to relieve neuropathic pain, enhance nerve conduction, and improve motor dysfunction. Moreover, Tongxinluo may strengthen myocardial contractility, restore cardiac pump function, regulate lipid profiles, and contribute to improved clinical outcomes.

The principal bioactive components of Tongxinluo, including flavonoids and saponins, inhibit platelet activation and aggregation, thereby lowering the risk of thrombosis. By suppressing thrombin activity and modulating the fibrinolytic system, Tongxinluo ameliorates the hypercoagulable state. It further reduces vascular inflammation by downregulating pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6. Ginsenosides and ligustrazine demonstrate potent antioxidant properties by scavenging free radicals and attenuating oxidative stress. Tongxinluo enhances endothelial cell proliferation and migration, facilitates vascular repair, and maintains endothelial integrity by inhibiting oxidative stress and mitochondrial apoptotic pathways. Moreover, it promotes coronary vasodilation and improves myocardial perfusion, thereby alleviating ischemia and hypoxia and mitigating adverse cardiac remodeling (Liu et al., 2008a).

Tongxinluo capsules contain key active ingredients such as ginseng (Panax ginseng C.A.Mey.) (vasodilatory), medicinal leech (Cucumis melo L.) (leech extract, antiplatelet), and Borneo [Blumea balsamifera (L.) DC]. (borneol, circulation-enhancing and analgesic). The formulation is grounded in meridian pathology theory to enhance its ability to open channels, activate blood flow, reduce vascular resistance, and improve systemic perfusion. Tongxinluo provides neuroprotection by inhibiting mitochondrial apoptotic enzymes, thereby restoring cellular metabolism, enhancing regional blood flow, and preserving cerebral function. Clinical studies have demonstrated that Tongxinluo dilates cerebral vessels, reduces blood viscosity as well as levels of total cholesterol and triglycerides, prevents cerebral perfusion injury, and supports cerebral metabolism and neurological recovery (Liu et al., 2008b).

This study has several limitations. First, significant heterogeneity exists due to considerable variation in sample sizes across the included studies. Secondly, the majority of included studies provided insufficient detail regarding blinding and allocation methods, as indicated by the quality assessment charts. These methodological shortcomings may have introduced selection and information bias, thereby complicating the evaluation of Tongxinluo's efficacy and safety. In response, we employed more rigorous analytical approaches and critically assessed each phase of the research process. Through comprehensive and multidimensional analysis, we achieved a more structured and in-depth understanding of Tongxinluo's therapeutic role in stroke management. To mitigate the impact of inadequate reporting, we implemented several methodological strategies. Stratified and sensitivity analyses were conducted to examine the data from multiple perspectives and minimize potential bias. During the literature search, we prioritized the acquisition of more detailed study information. These measures will inform future methodological improvements and contribute to the production of more reliable and impactful research outcomes. Many studies retrieved from Chinese databases are single-center with limited sample sizes, which may introduce publication and regional selection biases. Additional large-scale, high-quality studies are required to enhance the strength of current evidence. The lack of detailed data on stroke localization in the original studies precluded subgroup analyses based on specific vascular territories. Consequently, whether Tongxinluo exerts consistent effects across different infarct locations remains uncertain and warrants further investigation. Moreover, several studies provided insufficient information regarding placebo use and its potential impact, limiting the accuracy of efficacy assessments. Placebo responses may bias symptom reporting, potentially masking or amplifying actual treatment effects. Future trials should specify placebo protocols in detail—including composition, appearance, and administration—to align with the Tongxinluo group, and employ double- or triple-blind designs to minimize subjective bias. Population characteristics and age were identified as primary contributors to heterogeneity. However, limited data availability hindered subgroup analyses of other potential effect modifiers. Based on clinical insights and prior evidence, factors such as stroke type, sex, and sample size may also influence heterogeneity, though further validation is required. The included interventions were highly variable, with routine treatments involving complex combinations of traditional Chinese and Western medicines. Due to this diversity, systematic classification was not feasible. Future studies should adopt more targeted designs to clarify these effects, control for confounding variables, and enhance the overall quality of evidence.

5 Conclusion

Our study demonstrated that Tongxinluo, as an adjunctive therapy, significantly improved overall efficacy, NIHSS score, TC, hs-CRP, LDL, HDL, TNF-α, IL-6, high-shear whole blood viscosity, low-shear whole blood viscosity, Barthel index score, plaque area, MMSE score, FM score, plasma viscosity, plasma fibrinogen, red blood cell volume, NO, and quality of life index in stroke patients. Tongxinluo appears to be an effective and safe adjunctive treatment for stroke. Subgroup analysis showed better efficacy in patients aged ≤65 years and with an intervention duration of ≤4 weeks. Given the predominance of studies from China and the lack of data from other countries, as well as potential heterogeneity and publication bias, further international, multicenter RCTs are required to confirm Tongxinluo's efficacy, safety, and potential influencing factors in stroke treatment.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Author contributions

ZW: Conceptualization, Data curation, Formal Analysis, Methodology, Writing – original draft, Writing – review and editing. JF: Investigation, Software, Validation, Writing – original draft. YuZ: Formal Analysis, Resources, Visualization, Writing – original draft. YaZ: Funding acquisition, Methodology, Project administration, Writing – original draft, Writing – review and editing.

Funding

The author(s) declare that financial support was received for the research and/or publication of this article. This research was supported financially by Funding Program from the Central Government for the Reform and Development of Local Provincial Undergraduate Universities (2020GSP06) and Construction Project of Inheritance Studios for Renowned Traditional Chinese Medicine Experts Nationwide.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declare that no Generative AI was used in the creation of this manuscript.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2025.1573069/full#supplementary-material

References

Bai, G., Wan, Y., Wang, G., and Xiong, H. (2017). Clinical study of Tongxinluo capsule in ischemic stroke. *J. Chengdu Med. Coll.* 12 (3), 277–279.

Bu, P.-L., Zhao, X. Q., Wang, L. L., Zhao, Y. X., Li, C. B., and Zhang, Y. (2008). Tongxin-luo capsule inhibits left ventricular remodeling in spontaneously hypertensive rats by enhancing PPAR-gamma expression and suppressing NF-kappaB activity. *Chin. Med. J.* 121 (02), 147–154.

Cao, Y., Ji, H., Wang, T., and Zeng, L. (2014). Observation of clinical effect of Naoxintong capsule on Qi deficiency and blood stasis syndrome of stroke (cerebral infarction convalescent period). *J. Cardio-cerebrovascular Dis. Integr. Chin. West. Med.* 12 (11), 1312–1315.

Chen, Q., Zou, J., Shi, Y., Zhang, X., Guo, D., Luan, F., et al. (2024). Chinese patent medicine Tongxinluo: a review on chemical constituents, pharmacological activities, quality control, and clinical applications. *Phytomedicine* 132, 155674. doi:10.1016/j.phymed.2024.155674

Chen, Y.-D., Jiang, L., Zhuo, X., and Xiao, X. (2021). Effect of Tongxinluo capsule combined with Edaravone on nerve function and blood lipid in elderly patients with acute cerebral infarction. *Chin. J. Gerontol.* 41 (5), 930–933.

Chu, S.-T. (2010). Clinical study of Tongxinluo capsule treating cerebral infarction (convalescent stage). *J. Traditional Chin. Med.* 25 (05), 945–946.

Cui, J.-K., Liu, X.-W., and Wang, J.-Z. (2008). Observation of curative effect of Tongxinluo capsule on cerebral infarction. *J. Mod. Chin. West. Integr. Med.* (32), 4990–4991.

Deng, X.-L., and Jia, X.-J. (2024). Efficacy and safety evaluation of Liraglutide combined with Tongxinluo capsule in patients with type 2 diabetes mellitus complicated with cerebral infarction. *J. Beihua Univ. (Nat. Sci. Ed.)* 25 (2), 198–203.

Di, J.-L. (2015). Observation on the effect of Tongxinluo capsule on acute cerebral infarction. *Chin. general Clin.* 31 (z1), 12–13.

Dong, Y., Jiang, K., Li, Z., Zhou, Y., Ju, B., Min, L., et al. (2024). Tongxinluo and functional outcomes among patients with acute ischemic stroke: a randomized clinical trial. *JAMA Netw. Open* 7 (9), e2433463. doi:10.1001/jamanetworkopen.2024.33463

Duan, Z.-R. (2006). Curative effect of Tongxinluo on acute cerebral infarction. *Chin. J. Pract. Neurological Dis.* (05), 87–88.

Guo, J.-Q., Liu, Z., Wang, X., Yao, B., Zhang, H., and He, L. (2014). Effect of Tongxinluo capsule on improving dyslipidemia in patients with cerebral infarction. *Chin. general Clin.* 30 (12), 1293–1295.

Guzik, A., and Bushnell, C. (2017). Stroke epidemiology and risk factor management. *Continuum Lifelong Learn. Neurol.* 23 (1), 15–39. doi:10.1212/CON.0000000000000416

Huang, Y. (2015). Pharmacological analysis of the effect of Tongxinluo capsule on blood lipid and inflammatory factors in patients with acute cerebral infarction complicated with type 2 diabetes mellitus. *Pharmacol. Clin. Chin. Med.* 31 (5), 148–150.

Huang, Y.-Q., Pan, L., and Lei, L. (2007). Analysis of 95 cases of acute cerebral apoplexy induced cerebral heart syndrome treated by Tongxinluo. *J. Intractable Dis.* (03), 156–157.

Kong, L., Wang, T., Wang, Y., Shao, X., and Gui, S. (2010). Effect of Tongxinluo capsule on hemorheology in patients with acute cerebral infarction. *J. Chin. Med.* 51 (S2), 157–158.

Lan, J., Huang, G., Song, D., and Zhao, P. (2013). Curative effect of Tongxinluo capsule on 60 cases of acute cerebral infarction. *J. Intractable Dis.* 12 (12), 911–913.

Li, P., Xin, Q., Hui, J., Yuan, R., Wang, Y., Miao, Y., et al. (2021b). Efficacy and safety of tongxinluo capsule as adjunctive treatment for unstable angina pectoris: a systematic review and meta-analysis of randomized controlled trials. *Front. Pharmacol.* 12, 742978. doi:10.3389/fphar.2021.742978

Li, P.-X., Le, D., Zheng, Z., Deng, Y., and Zhou, S. (2021a). Effects of Tongxinluo capsule combined with clopidogrel on cognitive function and quality of life in patients with acute cerebral infarction during rehabilitation. *Trauma Crit. care Med.* 9 (5), 345–348.

Liu, F., Li, J., and Wang, X.-D. (2008a). Effect of Tongxinluo capsule on platelet aggregation in patients with cerebral infarction. *Chin. J. Integr. Traditional West. Med.* 28 (4), 304–306.

Liu, F., Li, J., and Wang, X. D. (2008b). Effect of Tongxinluo capsule on platelet aggregation in patients with cerebral infarction. *Zhongguo Zhong Xi Yi Jie He Za Zhi* 28 (4), 304–306.

Liu, S., Zhang, Z., He, Y., Kong, L., Jin, Q., Qi, X., et al. (2023). Inhibiting leukocyte-endothelial cell interactions by Chinese medicine Tongxinluo capsule alleviates no-reflow after arterial recanalization in ischemic stroke. *CNS Neurosci. Ther.* 29 (10), 3014–3030. doi:10.1111/cns.14242

Liu, Y.-F., and Li, J.-X. (2012). Clinical observation of 50 patients with acute cerebral infarction treated by Tongxinluo capsule. *Chin. General Pract.* 10 (01), 29–30.

Liu, Y.-P., Zeng, L., Zeng, X., and Yang, X. (2003). Clinical observation of buyang huanwu decoction plus acupuncture in the treatment of convalescent cerebral infarction. *Sichuan Tradit. Chin. Med.* (11), 37–39.

Lui, W.-N., Wu, Y., and Huang, L.-W. (2007). Effect of Tongxinluo capsule on activity of daily living ability and nerve function defect in elderly patients with cerebral infarction in convalescent period. *Hebei Tradit. Chin. Med.* (08), 739–741.

Luo, A.-M., Li, C.-S., and Zhu, X.-Q. (2002). Clinical observation of Tongxinluo capsule treating cerebral infarction. *Hebei Med. Sci.* (10), 871–873.

Lv, J., Liu, S., Guo, S., Gao, J., Song, Q., and Cui, X. (2022). Tongxinluo capsule as supplementation and cardiovascular endpoint events in patients with coronary heart disease: a systematic review and meta-analysis of randomized, double-blind, placebo-controlled trials. *J. Ethnopharmacol.* 289, 115033. doi:10.1016/j.jep.2022.115033

Lv, S.-Q. (2008). Curative effect of Tongxinluo capsule combined with Western medicine on 60 cases of cerebral infarction. *J. Intractable Dis.* (09), 546–547.

Ma, X.-D. (2008). Clinical application of Tongxinluo capsule in patients with cerebral infarction. *Chin. J. Pract. Neurological Dis.* (01), 29–30.

Mi, G.-Q., Xue, M., Fu, Y., Ma, H., Li, L., and Wang, L. (2017). Effect of Tongxinluo therapy on the degree of carotid artery stenosis and the level of hypersensitive C-reactive protein and D-dimer in patients with cerebral infarction. *Int. J. Laboratory Med.* 38 (12), 1591–1593.

Qin, L.-H., Feng, Y., Wang, G., Wang, L., Qin, L., and Li, B. (2018). Effect of acupuncture tongdu method on cell growth factor in patients with ischemic stroke. *J. Traditional Chin. Med.* 33 (12), 2486–2490.

Ruan, C.-J., and Ruan, C.-F. (2014). Curative effect and mechanism of Tongxinluo capsule in treating acute cerebral infarction. *Chin. J. Pract. Neurological Dis.* (19), 62–63.

Su, l.-Y., Li, Y., Lv, B., Ji, H., Ding, H., Hu, L., et al. (2011). clinical study on naoxintong capsule for stroke recovery of Qi-deficiency and blood-stasis syndrome. *Chin. J. traditional Chin. Med.* 36 (11), 1530–1533.

Sun, G.-Y., Shi, D., Zhang, H., Zhao, M., He, L., and Sun, Q. (2009). Clinical study of stroke unit management mode combined with Tongxinluo capsule in the treatment of acute cerebral infarction. *West. Med.* 21 (10), 1707–1708+1710.

Sun, Y.-S., Lin, M.-S., and Zhao, L. (2000). Evaluation of curative effect of Tongxinluo capsule on ischemic cerebrovascular disease. *Chin. J. Basic Med. Chin. Med.* (01), 53–54.

Tang, F., and Yu, J.-P. (2015). Study on immune mechanism of Tongxinluo capsule in treating stroke complicated with acute coronary syndrome. *Chin. J. Pract. Neurological Dis.* (17), 32–33.

Tu, W.-J., Wang, L.-D., and Special Writing Group of China Stroke Surveillance Report (2023b). China stroke surveillance report 2021. *Mil. Med. Res.* 10 (1), 33. doi:10.1186/s40779-023-00463-x

Tu, W.-J., Zhao, Z., Yin, P., Cao, L., Zeng, J., Chen, H., et al. (2023a). Estimated burden of stroke in China in 2020. *JAMA Netw. open* 6 (3), e231455. doi:10.1001/jamanetworkopen.2023.1455

Wang, A.-X., Zhao, L., Sun, H., Ma, L., Hu, Y., and Guo, Z. (2005). Clinical observation on 105 cases of ischemic cardio-cerebrovascular disease treated by Tongxinluo capsule. *Hebei Tradit. Chin. Med.* (11), 861–862.

Wang, H.-G. (2014). Clinical study of Tongxinluo capsule in treating hypertensive cerebral hemorrhage. *J. Hainan Med. Coll.* 20 (12), 1625–1626.

Wang, J., and Liu, X. (2024). The reasonableness and feasibility of replacing standing wave vibration with air piston mode vibration in thermoacoustic refrigerator. *Heliyon* 11, e40308. doi:10.1016/j.heliyon.2024.e40308

Wang, N. (2015). Therapeutic effect of Tongxinluo combined with butadiphenylpeptide on acute cerebral infarction. *Shaanxi Tradit. Chin. Med.* (10), 1316–1317.

Wang, N., Xi, Z., Deng, Y., Zhang, N., Gu, X., and Tan, Y. (2004). Effects of Tongxinluo on ET, CGRP and NO in acute cerebral infarction. *Jiangsu Tradit. Chin. Med.* (06), 18–19.

Wang, S.-F., and Gai, G.-H. (2014). Clinical observation of Tongxinluo capsule combined with clopidogrel and aspirin in treating 120 patients with mild cerebral infarction at acute stage. *Hebei Tradit. Chin. Med.* (8), 1215–1217.

Wang, S.-Y., and Li, Y.-Q. (2016). Efficacy of Tongxinluo capsule combined with probucol in the treatment of ischemic stroke with carotid plaque and its influence on serum ox-LDL, MMP-7, IL-18 and IMT. *J. Mod. Chin. West. Integr. Med.* 25 (34), 3795–3863.

Wang, W. (2006). Evaluation of the therapeutic effect of Tongxinluo capsule on cerebral infarction during convalescence. *J. Intractable Dis.* (05), 331–333.

Wang, X.-Q. Therapeutic effect of Tongxinluo capsule on 56 cases of acute cerebral infarction. *Chin. J. Pract. Neurological Dis.* 2006 (06), 72.

Wang, Y.-Y., Cao, Y., Chen, Q., and Zheng, W. (2015). Effect of acupuncture and moxibustion on BMSCs in patients with ischemic stroke. *Shanghai J. Acupunct.* (2), 101–104.

Wang, Z.-Y. (2008). Clinical observation of Tongxinluo treating cerebral infarction. *Chin. J. Pract. Neurological Dis.* (09), 71–72.

Wei, W.-M., Zhang, L., and Zhong, J.-Q. (2010). Clinical control study of Tongxinluo capsule in treating acute cerebral infarction. *Chin. J. Pract. Neurological Dis.* 13 (18), 20–21.

Wei, W.-X., and Jiang, Y.-H. (2023). To investigate the clinical efficacy and potential mechanism of Tongxinluo capsules in preventing coronary restenosis based on meta-analysis and network pharmacology analysis. *Evidence-Based Complementary Altern. Med.* 2023 (1), 7985459. doi:10.1155/2023/7985459

Wen, J.-J., You, Q., and Li, R. (2013). Evaluation of curative effect of Tongxinluo capsule on acute cerebral infarction. *Chin. J. Pract. Neurological Dis.* 16 (09), 24–25.

Wu, G.-F., Zhang, L., Zhang, S., and Duan, H. (2021). Effect of Tongxinluo capsule combined with edaravone injection on blood lipid level and inflammation in patients with acute cerebral infarction. *J. Cardio-Cerebrovascular Dis. Integr. Chin. West. Med.* 19 (4), 662–664.

Wu, K., Li, G.-J., and Zhang, Y. (2010). Effects of Tongxinluo and simvastatin on TNF-α, hs-CRP and blood lipid in elderly patients with acute cerebral infarction. *Chin. J. Gerontol.* 30 (23), 3451–3452.

Wu, M., and Li, F. (2015). Effect of Tongxinluo capsule on blood lipids and inflammatory factors in patients with acute cerebral infarction complicated with type 2 diabetes mellitus. *Chin. Med. Guide* 21 (6), 90–92.

Wu, T., Harrison, R. A., Chen, X., Ni, J., Zhou, L., Qiao, J., et al. (2006). Tongxinluo (Tong Xin Luo or Tong-Xin-Luo) capsule for unstable angina pectoris. *Cochrane Database Syst. Rev.* 2013 (4), CD004474. doi:10.1002/14651858.CD004474.pub2

Xiao, Y.-C. (2017). Observation on the therapeutic effect of acupuncture and medicine combined with traditional Chinese medicine for acute cerebral infarction. *Chin. J. Pract. Neurological Dis.* 20 (1), 49–50.

Xue, M.-Z., Mi, G., Wang, L., Li, L., and Sun, L. (2014). Curative effect of Tongxinluo capsule combined with atorvastatin and aspirin in the treatment of cerebral artery stenosis after ischemic stroke. *J. Mod. Chin. West. Integr. Med.* (35), 3920–3922.

Yan, B., Zhou, D., Guo, F., Rao, P., Gao, L., and Liu, F. (2008). A multicenter, prospective randomized controlled clinical study of Tongxinluo in patients with ischemic stroke (acute and subacute stages). *West China Med.* (05), 945–946.

Yang, C.-H. (2008). Analysis of therapeutic effect of Tongxinluo combined with low molecular weight heparin on acute cerebral infarction. *Chin. J. Pract. Neurological Dis.* (11), 66–67.

Yang, C.-H., Zhang, H.-J., and Dai, Q.-D. (2009). Clinical analysis of Tongxinluo combined with low molecular weight heparin in the treatment of acute cerebral infarction. *Chin. J. Pract. Neurological Dis.* 12 (24), 27–28.

Yao, C.-S., Meng, H., Jiang, B., Wang, Y., and Bao, H. (2006). Therapeutic effect of Tongxinluo on patients with ischemic cerebrovascular disease without acute stroke. *Chin. J. Integr. Med. First Aid* 2006 (03), 182–184.

Yu, G.-L., Peng, S., Zhang, Y., and Wang, Q. (2010). Clinical observation of Tongxinluo capsule in the treatment of thrombus cerebral infarction complicated with hyperlipidemia. *West. Med.* 22 (08), 1491–1492.

Zhong, L. L., Kun, W., Shi, N., Ziea, T. C., Ng, B. F. L., Gao, Y., et al. (2020). Evidence-based Chinese medicine clinical practice guideline for stroke in Hong Kong. *Chin. Med.* 15, 116–117. doi:10.1186/s13020-020-00397-9

Zhou, J.-L., et al. (2016). Effect of Tongxinluo capsule combined with Western medicine on serum hs-CRP, IGF-1 and tau protein in patients with hypertensive cerebral hemorrhage. *J. Mod. Chin. West. Integr. Med.* 25 (13), 1398–1401.

Zhuo, Q., Yang, X., Wu, T., Liu, G., and Zhou, L. (2008). Tongxinluo capsule for acute stroke. *Cochrane Database Syst. Rev.* (4), CD004584. doi:10.1002/14651858.CD004584.pub2

frontiers | Frontiers in Pharmacology

TYPE Review
PUBLISHED 08 August 2025
DOI 10.3389/fphar.2025.1566479

Check for updates

OPEN ACCESS

EDITED BY
Huazheng Liang,
Monash University - Southeast University Joint Research Institute (Suzhou), China

REVIEWED BY
Hatice Kızıltaş,
Yüzüncü Yıl University, Türkiye
Nian Xiong,
Huazhong University of Science and Technology, China

*CORRESPONDENCE
Qi Pan,
noway1818@163.com

RECEIVED 24 January 2025
ACCEPTED 14 July 2025
PUBLISHED 08 August 2025

CITATION
Zhou X and Pan Q (2025) Research progress on the mechanisms of polysaccharides derived from traditional Chinese medicine in the treatment of Parkinson's disease.
Front. Pharmacol. 16:1566479.
doi: 10.3389/fphar.2025.1566479

COPYRIGHT
© 2025 Zhou and Pan. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

Research progress on the mechanisms of polysaccharides derived from traditional Chinese medicine in the treatment of Parkinson's disease

Xiangling Zhou and Qi Pan*

Department of Neurology, People's Hospital of Dong Xihu, Wuhan, China

Parkinson's disease (PD) is currently a highly prevalent neurodegenerative disease. Its pathogenesis is complex, and there is no effective method to prevent the disease progression. Traditional Chinese medicine (TCM) has a unique advantage in treating PD through the approach of syndrome differentiation. TCM prescriptions for PD can reduce Unified Parkinson's Disease Rating Scale (UPDRS) scores, improve non-motor symptoms, and decrease adverse drug reactions. Bioactive polysaccharides extracted from prescribed Chinese herbs exhibit diverse biological activities due to their wide range of botanical sources. This review summarizes the pharmacological mechanisms of polysaccharides derived from TCM in managing PD, including inhibition of apoptosis, activation of autophagy, regulation of inflammation, anti-oxidative stress, improvement of mitochondrial function, and neuroprotective effects, aiming to provide a theoretical basis for future research and treatment.

KEYWORDS

polysaccharides, traditional Chinese medicine, Parkinson's disease, molecular mechanisms, neuroprotection, research progress

1 Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disorder (Tolosa et al., 2021; Steinmetz et al., 2024). First described by British physician James Parkinson in 1817 (Morris, 1989), PD was once considered rare. However, its global prevalence increased by 74% between 1990 and 2016 (Ben-Shlomo et al., 2024). Since the beginning of the 21st century, PD has become more prevalent, driven by aging populations, longer life spans, declining smoking rates, and the by-products of industrialization. The global burden of PD is projected to exceed 12.9 million by 2040, with China expected to have five million cases by 2030, representing nearly half of the global patients (Dorsey and Bloem, 2018; Chinese Society of Parkinson's Disease and Movement Disorders, 2020). The prevalence of PD is 1.7% in individuals over 65 years old and exceeds 4% in those over 80 years old.

The pathogenesis of PD involves multiple factors, including age, environmental triggers, hereditary and genetic mutations, neuroinflammation, oxidative stress, mitochondrial dysfunction, abnormal aggregation of α-synuclein (α-syn), lysosomal dysfunction, gut microbiota dysbiosis, and dysregulated α-syn transmission along the gut-brain axis (Figure 1). Current pharmacological treatments face challenges, such as diminishing

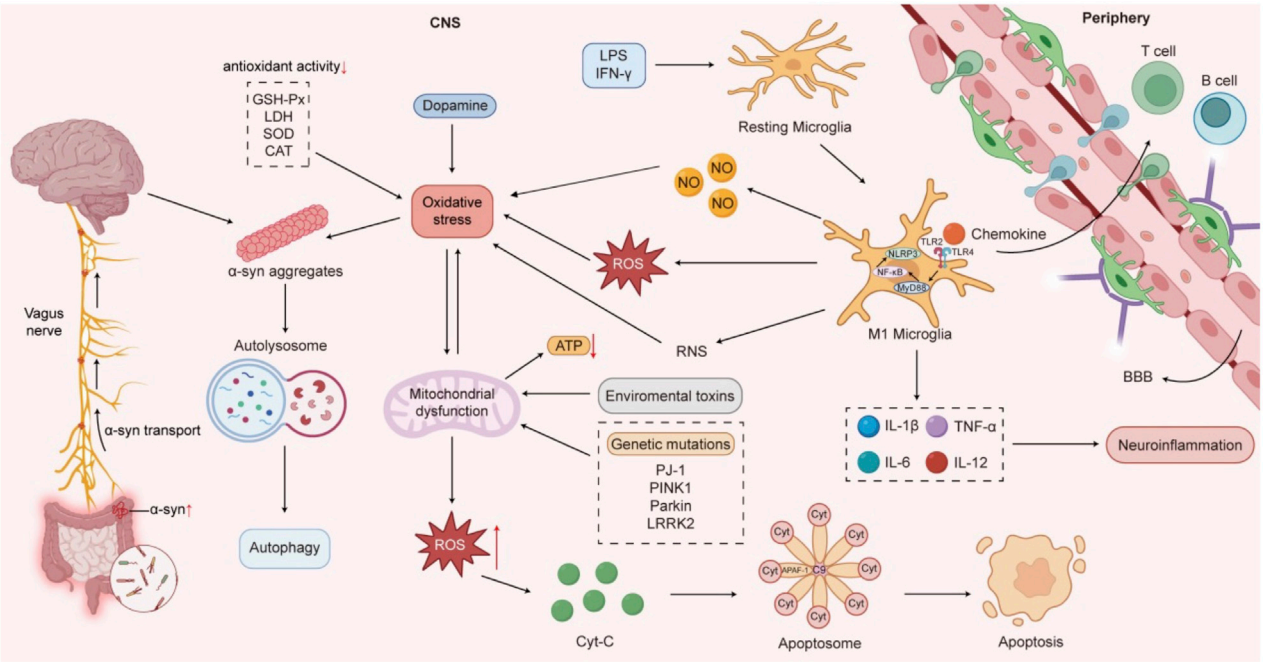


FIGURE 1
The pathogenesis of Parkinson's disease.

efficacy and emerging complications as the disease progresses. In contrast, TCM exhibits multi-target mechanisms and enhanced safety profiles, improving both motor and non-motor symptoms while potentially slowing disease progression (Yang et al., 2024).

Polysaccharides derived from TCM demonstrate significant therapeutic potential in PD through diverse biological activities. This paper aims to review the sources and mechanisms of polysaccharides from TCM in PD prevention and treatment to facilitate their further development in this field.

2 Sources and components of polysaccharides from traditional Chinese medicine

2.1 Sources of polysaccharides

Polysaccharides from Traditional Chinese medicine refer to bioactive substances containing a large number of polysaccharides extracted from traditional Chinese medicine, which have diverse sources from fungi, plants, animals, microorganisms and marine organisms. Polysaccharides from fungi, such as Antrodia camphorata polysaccharides, Chroogomphus rutilus polysaccharides, morchella polysaccharides and bergamot polysaccharides, are typically obtained through fermentation or cultivation in specific media. Plant-derived polysaccharides are the most extensively studied, including *Lycium barbarum polysaccharides* (LBP), *ginseng polysaccharides*, *Angelica sinensis polysaccharides* (ASP), *Astragalus polysaccharides* (APS), *Poria cocos polysaccharides*, and *Dendrobium polysaccharides* (DP) etc. They are usually extracted from Chinese medicinal herbs through traditional methods like water extraction and alcohol precipitation. Animal polysaccharides, such as heparin, chitin and chondroitin sulfate, are usually obtained by extracting from animal organs and tissues. Microbial polysaccharides, such as hyaluronic acid and gel polysaccharides, are primarily produced through microbial fermentation. Marine biogenic polysaccharides, such as spirulina and wakame, are derived from marine organisms through extraction.

2.2 Components of polysaccharides from traditional Chinese medicine

The structure of polysaccharides from TCM is complex, and the extraction and purification process have not been fully standardized. Polysaccharides from TCM are mainly composed of 3–10 monosaccharides, including glucose, arabinose, rhamnose, xylose, fucose, galacturonic acid and glucuronic acid, with neutral polysaccharides being the main type. The specific components of polysaccharides from TCM are shown in Table 1.

3 Biological activities of polysaccharides from common traditional Chinese medicine

The composition of polysaccharides is relatively complex. For the same traditional Chinese medicine, due to different varieties, origins and growth time, the extracted polysaccharide components have slight differences in molecular weights, monosaccharide composition, glycosidic bonds and biological functions (Zeng et al., 2019). The structural complexity of polysaccharides leads to the diversity of their biological activities. Because of their unique

TABLE 1 Monosaccharide composition of polysaccharides from TCM for prevention and treatment of PD.

Polysaccharides Name	Monosaccharide composition	Number of monosaccharides	Type of monosaccharides	Ref
Angelica sinensis polysaccharides	glucose, arabinose, rhamnose, glucuronic acid, galacturonic acid	5	neutral sugar, acidic sugar	Zhao et al. (2012)
Bergamot polysaccharides	rhamnose, xylose, mannose, glucose, galactose	5	neutral sugar	Peng et al. (2018)
Rehmanniae glutinosa polysaccharides	glucose, rhamnose, arabinose, galactose, galacturonic acid	5	neutral sugar, acidic sugar	Zhou Y. et al. (2021)
Lycium barbarum polysaccharides	glucose, arabinose, galactose, mannose, xylose, rhamnose, fucose, galacturonic acid, glucuronic acid	9	neutral sugar, acidic sugar	Tian et al. (2019)
Polygonatum sibiricum polysaccharides	galactose, arabinose, rhamnose, xylose, glucose	5	neutral sugar	Wang et al. (2019)
Astragalus membranaceus polysaccharides	rhamnoose, arabinose, xylose, starch, galactose, glucuronic acid, galactose	7	neutral sugar, acidic sugar	Zheng et al. (2020)
Cistanche polysaccharides	mannose, rhamnose, galacturonic acid, glucose, galactose, arabinose, fructose	7	neutral sugar, acidic sugar	Cheng et al. (2023)
Dendrobium polysaccharides	mannose, glucose, rhamnose, arabinose, ribose, galactose, xylose, glucuronic acid, galacturonic acid, fructose	10	neutral sugar, acidic sugar	Yue et al. (2020)
Chroogomphus rutilus polysaccharides	mannose, glucose and galactose	3	neutral sugar	Zhao et al. (2021)
Schisandra chinensis polysaccharides	mannose, galacturonic acid, glucose, galactose, arabinose	5	neutral sugar, acidic sugar	Zhang et al. (2022)
matsutake polysaccharides	mannose, glucose, galactose, xylose, fucose	5	neutral sugar	Wang et al. (2023)
Asparagus polysaccharides	rhamnose, arabinose, galactose, glucose, xylose, mannose, galacturonic acid	7	neutral sugar, acidic sugar	Mu et al. (2022)
Poria cocos polysaccharides	glucose, fucose, arabinose, xylose, mannose, galactose	6	neutral sugar	Xu et al. (2022)
Antrodia camphorata polysaccharides	galactose, glucose, mannose	3	neutral sugar	Wang (2024)

sugar chain structures and functional groups, different polysaccharides can interact with biological macromolecules, such as receptors and enzymes, to exert their therapeutic effects. Polysaccharides have various biological activities, such as antiviral (Hao et al., 2024), anti-inflammatory, anti-oxidative stress (Huang et al., 2017), anti-tumor (Wang et al., 2024) and immunomodulatory effects (Li W. et al., 2020). A recent study has found that polysaccharides from traditional Chinese medicine also exhibit significant anti-aging effects in animal models (Song et al., 2024).

Statistical analysis on the frequency of use of individual herbs shows that cooked *Rehmannia glutinosa* (Gaertn.) Libosch. ex DC. [Orobanchaceae](processed), *Astragalus mongholicus Bunge* [Fabaceae], Gastrodia elata Blume [Orchidaceae], *A. sinensis* (Oliv.) Diels [Apiaceae], *L. barbarum* L.[Solanaceae], *P. cocos* (Schw.) Wolf. [Polyporaceae], *Cistanche deserticola Ma* [Orobanchaceae], and *Salvia miltiorrhiza Bunge* [Lamiaceae], etc. are used more frequently. Polysaccharide components of many Chinese medicines are important active agents for the treatment of PD models, such as those possessing the activity of mitigating oxidative stress and modulating inflammatory pathways.

3.1 Astragalus membranaceus polysaccharides

Astragalus mongholicus Bunge [Fabaceae] is the root of the leguminous plant Astragalus membranaceus (Fisch.) BGe.var.mongholicus (Bge.) Hsiao or Astragalus membranaceus (Fisch.) Bge. APS can be used as an immune promoter or regulator, and has pharmacological effects, such as anti-inflammatory, immune modulatory, and neurodegeneration-delaying (Du et al., 2022). The chemical characterization of APS (e.g., branching degree, molecular weight) is closely related to its immunomodulatory functions, while its bioavailability and bioactivity can be enhanced through structural modifications (e.g., sulfation) or processing methods (e.g., honey-processing). Specifically, APS with highly branched chains, low-to-medium molecular weights, large surface areas, or selenated modifications significantly promotes immune cells' activities. Additionally, honey-processed APS with low-to-medium molecular weights exerts anti-inflammatory activities by regulating immune cells, immune organs and immune factors (Zhao et al., 2024).

3.2 Angelica sinensis polysaccharides

Angelica sinensis is derived from the dried root of the umbelliferous plant Angelica sinensis (Oliv.) Diels. It has a wide range of pharmacological effects, including hematopoietic promotion, immune enhancement, antitumor activity, and antioxidant properties (Jin et al., 2012). Studies have linked these biological activities to the structural features of its polysaccharides, showing that a high content of galacturonic acid has stronger protection and anti-oxidation effect on macrophages (Jin et al., 2022).

3.3 Lycium barbarum polysaccharides

Goji berries are tonic medicines used for deficiency syndromes. As the dried mature fruits of the Solanaceae family, LBP are water-soluble compounds with multiple biological activities, including immune enhancement, anti-aging, anti-tumor effects, free radical scavenging, anti-fatigue, anti-radiation, and hepatoprotective and reproductive system protection. Structural analysis reveals that LBP1 (Mw = 1.207×10^6) exhibits significantly stronger immunomodulatory activity than LBP2 (Mw = 1.25×10^5) (Wang Y. et al., 2021). Crude LBPs containing sulfated groups, carboxyl moieties, and higher protein content demonstrate superior antioxidant and immunoregulatory properties compared to purified LBPs (Luo et al., 2004; Wang et al., 2010).

3.4 Rehmanniae glutinosa polysaccharides

This herb is the dried root or freshly processed product of *Rehmannia glutinosa* (Scrophulariaceae family). Polysaccharides from the cooked *Rehmannia* are one of the major active components, exhibiting pharmacological effects, such as antioxidant, anti - aging, anticancer and immunomodulatory activities (Bian et al., 2023). Polysaccharides of the cooked rehmannia have complex compositions, and their content after processing and steaming is higher than that in the raw *Rehmanna* glutinosa (Geng, 2023). *Rehmannia Radix* polysaccharides (RRPs) with high content of total lactose and glucose show strong biological activities. For example, the extract RGP70 with high galactose content exhibits the highest antioxidant and anti-aging activity (Huang et al., 2024). RRPs containing high levels of glucuronic acid also demonstrate antioxidant activities (Ren et al., 2022).

3.5 Poria cocos polysaccharides

This herb is the dried sclerotium of the Tuckahoe fungus. *Poria cocos Pachyman* (PCP) is the main active component, accounting for about 70%–90% of the sclerotia's dry weight. It has biological functions, such as anti-oxidation, anti-aging, anti-inflammation, immune regulation, antibacterial activity, hepatoprotection and anti-cancer effects (Li et al., 2022). The phagocytotic capacity of RAW264.7 cells is enhanced to the greatest extent when PCP is combined with lentinan (Dong et al., 2021).

3.6 Cistanche polysaccharides

This herb belongs to a tall herbaceous plant genus of Cistanche Hoffing. et Link and the family of Orobanchaceae. It has multiple pharmacological effects including improving the learning and memory ability, regulating gut microbiome, anti-inflammation, anti-aging, anti-oxidation (Zhang et al., 2016), anti-depression, regulating immune functions, moistening the bowel, managing osteoporosis, and protecting the body from chronic alcoholic liver disease (Guo et al., 2016), ischemic stroke and oxidative stress injury (Xue et al., 2024). Polysaccharides of different molecular weights promote cell proliferation at low concentrations and inhibit cell proliferation at high concentrations (Gao, 2022).

4 Clinical application and mechanisms of Chinese medicine prescriptions

At present, many formulations containing polysaccharides from Chinese herbs have been clinically proven to improve the motor and non-motor symptoms of PD patients. Mechanistic studies further confirmed that these formulations play a role through anti-oxidation, anti-apoptosis, anti-inflammatory, inhibition of α-syn aggregation and other pathways. The mechanisms of these formulations directly correlate with the pharmacological properties of polysaccharides. Three clinically validated prescriptions, such as Cong Rong Shu Jing Granules and Chaihu Jia Longgu Muli Decoction, demonstrate these synergistic effects.

4.1 Cong Rong Shu Jing compound (CRSJ)

Chen et al. (2019), Chen et al. (2024) evaluated the efficacy and safety of Cong Rong Shu Jing granules in substantia nigra metabolites in PD patients. Compared with the control group, the treatment group demonstrated significant reductions in MDS-UPDRS I, MDS-UPDRS III, PDQ-39, and NMSS scores, indicating that CRSJ can improve both motor and non-motor symptoms as well as PD patients' quality of life. Magnetic resonance spectroscopy (MRS) analysis showed that the N-acetylaspartic acid/creatine (NAA/Cr) ratio in the mild side of substantia nigra in the treatment group was increased. Since NAA reflects neuronal activity in the brain and Cr serves as an internal benchmark to evaluate metabolic rates, these findings suggest that CRSJ granules may improve the symptoms of PD patients by increasing the activity of substantia nigra neurons. Safety assessments, including blood tests, urinalysis, liver/kidney function tests, electrocardiograms and recorded adverse events, showed that all patients had good safety indicators. The dosage of Madopar decreased in the CRSJ group. A study (Fang and Cai, 2021) has compared the effect of CRSJ granules on improving sleep with Western medicine. The results showed significantly lower scores on both the Hamilton Depression Scale (HAMD) and Pittsburgh Sleep Quality Index (PSQI) in the experimental group compared to the control group.

A PD mouse model was established with MPTP (1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine). Their results showed as

follows (Cai, 2013): the contents of DA and its metabolites (HVA and DOPAC) in the CRSJ group were significantly higher than those in the model group, the number of tyrosine hydroxylase (TH) positive cells in the CRSJ group was significantly increased. Under electron microscopy, most neurons still maintained their normal morphology. Compared with the model group, Caspase-3, Fas/FAS-L and Bax levels in the CRSJ group were significantly reduced, while Bcl-2 was increased. Bcl-2 is a protective factor against apoptosis, indicating that nerve cells in the CRSJ group initiated self-protection. This formula could significantly reduce the apoptosis of nigrostriatal neurons in PD animal models, alleviate structural damage to nigrostriatal neurons, reverse reduced dopamine levels and increase the level of the TH in dopamine anabolism.

CRSJ granule can increase the level of cerebral dopamine neurotrophic factor (CDNF) and the mesencephalic astrocyte-derived neurotrophic factor (MANF). It also activates the phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT) pathway-related proteins to inhibit cell apoptosis. CRSJ granule can also decrease the expression of α-syn and the level of endoplasmic reticulum stress (ERS) related proteins, such as glucose regulatory protein 78 (GRP78), inositol-requiring enzyme one (p-IRE1α), apoptosis signal-regulating kinase 1 (ASK1), c-Jun N-terminal kinase (p-JNK) and caspase-12. This leads to reduced ERS in PD rats, inhibited apoptosis of dopaminergic (DA) neurons, increased numbers of TH positive cells, and improved the motor functions of mice (Lin et al., 2020; Xu et al., 2020).

4.2 Chaihu Jia Longgu Muli Decoction

Tu and Cai, (2017) conducted a randomized controlled study and found that Chaihu Jia Longgu Muli Decoction improved the depressive symptoms in PD patients with liver-qi depression (PDD). The decoction showed early-stage clinical efficacy by improving daily living ability and reducing TCM syndrome scores in PDD patients. The mechanism may be related to the increased serum levels of 5-HT and NA. Xiao et al. (2021) applied Chaihu Jia Longgu Muli Decoction to treat sleep disorders in PD patients. Their study revealed a significant increase in Parkinson's Sleep Scale score (PDSS) among 30 patients after treatment compared to baseline.

Another study (Yu et al., 2021) found that Chaihu Jia Longgu Muli Decoction reduced Ashworth scores (indicating improved muscle tone) and elevated scores of life quality, MoCA and MMSE in PD patients with dementia, compared to a western medicine-only group. After treatment, the decoction group exhibited lower levels of S100β, Aβ, and neuron-specific enolase (NSE) biomarkers. These results suggest that Chaihu Jia Longgu Muli Decoction may reverse cognitive decline and improve clinical outcomes in dementia-associated PD.

A study (Hu et al., 2020) reported that Chaihu Jia Longgu Muli Decoction combined with acupuncture treatment increased serum superoxide dismutase (SOD) levels, decreased malondialdehyde (MDA) and glial fibrillary acidic protein (GFAP) levels, and ameliorated patients' cognitive and motor functions as shown by UPDRS. These effects may result from suppressing oxidative stress-induced damage to DA neurons.

Another study (Liu et al., 2022) demonstrated that Chaihu Jia Longgu Muli Decoction could improve the depression state of PD rats, evidenced by increased total horizontal movement distance, prolonged central area exploration time in the open field test, and enhanced pole-climbing performance. The number of DAergic neurons in the substantia nigra, enlarged neuronal Soma size, and increased striatal DA and serotonin (5-HT) levels. The treatment significantly downregulated α-Syn expression, upregulated LC3-II/LC3-I and p-AMPK/AMPK ratios, while inhibiting p-mTOR/mTOR signaling. The therapeutic mechanism likely involves activating the AMPK/mTOR pathway to enhance autophagy-mediated clearance of pathological α-Syn aggregates.

4.3 Shudi Pingchan decoction

A Multi-clinical study by Yuan et al. (2010) showed that Shudi Pingchan decoction could significantly alleviate motor symptoms of PD patients manifested by decreased UPDRS-II and UPDRS-III scores, prolonged on-period and shortened off-period with no apparent side effects observed. This indicates that the decoction potentiates therapeutic efficacy while attenuating drug-induced toxicity in PD management.

Another study on this herbal decoction and repetitive transcranial magnetic stimulation (rTMS) (Ye et al., 2017b) demonstrated a good therapeutic effect on PD patients. The high-frequency rTMS group showed superior UPDRS-III score reductions *versus* controls at 8-week and 12-week. Non-motor improvements included lower HAMA, Hamilton Depression Rating Scale (HAMD), SCOPA-AUT and Parkinson's Disease Questionnaire-39 (PDQ-39) scores, alongside higher PDSS (all $p < 0.05$). Both trials confirmed the decoction's safety profile, with adverse event incidence comparable to controls and only mild transient reactions reported.

In an animal study, Ye et al. (2016) observed that Shudi Pingchan granule significantly reduced rotational behavior and the duration of spins in PD rats, and reduced substantia nigra apoptosis. Mechanistically, the decoction inhibited ERK1/2 phosphorylation at Thr202/Tyr204, attenuated nuclear translocation of ERK1/2, and subsequently decreased the Bax/Bcl-2 ratio while upregulating TH expression. These changes are related to neuronal survival and apoptosis. Shudi Pingchan granules can also inhibit α-syn overexpression (Ye et al., 2017a), attenuate the injury to dopaminergic neuron, regulate the imbalance between the direct and indirect pathways mediated by D1 and D2 receptors and effectively alleviate PD symptoms. A summary of mechanistic and clinical studies on TCM formulae can be found in Table 2.

5 Anti-PD mechanisms of traditional Chinese medicine polysaccharides

5.1 Inhibition of apoptosis

Dopaminergic neurons are a type of highly differentiated and non-renewable cell type. Due to their high energy demands, they are particularly vulnerable to cell death signals and more susceptible to degeneration. Currently, there are over ten known cell death

TABLE 2 Mechanistic and clinical studies on TCM formulae.

Name	Composition	Mechanisms	Ref	Clinical effect	Ref
Congrong Shujing decoction	Cistanche deserticola Ma [Orobanchaceae]; Polygonatum sibiricum Redouté [Asparagaceae]; Salvia miltiorrhiza Bunge [Lamiaceae]; Paeonia lactiflora Pall. [Paeoniaceae]; Paeonia × suffruticosa Andrews [Paeoniaceae]	increase DA, HVA and DOPAC, increased number of TH-positive cells, decrease Caspase-3, Fas/Fas-L and Bax, increase Bal-2 and activate the PI3K/AKT signaling pathway	Cai, (2013)	decrease MDS-UPDRS I, MDS-UPDRS III, PDQ-39, and NMSS scores, increase NAA/Cr ratio in less inflicted SN on MRS, decrease dosage of Madopar	Chen et al. (2019); Chen et al. (2024)
		decrease GRP78, p-IRE1α, ASK1, p-JNK, and caspase-12	Lin et al. (2020) Xu et al. (2020)	decrease HAMD and PSQI scores	Fang and Cai (2021)
Chaihu plus Longggu Muli decoction	Bupleurum chinense DC. [Apiaceae]; Scutellaria baicalensi Georgi [Lamiaceae]; Codonopsis pilosula (Franch.) Nannf.[Campanulaceae]; Neolitsea cassia (L.) Kosterm.[Lauraceae]; Wolfiporia cocos (F.A. Wolf) Ryvarden & Gilb; Pinelliae Rhizoma Praeparatum Cum Alumine [Araceae]; Rheum palmatum L. [Polygonaceae](processed); Mastodi Ossis Fossilia; Ostrea gigas Thunberg; Haematitum; Ziziphus jujuba Mill.[Rhamnaceae]; Zingiber officinale Roscoe [Zingiberaceae]	activate the AMPK/mTOR signaling pathway to promote degradation of abnormally aggregated α-Syn	Liu et al. (2022)	improve the ADL of PDD patients, decrease TCM syndrome score	Tu and Cai, (2017)
		decrease S100β, β-amyloid and NSE	Yu et al. (2021)	increase PDSS score	Xiao et al. (2021)
		upregulate cAMP/PKA/CREB/BDNF signaling pathways, restore expression of BDNF and its receptor tyrosine kinase B	Zhao et al. (2023)	increase MMSE and MoCA scores	Yu et al. (2021)
				increase serum SOD, and decrease MDA and GFAP	Hu et al. (2020)
				Decrease HAMA score, increase serum 5-HT and MHPG	Jia and Li, (2022)
Shudi Pingchan granule	Rehmannia glutinosa (Gaertn.) Libosch. ex DC. [Orobanchaceae]; Lycium barbarum L.[Solanaceae]; Taxillus chinensis (DC.) Danser [Loranthaceae]; Gastrodia elata Blume [Orchidaceae]; Paeonia lactiflora Pall. [Paeoniaceae]; Bombyx Batryticatus; Complete Scorpio; Scolopendra subspinipes mutilans L.Koch[Scolopendridae]; Arisaema amurense Maxim. [Araceae]; Acorus gramineus Aiton [Acoraceae]; Polygala tenuifolia Willd. [Polygalaceae]; Salvia miltiorrhiza Bunge [Lamiaceae]	decrease apoptosis in SN, inhibit activation of the ERK signaling pathway	Ye et al. (2016)	decrease MDS-UPDRS score, HAMA, HAMD, PDQ-39, SCOPA-AUTscores,improve PDSS score	Yuan et al. (2010); Ye et al. (2017b)
		inhibit overexpression of α-syn	Ye et al. (2017a)		

MDS-UPDRS, Movement Disorder Society- Unified Parkinson Disease Rating Scale; NMSS, non-Motor Symptom Rating Scale; PSQI, Pittsburgh Sleep Quality Scale; SCOPA-AUT, autonomic symptoms scale; PDSS, Parkinson's disease Sleep Scale; HAMA, Hamilton Anxiety Scale; HAMD, Hamilton Depression Scale; MMSE, Mini Mental State Scale; MoCA, Montreal cognitive assessment; PDQ-39, the quality of the survival questionnaire to evaluate Parkinson's disease; ADL, Activity of Daily Living.

pathways, among which the apoptosis of dopaminergic neurons is considered an important mechanism underlying PD. A notable feature of apoptosis is the formation of apoptotic bodies. Apoptosis primarily occurs via three pathways (Yang X. et al., 2018): the death receptor pathway, the mitochondrial pathway, and the endoplasmic reticulum pathway. The mitochondrial pathway, also known as the intrinsic apoptotic pathway, operates as follows: under the pro-apoptotic stimulation, the permeability of the outer mitochondrial membrane changes, and the mitochondrial permeability transition pore opens, leading to the release of cytochrome C (Cyt C) from the mitochondrial intermembrane space into the cytosol. The released Cyt C combines with apoptotic protease activating factor-1 (Apaf-1) and procaspase-9 to form an apoptotic complex, thereby activating caspase-9 and caspase-3, ultimately triggering apoptosis (Wu and Bratton, 2013). The BCL-2 protein family plays a crucial role in regulating mitochondrial membrane permeability and is a core factor in modulating the mitochondrial apoptotic pathway. Key members include pro-apoptotic proteins, such as BAX and anti-apoptotic proteins, such as BCL-2. When apoptotic signals are activated, BAX undergoes mitochondrial translocation, inserts into the mitochondrial membrane, and polymerizes to form transmembrane channels. This process increases mitochondrial membrane permeability, facilitating the release of Cyt C. BCL-2 inhibits the homodimerization of BAX and its impact on mitochondrial membrane permeability by forming heterodimers with BAX.

The apoptotic pathway is precisely regulated by multiple signaling pathways (He et al., 2022), including the p38 MAPK, JNK/SAPK, Notch, PI3K/AKT/mTOR, and Nrf2/ARE pathways. These pathways form an intricate regulatory network that collectively controls the initiation and progression of apoptosis.

Polysaccharides, such as LBP and ASP, enhance anti-apoptotic mechanisms through reducing reactive oxygen species (ROS) accumulation, inhibiting Cyt-C release, thereby suppressing activation of the intrinsic apoptotic pathway. The neurotoxin MPP^+ inhibits mitochondrial complex I, elevating ROS accumulation and triggering Cyt-C release. This cascade triggers Cyt-C release from mitochondria, which sequentially activates Caspase-9 and Caspase-3, while dysregulating Bcl-2 family proteins: pro-apoptotic Bax increases, whereas anti-apoptotic Bcl-2 decreases. LBP scavenges ROS and stabilizes mitochondrial

integrity, thereby blocking Cyt-C translocation to the cytoplasm and subsequent caspase activation (Wang and Han, 2021). After pretreating SH-SY5Y cells with LBP for 1 h, it was observed that LBP downregulated MPP^+-triggered overexpression of pro-apoptotic proteins, ultimately, significantly reversed MPP^+-induced apoptosis. This study utilized SH-SY5Y cells but lacked validation using primary neurons or co-culture with glial cells. LBP pretreatment lasted only 1 h and whether longer pretreatment durations would enhance the protective effect was not verified. Similarly, ASP treatment (Zhang et al., 2024) attenuated astrocytic damage and apoptosis in MPTP-injected mice by modulating Bax/Bcl-2/caspase-3, thereby mitigating MPTP-induced PD-like behavioral and molecular alterations. Consistent with molecular changes, behavioral tests demonstrated that ASP significantly improved limb grip strength and latency time in MPTP-treated mice, alleviating motor deficits in PD models. This experiment evaluated motor function only using a grip strength meter and rotarod apparatus, core behavioral phenotypes for PD, such as gait analysis or open field test, were not included.

The PI3K/Akt pathway is an important pathway of anti-apoptotic signal transduction mechanism, which can protect cells from being stimulated by many apoptotic signals. When PI3K is activated, it phosphorylates PIP2 to PIP3 and promotes the phosphorylation of Akt. Phosphorylated Akt can inhibit the expression and activation of various pro-apoptotic proteins, thereby exerting anti-apoptotic effects. It was shown that LBP (Li P. et al., 2020) could reduce the apoptosis rate of PC12 cells induced by MPP+. After pretreatment with a PI3K inhibitor LY 294002, the protective effect of LBP was blocked. These findings indicate that LBP activates the PI3K/Akt pathway to reduce apoptosis and protect neurons. Mechanisms underlying the apoptosis effect of LBP is presented in Figure 2.

Another study showed (Li et al., 2016) that ASP alleviated the degeneration of dopaminergic neurons and defects in food sensing behavior in nematodes treated with 6-OHDA, and also prolonged the lifespan of nematodes exposed to 6-OHDA. Real time quantitative PCR analysis showed that among programmed cell death related proteins like CED-4, CED-3, CED-9, and EGL-1, ASP significantly reduced the mRNA level of EGL-1 in *Caenorhabditis elegans*, suggesting the role of ASP in regulating the expression of programmed cell death genes. Research has shown that (Wang and Han, 2021) hypoxia induces an increase in the expression of urothelial cancer associated antigen 1 (UCA1) in neurons. UCA1 can promote the progression of PD by regulating the SNCA gene, and its small interfering RNA (si-UCA1) is used for gene silencing. LBP + si-UCA1 is more effective in improving cell survival and reducing apoptosis rate than LBP or si-UCA1 alone, indicating a synergistic anti apoptotic effect of LBP and si-UCA1.

5.2 Anti-oxidative stress

During normal life activities, free radicals are constantly produced in the body. Cellular antioxidant defense systems efficiently neutralize these radicals, thereby protecting cells from oxidative damage. However, when the dynamic balance between the

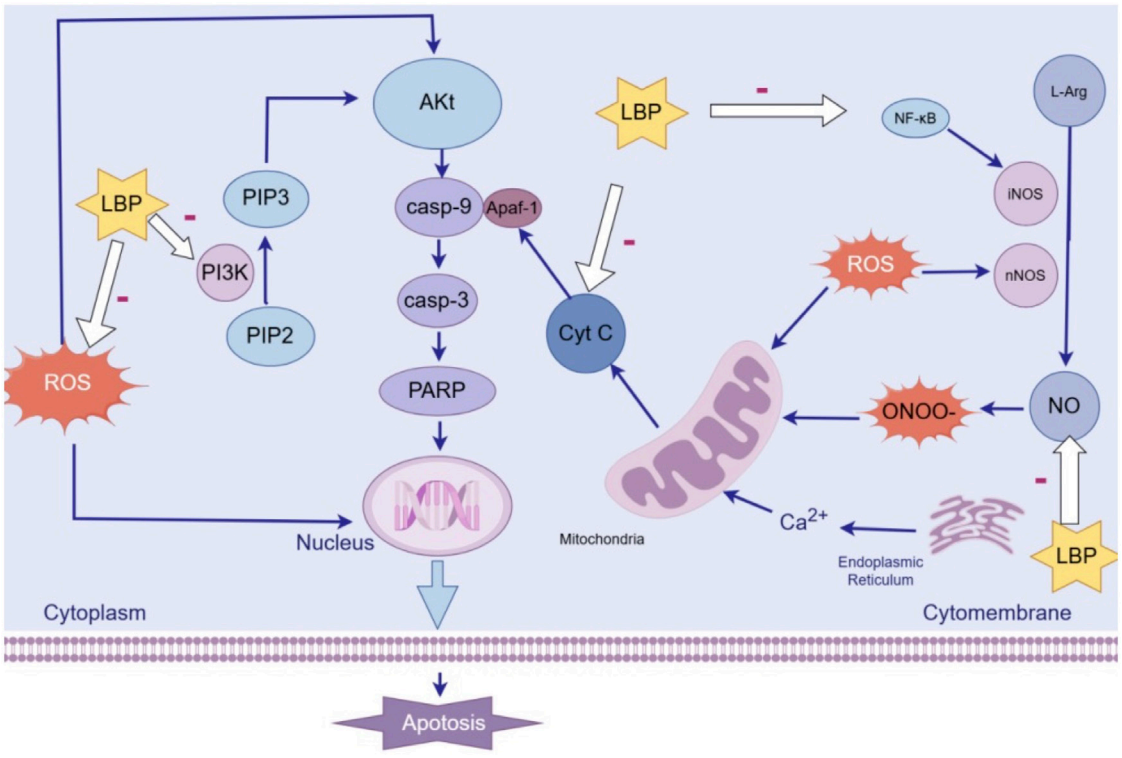


FIGURE 2
Mechanisms underlying the anti-apoptosis effect of LBP.

oxidation and antioxidation systems is disrupted-particularly when excessive reactive oxygen species (ROS) production overwhelms the antioxidant clearance capacity, oxidative stress occurs. Excessive ROS induces macromolecular structural damage, ultimately leading to cell dysfunction. Notably, dopaminergic neurons in the substantia nigra are highly susceptible to ROS-mediated oxidative injury, a key pathological mechanism in PD.

Studies have shown that the oxidative stress factors leading to PD mainly include the following aspects (Zhu et al., 2019): Firstly, mitochondrial dysfunction, impaired mitochondrial complex I activity substantially increases ROS generation; Secondly, neuroimmune inflammatory responses induce the massive release of pro-inflammatory cytokines (e.g., TNF-α, IL-1β, IL-2), enhances iNOS activities, promoting ROS and ONOO- formation (Przedborski and Dawson, 2001). In addition, abnormal dopamine metabolism, iron accumulation, calcium ion overload, and compromised antioxidant defense system - manifested as reduced activity of antioxidant key enzymes, such as glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), catalase (CAT), lactate dehydrogenase (LDH), and vitamin C (VC).

From a genetic perspective, mutations in mitochondrial quality control genes (e.g., DJ-1, PINK1, and Parkin) disrupt dopaminergic neuron mitochondrial homeostasis (Hu et al., 2021), initiating oxidative cascades. Clinical studies also show that levels of inflammatory factors (IL-6) and oxidative stress markers (SOD, MDA, NOS) in patients with PD are significantly higher than healthy controls (Nagatsu et al., 2000). These results further confirm the role of oxidative stress in the pathogenesis of PD.

Treatment of PC12 cells (a cell line derived from rat pheochromocytoma) with 6-OHDA significantly reduced cell survival and induced typical apoptotic features, such as pyknosis, karyorrhexis, and formation of apoptotic bodies. Excessive ROS accumulation occurs intracellularly. ROS, as the initial trigger of apoptosis, activates p38 mitogen-activated protein kinase (MAPK), induce scaspase-9 and caspase-3 activation, inhibits Akt phosphorylation, and ultimately induces apoptosis. LBP effectively suppressed intracellular ROS generation, blocked p38 activation, reduced 6-OHDA-induced cleavage of caspase-3, and thereby attenuated apoptotic cell death (Gao et al., 2014).

Polysaccharides from TCM significantly inhibit nitric oxide (NO) accumulation and NF-κB activation. The peroxynitrite ($ONOO^-$) generated from NO metabolism can Inhibit mitochondrial complexes, induce Ca^{2+} release from the endoplasmic reticulum, trigger Cyt C release, activate apoptosis. Neuronal nitric oxide synthase (nNOS) and inducible nitric oxide synthase (iNOS) are the catalytic enzymes for NO production. 6-OHDA causes overexpression of nNOS and iNOS in PC12 cells, increases intracellular NO and its metabolites, raises Ca^{2+} levels, activates NF-κB in apoptosis-associated signaling pathways, and upregulates p53. After pretreating PC12 cells with different concentrations of LBP, intracellular Ca^{2+} levels decreased, and the expression of nNOS and iNOS as well as the activation of NF-κB were suppressed in a dose-dependent manner (Gao et al., 2014). This indicates that LBP prevents 6-OHDA-induced cell apoptosis by blocking nNOS and iNOS expression, and downregulating the intracellular $Ca^{2+}$and NF-κB pathways. This study used ELISA to measure protein-bound compound to assess the level of nitrosative stress mediated by nitrogen oxides, which is susceptible to non-specific interference. If validated with immunohistochemistry or Western blotting, the conclusion would have greater specificity.

Chen et al. (2018) used LBP to study the impact on antioxidant capacity of brain tissue in Parkinson's model mice. Their findings revealed a paradoxical dissociation between behavioral outcomes and neuroprotective efficacy: LBP-treated (50 mg/kg, 100 mg/kg) mice exhibited a significant improvement in pole-climbing scores compared to the MPTP group, the pole - climbing scores of LBP - treated mice remained lower than those of the L - DOPA group. This suggests that while LBP partially alleviates motor deficits, its symptomatic efficacy is statistically inferior to conventional L-DOPA therapy. However, the number of tyrosine hydroxylase-positive (TH+) neurons in the substantia nigra pars compacta (SNpc) of the LBP group was larger than that of the MPTP model and the L-DOPA group, suggesting direct neuroprotection. The activities of antioxidant enzymes SOD, GSH Px, and CAT in the LBP group were significantly increased compared to the MPTP group or the L-DOPA group, while the content of oxidative product MDA, a lipid peroxidation marker, was significantly decreased compared to the above two groups. These results highlight LBP's unique disease-modifying potential through antioxidant and neuroprotective synergy, contrasting with L-DOPA's transient symptomatic relief. However, the incomplete behavioral recovery implies that dose optimization or combinatorial therapies may be required for clinical translation. The article proposes that LBP protects neurons through antioxidant effects, but it does not specify the pathways and lacks evidence at the molecular level.

When APS was used in combination with L-dopa, the content of glutathione (GSH), activities of the GSH-Px and SOD in L-dopa-induced astrocytes were similar to those of the blank control group, and the content of MDA was not significantly higher than that in the blank control group, and both were better than those in the L-dopa alone group. These reactions were evident 4 h after administering these compounds (Li et al., 2006), indicating that the protective effect of APS was time-dependent. Mechanism studies have shown that L-DOPA produces a large number of free radicals during metabolism, leading to oxidative stress and toxic effects on various neurons. ASP can effectively eliminate free radicals and protect astrocytes treated with L-DOPA from injury by increasing the activity of antioxidant enzymes. Similarly, this study has not explored the specific signaling pathway through which APS modulates oxidative stress levels, which would require further investigation. It is recommended that subsequent research designs quantitatively analyze the key molecular targets of APS through Western blotting and ROS fluorescent probes.

When Gao et al. (2015b) used sulfated Poria polysaccharides (SP) to treat MPP + pretreated cells, it was observed that polysaccharides significantly increased the activity of intracellular antioxidant enzymes (such as GSH Px, SOD, and CAT), while reducing the content of oxidative products (such as hydrogen peroxide-H_2O_2 and MDA), thereby reducing the degree of cellular oxidative injury. Further *in vivo* experiments showed that injecting MPTP with SP (Gao et al., 2015a) or Ganoderma lucidum polysaccharides (Yang et al., 2017) significantly increased the activity of antioxidant enzymes and the content of superoxide anions, such as SOD, GSH Px, CAT, LDH in the striatum of Parkinson's mice, while reducing the content of H_2O_2, MDA,

etc., indicating that SP also has strong antioxidant activity *in vivo*. Histologically, a significant increase in the number of neurons was observed in hippocampal slices of the mouse brain tissue, with larger cell bodies, denser and more organized arrangement, reduced intercellular gaps, and significant restoration of adhesion ability, indicating good recovery of cell morphology and function. Although SP was confirmed to reduce oxidative stress markers, like SOD, GSH-Px, CAT, MDA, etc. in both *in vitro* and *in vivo* experiments, the study failed to detect core pathological markers of PD, such as the loss of dopaminergic neurons in the substantia nigra, thereby rendering the neuroprotective conclusions indirect. In summary, polysaccharides can enhance the antioxidant enzyme activity in the striatum of mice and protect dopamine neurons in the brain.

In a study on crude polysaccharides from Chroogomphis rutilus (Zhang et al., 2011) showed that they can inhibit lipid peroxidation in the liver of MPTP injected PD model mice. The results showed that the minimum antioxidant activity of crude polysaccharides from Chroogomphis rutilus was between 2 and 4 mg/mL. With increasing concentration, the antioxidant activity of polysaccharides from Chroogomphis rutilus was significantly enhanced, even reaching 70% of the antioxidant activity of vitamin C. The number of TH positive cells in the substantia nigra of mice in the Chroogomphis rutilus group increased with the increase of polysaccharide concentration, significantly higher than that in the PD model group. Behavioral observations showed significant improvement in the climbing, swimming, and hanging tests compared to the PD group. These results indicate that polysaccharides from Chroogomphis rutilus have good antioxidant activity, ameliorate injury to dopaminergic neurons, and improve the behavioral status of experimental animals. However, the article did not further explore the specific antioxidant mechanism of polysaccharides from Chroogomphis rutilus. This study conducted detailed behavioral observations and comparisons in mice, but the small sample size may compromise the statistical power. Additionally, the assessment of antioxidant capacity relied solely on hepatic lipid peroxidation assays, without evaluating critical antioxidant enzyme activities or intracerebral ROS levels.

5.3 Improve mitochondrial functions

Mitochondria play a central role in cellular energy metabolism (Morris, et al., 2024), providing energy to cells through oxidative phosphorylation. Their regulation of cellular bioenergetic states critically determines whether cells survive or undergo apoptosis. Notably, mitochondrial dysfunction constitutes one of the early pathogenic drivers in PD progression. Experimental models demonstrate that MPTP undergoes bioactivation to MPP^+, which is selectively internalized via dopamine transporters into dopaminergic neurons. This metabolite potently inhibits mitochondrial complex I activity, precipitating bioenergetic failure and ultimately provoking dopaminergic neurodegeneration with PD-like symptoms. Under physiological conditions, mitochondrial-derived ROS maintain cellular homeostasis. However, pathological ROS accumulation during cellular stress induces oxidative damage to biomolecules, such as proteins, DNA, and RNA, thereby exacerbating mitochondrial impairment. Crucially, mitochondrial morphology and functionality exhibit tight coupling. Both exogenous stressors and intracellular oxidative stress disrupt mitochondrial morphology and bioenergetic function. The reticular network within mitochondria represents the integrity and damage of organelle health manifests as: (1) abnormal grid morphology, (2) elevated membrane potential ($\Delta\psi m$), (3) aberrant opening of mitochondrial permeability transition pores (mPTP), (4) altered inner membrane permeability, and (5) depressed enzymatic activity, collectively culminating in functional deficits. The mitochondrial membrane potential is also affected by the stimulation of cell apoptosis. After receiving the apoptosis signal, a decrease in mitochondrial membrane potential can be observed in the early stage, and membrane permeability increases, thereby establishing a pro-apoptotic cascade. The excessive accumulation of ROS leads to structural damage marked by mitochondrial membrane potential collapse, which in turn triggers the release of Cyt C from mitochondria, ultimately inducing apoptosis.

Mitochondrial function is finely regulated by various upstream regulatory factors (He et al., 2023), including: AMPK, p38 MAPK, SIRT1. Peroxisome proliferators activated receptors gamma coactivator one alpha (PGC-1α) is key gene regulating mitochondrial biogenesis. It can activate various transcription factors, such as nuclear respiratory factor-1/2 (NRF-1/2), estrogen-related receptors (ERRs), and PPARγ, promoting mitochondrial function and energy metabolism.

Polysaccharides from TCM protect mitochondrial morphology and structure. A study on tricholoma matsutake polysaccharides (TMP) (Lv et al., 2024) demonstrated that the TMP-H (high concentration of TMP) group and TMP-M (medium concentration of TMP) group showed an increase in the average length and number of mitochondrial network branches, as well as an increase in mitochondrial membrane potential ($\Delta\Psi m$), which stabilized the morphology of the mitochondrial network and significantly increased the cell survival rate and the number of cell body protrusions. This indicates that TMP can repair MPP + - induced injury to the mitochondrial network morphology in PC12 cells, stabilize mitochondrial membrane potential, and protect mitochondrial morphology and structure.

6-OHDA causes an increase in ROS, which leads to damage and a decrease in mitochondrial membrane potential ($\Delta\ \psi\ m$), while sea cucumber polysaccharides (Cui et al., 2016) can effectively reduce ROS levels, stabilize mitochondrial membrane potential, and protect mitochondrial structure from damage. It can also activate the PI3K/Akt signaling pathway, which plays a crucial role in maintaining mitochondrial function and cell survival. Activation of this pathway helps to inhibit cell apoptosis and protect normal mitochondrial function. In addition, sea cucumber polysaccharides (Cui et al., 2016) can inhibit the activation of NF- κB, thereby reducing the expression of iNOS and the production of NO, which helps protect mitochondria from oxidative stress injury.

Fucoidan protected mitochondrial function through the PGC-1 α/NRF2 signaling pathway, which enhances antioxidant enzyme activity to improve mitochondrial antioxidant capacity and maintain normal mitochondrial function. Mitochondrial respiratory capacity is a critical determinant of cell survival. Rats treated solely with rotenone exhibited significantly reduced mitochondrial respiratory function, including basal respiration,

ATP production, and maximal respiration. Fucoidan at 140 mg/kg completely restored the levels of all three parameters (Zhang et al., 2018), indicating a protective effect on the mitochondrial respiratory chain. In a study on LBP (Dai et al., 2022), it was reported that LBP demonstrated a protective effect on mitochondrial respiratory function. It alleviated the increase of mitochondrial oxidative respiratory enzyme I in the striatum cells of PD rats induced by rotenone, causing only reversible and mild mitochondrial edema, and alleviating mitochondrial damage in the substantia nigra and striatum of PD rats.

Research on the mechanisms of polysaccharides from the sea cucumber is relatively comprehensive. However, the high cost of obtaining it may limit their clinical application. While these studies emphasize mitochondrial protection, deeper mechanistic links (e.g., direct binding targets of polysaccharides) and *in vivo* pharmacokinetics remain underexplored. Future studies could further investigate whether polysaccharides exert their effects by inhibiting mitophagy (via the PINK1/Parkin pathway, etc.) or modulating endoplasmic reticulum stress (through the PERK/eIF2α pathway, etc.). Comparative analyses of polysaccharide efficacy across models could strengthen translational relevance.

5.4 Induction of autophagy and inhibition of aggregation of abnormal proteins

Autophagy refers to the process in which cells engulf, degrade, and recycle damaged and aging organelles, clearing abnormal protein aggregates to maintain cellular homeostasis. Autophagy is crucial for maintaining neuronal metabolism. Dysfunction of autophagy can lead to the accumulation of pathological proteins that can not be cleared and cause neurotoxicity, which is considered one of the important mechanisms for inducing PD. The AMPK/mTOR signaling pathway is currently widely recognized as an autophagy regulatory signaling pathway. AMPK is an important energy receptor in cells. Under conditions, such as hunger and hypoxia, the AMP/ATP ratio increases, promoting AMPK phosphorylation and activation (Yang L. C. et al., 2018). Conversely, mTOR is a member of the PI3K protein kinase family and a negative regulator of autophagy. It can inhibit the activity of ULK1-Atg13-FIP200 initiation complex, a key target of cellular autophagy. Activated AMPK converts mTOR to an inactive p-mTOR state. Consequently, its inhibitory effect on autophagy is removed and autophagy is induced (Liang et al., 2024). The PI3K/AKT/mTOR signaling pathway is involved in the regulation of autophagy, with PTEN serving as a negative regulator of PI3K. After PI3K is activated, it promotes the conversion of PIP2 to PIP3 and phosphorylates Akt. The activated Akt directly activates the mammalian target of rapamycin complex 1 (mTORC1), which can inhibit the initiation stage of autophagy. The activated mTOR can inhibit autophagy by affecting the formation of the mTORC1 complex. LC3I/II and Beclin are autophagy markers and are often used to evaluate autophagy levels.

The experimental results of rotenone-induced PD rat model showed that the p-AMPK/AMPK ratio in the striatum of rats in the PD group was significantly decreased, and LBP significantly reversed this decrease in the p-AMPK/AMPK ratio in the striatum, increased the phosphorylation level of AMPK and the autophagy level (Dai et al., 2022). These indicate that LBP can regulate autophagy through the AMPK/mTOR signaling pathway, eliminate abnormal proteins, alleviate injury to neurons caused by the accumulation of abnormal proteins, and slow down the PD progression.

To determine whether LBP regulates autophagy through the AKT/mTOR pathway, Western blot analysis was conducted to assess the protein levels of p-AKT and p-mTOR in different groups. It was found that levels of phosphorylated AKT and mTOR in the substantia nigra of mice decreased after MPTP treatment. However, LBP attenuated this decrease of p-Akt and p-mTOR (Wang et al., 2018). Further studies found that the protein expression of PTEN also decreased after LBP treatment. This indicates that LBP treatment exerts its neuroprotective effect and alleviates neuronal damage caused by abnormally aggregated α-syn by inhibiting PTEN and regulating excessive autophagy of dopamine neurons through the AKT/mTOR pathway.

In a study on APS, it was reported that APS could promote the transformation from LC3-I to LC3-II, simultaneously downregulate the expressions of pAKT and pmTOR, upregulate the expression of PTEN, and increase cell viability and autophagy. When the PI3K protein in PC12 cells is knocked down (KD), the effect of APS is reversed in PI3K KD cells (Tan et al., 2020). It indicates that APS activates autophagy through the PI3K/AKT/mTOR pathway, thereby combating PD. Super-resolution microscopy (SIM) and transmission electron microscopy (TEM) observed that the formation of autophagosomes increased in PC12 cells treated with APS, while the formation of autophagosomes decreased in PI3K KD cells. This further confirms that APS regulates autophagy levels through the PI3K/AKT/mTOR pathway, improves cell proliferation and exerts anti-PD effects. These two conclusions seem to be contradictory. LBP suppresses autophagy, while APS activates it, yet both have been reported to possess anti-PD effects. This paradoxical phenomenon likely arises from the dual-phase role of autophagy in PD pathogenesis: moderate autophagy clears α-synuclein aggregates, whereas excessive autophagy triggers neuronal apoptosis. The regulatory direction (suppression vs activation) is contingent upon the cellular damage stage and microenvironmental context. However, the underlying mechanisms require further investigation to elucidate the precise molecular basis. LBP and APS regulate autophagy through the PI3K/AKT/mTOR pathway (Figure 3).

Oligomers formed by abnormal aggregation of α-syn manifest as the primary cytotoxic species targeting dopaminergic neurons (Sun et al., 2023). These insoluble aggregates preferentially accumulate within midbrain substantia nigra neurons, forming characteristic Lewy bodies - a cardinal histopathological hallmark of PD. Such pathological inclusions directly trigger caspase-mediated apoptotic cascades and neuronal demise. A study showed that the level of aggregated α-syn in the substantia nigra of mice injected with MPTP was significantly increased. LBP significantly reversed this increase (Wang et al., 2018), confirming that LBP can effectively inhibit the aggregation of α-syn.

The ThT fluorescence kinetics experiment is a classic experiment used for analyzing amyloid protein aggregation. The intensity of fluorescence is proportional to the number of protein β-folded structures. Li (2020) first incubated in the α-syn solution to explore the effect of lentinan (LNT) on the aggregation behavior of α-syn. The results showed that the ThT fluorescence signal of the α-syn group increased over time, indicating that the content of β-folding structures in the α-syn group increased with the extension of time. After adding

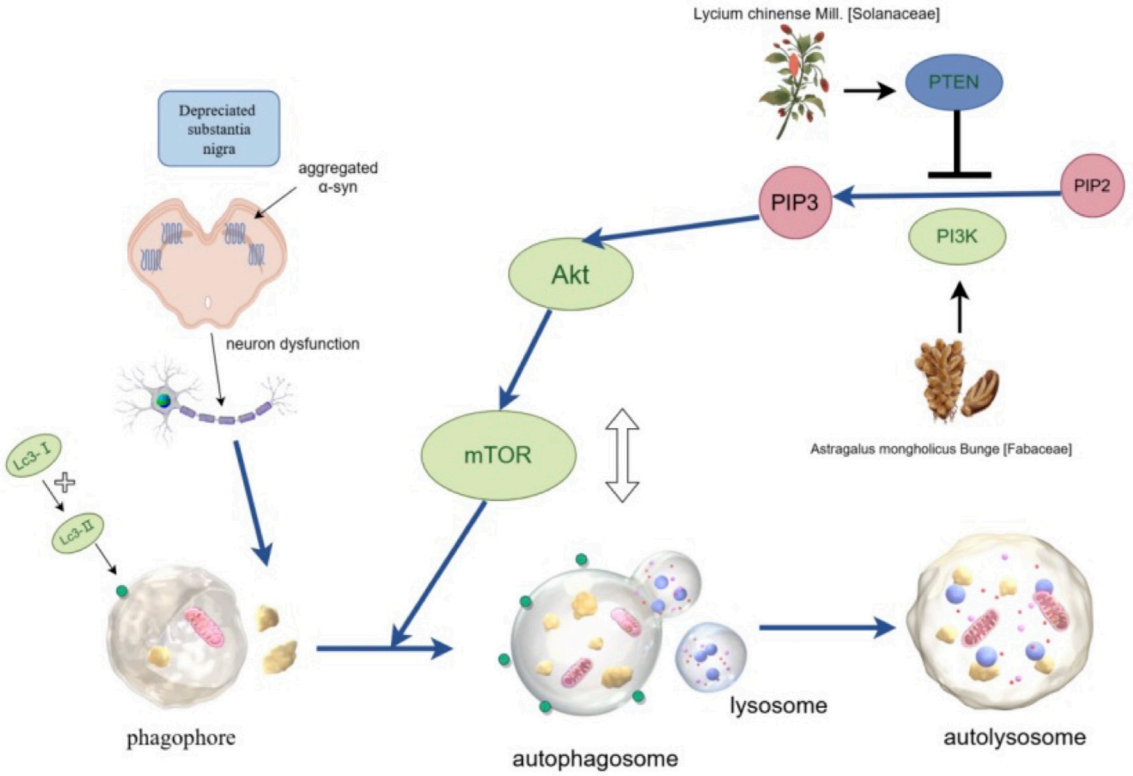


FIGURE 3
LBP and APS regulate autophagy through the PI3K/AKT/mTOR pathway. The activity of signaling pathway is regulated by phosphorylation of AKT and mTOR, and PTEN is a negative regulator. This pathway has a bidirectional regulatory effect on autophagy: it can maintain the basic level of autophagy while avoiding excessive autophagy.

LNT to the α-syn solution, the fluorescence signals of the samples increased only slightly over time. When the LNT concentration rose to 0.8 mg/mL, the fluorescence intensity was only 5% of that in the α-syn group. This result indicates that LNT can effectively inhibit the generation of β-folded structure substances by α-syn. Further research found that LNT can also trigger collective fragmentation and dispersion of the already aggregated α-syn fibers in the sample, and the protein fiber structure was gradually destroyed and finally disappeared, indicating that LNT can depolymerize α-syn fibers. When cells containing α-syn were incubated with a high concentration of LNT (0.8 mg/mL), the cell survival rate was increased by 30%, indicating that the high concentration of LNT reduced the cytotoxicity of the α-syn complex. In conclusion, LNT can counteract the toxicity of α-syn from three aspects: inhibiting the aggregation of α-syn, deaggregating α-syn, and reducing the toxicity of α-syn. This study pioneers the use of LNT to modulate α-synuclein (α-syn) aggregation and explores novel properties of LNT. However, whether LNT eliminates α-syn aggregates through autophagy induction requires further experimental validation.

5.5 Alleviation of neuroinflammation

Neuroinflammation plays an important role in the pathogenesis of PD. It mainly involves the activation of glial cells, the release of inflammatory factors, and the involvement of the peripheral immune system. Among them, inflammation of the central nervous system is mainly due to activation of microglia, as well as astrocytes. The peripheral immune response includes infiltration of T cells, dysfunction of regulatory T cells, and abnormal activation of neutrophils. Microglia exhibit two phenotypes after activation: pro-inflammatory M1 type and anti-inflammatory M2 type. It is worth noting that in PD patients, there is a significant increase in M1 type microglia, and activated M1 type cells can release various pro-inflammatory factors, including chemokines (Joers et al., 2016). These inflammatory mediators (such as TNF α, IL-1 β, IL-6, IL-2, IL-4) and enzymes (such as iNOS and COX-2) orchestrate a self-perpetuating cascade through NF-κB and JAK-STAT signaling pathways, simultaneously compromising blood-brain barrier (BBB) integrity via matrix metalloproteinase-9 (MMP-9) mediated tight junction degradation. Activated microglia release chemokines, facilitating more immune cells to participate in the inflammatory response. Peripheral immune cells (particularly T cells and B cells) migrate to the central nervous system. This process establishes bidirectional crosstalk between brain and peripheral inflammation, thereby synergistically promoting the progression of PD. These pro-inflammatory cytokines induce neuronal damage through the following pathways (Shao et al., 2020): pro-inflammatory cytokines (such as TNF - α and IL-1 β) can directly destroy the structure and function of neurons. Induced oxidative stress, excessive production of ROS and reactive nitrogen species (RNS) leads to oxidative damage to neurons. Inflammatory factors

activate the MAPK and NF-κB signaling pathways, promote the misfolding and aggregation of α-syn, and cause a decrease in the number of dopaminergic neurons. Combined effects reduce TH + neurons in substantia nigra pars compacta, accelerating Lewy pathology progression.

In an APS study on PD rats, the therapeutic effect and mechanism of APS on PD in a rat model induced by 6-OHDA was investigated. The study adopted a random grouping design, dividing the experimental rats into the following four groups: PD model group, PD model + L-DOPA group (positive control group), PD model + APS group and a normal control group. It was found that the number of rotations in the PD model + APS group and the PD model + L-DOPA group decreased by 20%, 11%, and 16%, respectively, on the first, seventh, and 14th day. Furthermore, the rotational speed shows non-uniformity, and the maximum rotational speed was reduced to 5–7 r/min (Chen and Zhou, 2015). This result indicates that APS exhibits good therapeutic effects in terms of behavior and are comparable to the efficacy of L-DOPA. Further exploration of the mechanism revealed that the expression levels of inflammatory cytokines IL-6, TNF and IL-2 in the brain tissue of rats in the PD model group + APS group were significantly lower than those in the PD model group on the 14th day. These suggest that APS may exert therapeutic effects on PD by reducing the levels of pro-inflammatory cytokines in the brain and improving the neuroimmune inflammatory response of the body.

It was reported by another study that Polygonatum sibiricum polysaccharide (PSP), when intragastrically administered for six consecutive weeks to 6-OHDA induced PD model rats, increased the expression of PPAR-γ in the brain tissue of PD model rats at the eighth week (Chen et al., 2010). PPAR-γ, a ligand-dependent nuclear hormone receptor, belongs to the nuclear hormone receptor superfamily and plays an important role in regulating gene expression. PPAR-γ has the potential to delay the course of neurodegenerative diseases, such as PD and Alzheimer's disease, by regulating the production of inflammatory factors. This study indicates that the therapeutic effect of PSP may be related to up-regulating PPAR-γ expression and inhibiting the generation of inflammatory factors.

Comparative analysis reveals both APS and PSP target neuroinflammation through distinct mechanisms: (1) APS directly suppresses cytokine production (IL-6/TNF-α/IL-2), whereas (2) PSP activates PPAR-γ-mediated transcriptional regulation. These findings position plant-derived polysaccharides as multi-target neuroprotective agents against PD-associated neuroinflammation.

The NLRP3 inflammasome plays an important role in neuroinflammation (Paik et al., 2021). NLRP3 is activated by sensing pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs). After activation, it recruits ASC and forms the NLRP3 inflammasome with caspase-1. The activated caspase-1 cleaves the precursors of IL-1β and IL-18, releasing mature pro-inflammatory factors, and induces pyroptosis. In PD model mice, Dendrobium huoshanense polysaccharide (DHP) (Shang et al., 2023) significantly improved the motor ability, including the performance in the balance beam experiment, suspension experiment and rod rotation experiment. The mechanisms include: alleviating the injury of dopaminergic neurons in the substantia nigra of the midbrain and increasing the expression of tyrosine hydroxylase (TH); inhibiting the inflammatory response by reducing the levels of pro-inflammatory factors IL-1β, TNF-α and IL-18 in microglia; reducing the expressions of NLRP3, ASC, Caspase-1 and IL-1β, thereby inhibiting the activation of the NLRP3 inflammasome and reducing the release of pro-inflammatory factors. Antrodia polysaccharides (Yang Y. et al., 2018) also had a similar effect through reducing the expression of pro-inflammatory factors IL-1β, TNF-α and IL-6, inhibiting the excessive activation of microglia, down-regulating the expression of NLRP3-Caspase1 inflammasome and its downstream inflammatory factors, and alleviating the injury to dopaminergic neurons.

These polysaccharides achieve their therapeutic benefits primarily by inhibiting the activation of the NLRP3 inflammasome pathway. Both studies employed ELISA to quantify inflammatory cytokines (IL-1β↑, IL-4↓, IL-6↑) in murine brain tissues, coupled with Western blot analysis of pro-caspase-1, activated caspase-1, and NLRP3 expression. These approaches provided reliable quantification of CNS neuroinflammation and inflammasome activation status. However, the mechanistic interpretations remain correlative, failing to elucidate whether polysaccharides directly bind to NLRP3 or modulate upstream signaling pathways (e.g., NF-κB priming or K^+ efflux).

Polysaccharides in TCM regulate signaling pathways and mitigate neuroinflammation. TNF-α, IL-1, and the NF-κB pathway can trigger the activation of the NLRP3 inflammasome, and the latter promotes the synthesis and secretion of pro-inflammatory factors. A high-dose of Pholidota chinensis lindl polysaccharides significantly reduced the expression levels of TNF-α, IL-1β and NF-κB in rats, and increased the number of TH-positive neurons (Zhou N. et al., 2021). These indicate that Pholidota chinensis lindl polysaccharides inhibit the activation of the NLRP3 inflammasome by regulating the NF-κB pathway and exerts anti-inflammatory and neuroprotective effects. Momordica charantia polysaccharides (MCPs) can reverse the increase of TLR4, MyD88, and p-p65 proteins induced by MPP+. MCPs inhibit the generation of NLRP3 inflammasome through the TLR4/MyD88/NF-κB signaling pathway and exert an anti-neuroinflammatory effect (Guo et al., 2021).

5.6 DA neuroprotection

In recent years, significant progress has been made in research on dopaminergic neuroprotection, particularly in revealing the relationship between cellular aging and neurodegenerative diseases. Research has shown that aging and death of dopaminergic neurons in the substantia nigra are the core pathological mechanisms of PD. Senescence markers, such as β-galactosidase (β-gal), telomerase, p16INK4a, matrix metalloproteinase-3 (MMP-3), and nuclear membrane protein Lamin B1, are closely related to the cellular aging process in PD (Zhang Y. et al., 2023). As a classic marker of cellular aging, β-gal elevation mirrors lysosomal stress in senescent neurons. Telomerase preserves telomeric TTAGGG repeats, delaying replicative senescence. p16INK4a enforces G1 cell cycle arrest via CDK4/6 inhibition, while MMP-3 degrades extracellular matrix components, disrupting neuron-glia crosstalk. Lamin B1 plays a

crucial role in maintaining nuclear structure and cell cycle regulation, and its reduction may be associated with neuronal aging and degeneration.

In terms of signaling pathways, the Wnt1 and β-catenin pathways in the substantia nigra are involved in processes, such as cell proliferation, differentiation, migration, and maintaining tissue homeostasis. In MPTP induced PD animal models, activation of the Wnt/β-catenin signaling pathway can improve nigrostriatal injury and reduce the death of dopaminergic neurons. *In vitro* experiments have also shown that activated Wnt signaling pathway can improve MPTP induced dopaminergic neuron cytotoxicity (Wang et al., 2007). GSK-3 β is a key regulatory factor in the Wnt/β - catenin signaling pathway, and its phosphorylation level directly affects the stability and pathway activity of β - catenin.

DHP may protect neurons by delaying the aging of astrocytes. Compared with the normal control group, mice treated with DHP exhibited the following characteristics: the proportion of cells in the G0/G1 phase decreased, the proportion of cells in the S phase increased, the positive rate of β-gal decreased, the expression of telomerase increased, the expressions of p16INK4a and MMP-3 decreased, and the fluorescence expression of Lamin B1 in mice of the DHP 100 mg/kg group and the DHP 350 mg/kg group increased (Zhang P. et al., 2023). All these were related to cellular senescence. These results indicate that DHP can inhibit astrocyte senescence through different mechanisms and pathways, thereby alleviating neuronal damage.

In another study, 6-OHDA induced PD rats were treated with Cistanches Herba polysaccharides (CDPS) (Yin et al., 2020). It was found that after CDPS intervention, the autonomous activity of SD rats was improved, shortened swimming static time and the decreased number of autonomous standing activities were restored, and the protein expression of Wnt1 and β-catenin in the substantia nigra was increased compared with the PD model group. The expression of GSK-3β mRNA was inhibited. It is speculated that CDPS may protect dopaminergic neurons by inhibiting the activity of GSK-3β, up-regulating the expression of β-catenin protein, activating the Wnt/β-catenin signaling pathway, promoting the proliferation and differentiation of dopaminergic cells. However, the mechanistic study did not conclusively prove the direct association between β-catenin and neuroprotection. It is necessary to verify the key nodes of the signaling pathway (such as detecting the nuclear translocation of β-catenin) to prove that the neuroprotective effect stems from the activation of the pathway. Moreover, the experimental design lacked reverse verification using a Wnt pathway inhibitor; for instance, employing a GSK-3β activator to counteract CDPS effects would strengthen the mechanistic argument.

Basic fibroblast growth factor (bFGF), an important neuroprotective factor, has been proven to inhibit the degeneration of dopaminergic neurons and promote their survival and functional recovery by activating the PI3K/Akt and ERK1/2 signaling pathways. A study found that on the 14th day, the TH content in the substantia nigra of rats in the PD model + APS group was significantly higher than that in the PD group (Chen and Zhou, 2015). In addition, the expression level of bFGF protein in substantia nigra was significantly lower than that in the PD model group. The authors interpreted the APS-mediated reduction of the 6-OHDA-induced bFGF increase as suggestive of a direct protective effect on dopaminergic neurons. Nevertheless, the observed decrease in bFGF expression presents a conceptual challenge. As bFGF is a well-established neuroprotective factor, its downregulation would typically be associated with exacerbated damage rather than protection. Therefore, the authors' speculation that the decrease in bFGF is linked to the neuroprotective effect of APS requires rigorous experimental verification to establish a plausible causal relationship.

In a cell culture study, SH-SY 5Y cells pretreated with crude polysaccharides from Chroogomphus rutilus, followed by a thorough wash and adding MPP + to verify whether they have direct protective effect on neural cells. The results showed that the survival rate of SH-SY 5Y cells pretreated with 800 μg/mL of crude polysaccharides from Chroogomphus rutilus was significantly higher than that of the control group (Zhang et al., 2013). This indicates that the protective effect of crude polysaccharides from Chroogomphus rutilus on MPP + damaged cells stems from the direct protective effect of polysaccharides on cells, rather than the direct combination of polysaccharides with MPP+. This study features a well-designed approach to eliminate interference. By thoroughly washing the cells after polysaccharide pretreatment and before adding the neurotoxin MPP^+, the study effectively rules out false positives arising from direct binding between the polysaccharides and MPP^+. However, the research utilizes crude polysaccharides. Without identifying the specific active monomers responsible for the effect (such as determining molecular weight or monosaccharide composition), standardization of the active component remains challenging. Future directions should include: isolating and purifying individual polysaccharide fractions, elucidating their structures using techniques like mass spectrometry (MS) and nuclear magnetic resonance (NMR), and screening these purified components to identify the core active ingredient.

LBP has a significant protective effect on PC12 cells induced by MPP+. A study showed that after 12 h and 24 h of LBP pretreatment, the cell survival rate increased significantly. The increase in cell survival rate was the most prominent after 24 h of pretreatment with 100 μmol/L LBP (Li P. et al., 2020). This indicates that LBP can counteract the neurotoxicity of MPP+ in a concentration-dependent manner and protect dopaminergic neurons.

In a PD silkworm model (Song et al., 2022), it was found that LBP coated on mulberry leaves significantly improved the motor symptoms of silkworms. UPLC detection showed that the dopamine content in the brains of PD silkworms significantly increased after LBP administration. ELISA results showed that LBP significantly enhanced the TH protein level of PD silkworms. Immunofluorescence quantification revealed an increase in the TH protein level, and the mRNA expression levels of downstream DDC and PRKN genes, indicating the protective effect of LBP on the gene expression and synthesis process of dopamine in dopaminergic neurons. This study demonstrates systematic model construction through comprehensive detection methodologies, including motor function assessment via behavioral trajectory analysis, dopamine metabolism quantification using UPLC, oxidative stress evaluation (T-AOC/MDA biomarkers), critical gene expression profiling (TH/PRKN/DDC). However, notable methodological limitations exist: insufficient LBP dosing,

with only two doses (50/100 mg/kg) tested, which fails to establish dose-response relationships and an unvalidated positive control, as the LD-CD (levodopa-carbidopa, 4 mM) group used a single concentration without a justification for the dose.

5.7 Intestinal flora imbalance and microbial-gut-brain axis regulation mechanism

Recent studies have shown that gut microbial dysbiosis may play an important role in the pathogenesis of PD. Metagenomic profiling reveals PD patients exhibit significant ecological shifts in gut microbiota composition, with a decrease in beneficial bacteria and an increase in harmful bacteria, as well as a decrease in the abundance of gut microbiota characterized by depletion of butyrate-producing taxa (e.g., Faecalibacterium prausnitzii) and concomitant enrichment of pro-inflammatory species (e.g., Enterobacteriaceae), alongside reduced alpha diversity indices. This disruption of gut microbiota may lead to increased permeability of the intestinal epithelial barrier, triggering bacterial and their products (such as lipopolysaccharides) translocation, activating TLR4/MyD88-dependent innate immunity and instigating systemic low-grade inflammation.

In addition, abnormal deposition of α-syn within enteric plexuses precede motor manifestations by 5–10 years, correlating with prodromal gastrointestinal dysfunction. Dysbiosis of gut microbiota can also elevate levels of inflammatory cytokines, such as TNF-α and IL-6, which in turn activate gut glial cells and promote misfolding of α-synuclein. Retrograde propagation studies validate prion-like α-synuclein trafficking along vagal afferents, culminating in nigrostriatal dopaminergic denervation.

Active polysaccharides can be used by intestinal flora as the main energy source, and the degraded monosaccharides promote the colonization of probiotics and maintain the homeostasis of intestinal flora. *In vitro* fermentation studies showed that Dendrobium officinale polysaccharides increased the abundance of some beneficial bacteria in the gut, including *Lactobacillus*, Faecalibacterium and Rummeliibacillus, while reducing the abundance of some harmful bacteria, such as Shewanella, Geodermatophilus, Peptostreptococcaceae, and *Mycobacterium* (Peng et al., 2023). Polygonum polysaccharide can regulate the composition of intestinal microorganisms in mice and enhance the integrity of intestinal barrier (Jiang et al., 2024). This regulatory effect helps to improve the balance of the gut microbiome, thereby promoting gut health.

In the process of polysaccharide degradation, intestinal flora produced a large amount of short-chain fatty acids (SCFA), which reduced the release of intestinal pro-inflammatory cytokines like TNF-α and IL-6, and inhibited intestinal inflammation. Dendrobium officinale polysaccharides increased the content of short-chain fatty acids, such as acetic acid, propionic acid and butyric acid in the gut, which improved intestinal health (Fu et al., 2019).

Mediated by enteric nervous system inflammation, microglia are abnormally activated, promoting the release of IL-1β, TNF-α, IL-6, NO and proteases in the brain, inducing neuroinflammation and eliciting injury to dopaminergic neurons. Dendrobium officinale polysaccharidesdown-regulated the expression of pro-inflammatory cytokines, such as TNF-α, IFN-γ, IL-4 and IL-6, and increased the expression of anti-inflammatory cytokine IL-10, improved gut microbiome mediated immune response and restored gut flora (Liang et al., 2022).

5.8 Multifunctional mechanisms of polysaccharides in PD treatment

5.8.1 Regulation of neurotransmitter systems

PD patients not only have degeneration of DA neurons, which affects motor regulation (such as gait and balance control), but also demonstrate strong associations with dementia-related cognitive decline. The cholinergic neurons also show reduced expression of N-acetylcholine receptor (nAChR) α4β2 subtypes along with upregulated M-acetylcholine receptor (mAChR) M1/M4 subtype activity (Golan et al., 2022; Ni, 2023).

Acetylcholine (ACh) plays a crucial role in regulating behaviors, such as movement, foraging, mating, and egg laying in *C. elegans*. The experiment on the regulatory effect of APS on acetylcholinesterase (AChE) activity in BZ555 nematodes showed that BZ555 nematodes exposed to 6-OHDA exhibited significant downregulation of AChE activity. However, when exposed to a solution containing 2.0 mg/mL APS, its AChE activity and behavioral activity was significantly normalized (Li et al., 2016). It is speculated that the protective effect of APS may be related to protecting AChE from damage and restoring acetylcholine system function. APS effectively inhibits the neurotoxicity caused by 6-OHDA, thus playing a neuroprotective role at the system level. This study showed concentration-dependent neuroprotection within the range of 0.5–4.0 mg/mL APS with 4.0 mg/mL being the optimal concentration. However, nematodes lack the blood-brain barrier, immune system, and complex neural circuits of mammals, making it impossible to directly translate these findings to human physiology.

5.8.2 Behavioral improvement in PD models

Accumulating preclinical evidence demonstrates that botanical polysaccharides exhibit significant motor improvement effects in a mouse model of PD. Among them, LBP ameliorates PD-like symptoms including hypophagia, gait instability, and static tremors, as well as motor coordination, such as pole climbing and grasping. LBP can significantly improve cognitive deficits in Morris Water Maze Test and significantly shorten their escape latency (Wang et al., 2007). ASP can significantly improve the limb grip and suspension dwell time of PD mice, and alleviate motor dysfunction (Zhang et al., 2024). PSP showed good performance in gait regulation and paw extension tests, while CDPS significantly increased the spontaneous activity and standing frequency of PD mice. The polysaccharides from Chroogomphis rutilus also showed significant improvement effects in suspension experiments, pole climbing, and swimming tests, further demonstrating the potential value of polysaccharides from TCM in PD treatment.

5.8.3 Suppression of astrocyte activation

The activation of astrocytes is a hallmark of neurodegeneration, mainly manifested by an increase in GFAP levels. MPTP injection into mice resulted in a significant increase in GFAP positive cells,

TABLE 3 Mechanisms underlying the therapeutic effect of TCM on PD.

Mechanism Classification	Polysaccharide Name	Test Object	Mechanism/Signaling pathway	Reference
Inhibition of Apoptosis	Lycium barbarum polysaccharides	SH-SY5Y	Downregulate Cyt C and Bax/Bcl-2, caspase-3 and caspase-9; activate the PI3K/Akt signaling pathway, upregulate p-Akt	Wang and Han, 2021; Li P. et al., 2020
	Lycium barbarum polysaccharides	PC12 cells	Inhibit NF-κB signaling pathway, downregulate nNOS and iNOS, Ca^{2+}, downregulateCyt C, Bax/Bcl-2, and p38	Gao et al. (2014)
	Angelica sinensis polysaccharides	Mice	Clear ROS, downregulate Bax/Bcl-2, caspase-3	Zhang et al., 2024
	Astragalus polysaccharides	BZ555 nematodes	Downregulate egl-1	Li et al. (2016)
	Sea cucumber polysaccharides	SH-SY5Y	Downregulate Bax/Bcl-2, cl-caspase-9/caspase-9, cl-caspase-3/caspase-3	Wang J. et al. (2021)
Antioxidative Stress	Lycium barbarum polysaccharides	Mice	Increase in SOD, GSH-Px, and CAT activity, decrease in MDA.	Chen et al. (2018)
	Chroogomphus rutilus polysaccharides	Mice	Increase in total antioxidant capacity	Zhang et al. (2011)
	Astragalus polysaccharides	Astrocyte cells	Increase in GSH, GSH-Px, and SOD activity, decrease in MDA.	Li et al. (2006)
	Sulfated Poria cocos polysaccharides	PC12 cells	Increase in SOD, GSH-Px, and CAT activity, decrease in MDA.	Gao et al. (2015a)
	Sulfated Poria cocos polysaccharides	Mice	Increase in SOD, GSH-Px, LDH, and CAT activity, decrease in MDA and H_2O_2	Gao et al. (2015b)
	Antrodia camphorata polysaccharides	Mice	Increase in SOD, GSH-Px, and CAT activity, decrease MDA and H_2O_2	Yang et al. (2017)
Induction of Autophagy	Lycium barbarum polysaccharides	Rats	Activate the AMPK/mTOR signaling pathway	Dai et al. (2022)
	Astragalus polysaccharides	PC12 cells	Activate the PI3K/AKT/mTOR signaling pathway, downregulate pAKT and pmTOR, upregulate PTEN, LC3-II/LC3-I	Tan et al. (2020)
Inhibition of α-Synuclein	Lycium barbarum polysaccharides	Mice	Inhibit α-synuclein aggregation	Wang et al. (2018)
	Lentinan	α-Syn solution	Inhibit β-sheet formation, depolymerize α-Syn fibers, reduce α-Syn toxicity	Li (2020)
Protection of Mitochondrial Morphology and Structure	Tricholoma matsutake polysaccharides	PC12 cells	Stabilize mitochondrial membrane potential, stabilize mitochondrial structure, downregulate Bax/Bcl-2	Lv et al. (2024)
	Sea cucumber polysaccharides	SH-SY5Y	Reduce ROS levels, stabilize mitochondrial membrane potential, activate the PI3K/Akt signaling pathway; inhibit the NF-κB signaling pathway, downregulate iNOS.	Cui et al. (2016)
Protection of Mitochondrial Respiration Function	Fucoidan	Rats	Enhance mitochondrial respiratory function, activate the PGC-1α/NRF2 signaling pathway	Zhang et al. (2018)
	Lycium barbarum polysaccharides	Rats	Reduce mitochondrial oxidative respiratory enzyme I, upregulate p-AMPK/AMPK.	Dai et al. (2022)
Alleviation of Neuroimmune Inflammation	Astragalus polysaccharides	Rats	Reduce IL-6, TNF, IL-2	Chen and Zhou (2015)
	Polygonatum polysaccharides	Rats	upregulate PPAR-γ expression	Chen et al. (2010)
	Dendrobium huoshanense polysaccharides	Mice	Reduce IL-1β, TNFα, IL-18, MDA, ROS; inhibit NLRP3 inflammasome; increase SOD.	Shang et al. (2023)
	Antrodia camphorata polysaccharides	Mice	Reduce IL-1β, IL-4, IL-6, inhibit NLRP3-Caspase1, NLRP3 inflammasome	Yang L. C. et al. (2018)
	Pholidota chinensis Lindl polysaccharides	Rats	Reduce TNF-α, IL-18, inhibit the NF-κB signaling pathway	Zhou N. et al. (2021)

(Continued on following page)

TABLE 3 (*Continued*) Mechanisms underlying the therapeutic effect of TCM on PD.

Mechanism Classification	Polysaccharide Name	Test Object	Mechanism/Signaling pathway	Reference
	Momordica charantia polysaccharides	Mice	Inhibit the TLR4/MyD88/NF-κB signaling pathway, reduce TNF-α, IL-1β, increase in *Bcl-2/Bax*	Guo et al. (2021)
Dopamine (DA) Neuroprotection	Dendrobium huoshanense polysaccharides	Mice	Downregulate the G0/G1 phase cell ratio, increase in the S phase cell ratio, B-gal, p16INK4a and MMP-3; upregulate telomerase expression, Lamin B1	Zhang et al. (2023)
	Cistanche deserticola polysaccharides	Rats	Activate the Wnt/β-catenin signaling pathway, upregulate β-catenin, Wnt1, inhibit GSK-3β	Yin et al. (2020)
	Astragalus polysaccharides	Rats	Downregulate bFGF expression	Chen and Zhou (2015)
	Chroogomphus rutilus polysaccharides	SH-SY5Y	Direct protective effect on cells	Zhang et al. (2013)
	Lycium barbarum Polysaccharides	PC12 cells	Direct protective effect on cells	Li P. et al., (2020)
Regulation of Gut Microbiota Imbalance	Dendrobium officinale polysaccharides	Mice	Increase in beneficial bacteria abundance, decrease in harmful bacteria abundance, downregulate TNF-α, IFN-γ, IL-4, and IL-6, increase in IL-10	Jiang et al., 2024; Liang et al., 2022
	Dendrobium officinale polysaccharides	*In vitro* fermentation	Increase in beneficial bacteria abundance, decrease in harmful bacteria abundance, increase in intestinal short-chain fatty acids	Fu et al. (2019)
Improvement of Neurotransmission	Astragalus polysaccharides	BZ555 nematodes	Activate acetylcholinesterase activity	Li et al. (2016)

enlarged cell spheres, and more cell protrusions in the ventral striatum. The LBP treatment group showed a decrease in GFAP, which alleviated the activation of astrocytes in PD mice and exerted its protective effect on dopaminergic neurons.

5.8.4 Multi-target mechanistic actions

Polysaccharides, composed of monosaccharides, usually have unique biological activities. These components often regulate multiple targets in the body and work synergistically. For example, the effect of Stichopus japonicus polysaccharide (SJP) on the SH-SY5Y PD model of human neuroepithelial tumor cells induced by 6-OHDA was investigated. It was demonstrated that SJP inhibited intracellular ROS generation and reduced oxidative stress damage. It inhibited the expression of cyclin D3, leading to the arrest of the cell cycle in the G1/S phase. In the meantime, it reversed the change in the Bax/Bcl-2 ratio and activated both p38/c-Jun N-terminal kinase (JNK)-MAPK and PI3K/Akt pathways, which play a crucial role in cell apoptosis. Furthermore, the activation of NF-κB, preventing the upregulation of iNOS and NO release in cells, and alleviating mitochondrial dysfunction were observed (Wang J. et al., 2021). SJP effectively protects dopaminergic neurons through the above five mechanisms.

Similarly, LBP can exert strong antioxidant effects, effectively suppress α-syn aggregation, and inhibit cell apoptosis through the PI3K/Akt pathway. As mentioned earlier, the mechanism of LBP in anti-PD involves multiple pathways, including: 1) eliminating ROS, reducing oxidative stress, reducing Cyt C release, and anti-apoptosis; 2) inhibiting the increase of mitochondrial oxidative respiratory enzyme I in cells and alleviating mitochondrial oxidative damage; 3) enhancing the activity of antioxidant enzymes to combat oxidative stress; 4) inhibiting the aggregation of α-syn and alleviating the damage to DA neurons caused by its toxic effects; 5) activating the PI3K/Akt pathway to resist apoptosis; 6) regulating autophagy in cells through the AMPK/mTOR signaling pathway.

Ganoderma lucidum polysaccharides not only reduces the generation of ROS, upregulates the activity of SOD, alleviates oxidative stress damage, but also reduces the expression of pro-inflammatory factors IL-1 β, TNF α, and IL-6, inhibits excessive activation of microglia, and exerts anti neuroinflammatory effects. They can also alleviate inflammation by downregulating the expression of NLRP3-Caspase1 inflammasome and its downstream inflammatory factors (Yang Y. et al., 2018).

Tricholoma matsutake polysaccharides can stabilize mitochondrial network morphology and membrane potential, protect mitochondrial function, and reduce cell apoptosis by reversing MPTP induced decrease in the Bax/Bcl-2 ratio.

Momordica charantia polysaccharides (MCPs) can reduce the expression of inflammatory factors like TNF - α and IL-1 β, increase the expression of anti-apoptotic protein Bcl-2, and regulate anti-apoptosis. MCPs can also exert anti-inflammatory and anti-apoptotic effects by regulating the TLR4/MyD88/NF-κB signaling pathway. A summary of mechanisms underlying the therapeutic effect of TCM on PD can be found in Table 3.

6 Application of polysaccharides from traditional Chinese medicine

As previously described, Chinese Medicine Prescriptions containing Astragalus, Lycium barbarum, Cistanche deserticola,

etc., have been proven to improve symptoms of PD in clinical trials. The mechanisms include regulating the PI3K/Akt/mTOR pathway to inhibit neuronal apoptosis, suppressing caspase cascade reactions, and inhibiting α-syn accumulation. These mechanisms overlap with the action mechanisms of polysaccharides derived from TCM, suggesting the universality of polysaccharide components in the treatment of PD. The strong preclinical evidence for polysaccharides from TCM indicates that they can alleviate motor and cognitive defects in PD mouse models. These findings collectively suggest that polysaccharides from TCM may exert their effects through synergistic mechanisms. Their ability to simultaneously relieve motor and non-motor symptoms in PD models makes them an important candidate in developing comprehensive treatment strategies for neurodegenerative diseases.

At present, hundreds of polysaccharides have been discovered, but due to their high molecular weight, complex structure, and difficulty in purifying bioactive compounds, only seven polysaccharides have been approved to enter the market. Among them, the most widely used are anticoagulant heparin and chondroitin sulfate for the treatment of osteoarthritis. ASP is used for diabetic nephropathy due to its anti-inflammatory effect (Zhou G. et al., 2021), which can ameliorate inflammatory reaction and reduce urinary protein with a good safety profile. Although there is currently no clinical research on polysaccharides in the treatment of PD, it has been confirmed in in vitro cell experiments and animal experiments that polysaccharides from TCM have multiple biological functions, such as immune regulation, antioxidant, lowering blood glucose, and protecting the nervous system. The safety and efficacy of traditional Chinese medicine polysaccharides have been verified.

7 Conclusion and future perspectives

In conclusion, polysaccharides derived from TCM demonstrate potential in treating PD through multi-target mechanisms. Although over a dozen of polysaccharides have been shown to enhance cell survival, improve motor function in animal models, and histologically delay PD pathological progression, there remains a notable absence of clinical trials validating their efficacy. Current research faces limitations, such as the vast majority of studies have a relatively small sample size; cellular investigations predominantly focus on signaling pathways, with insufficient depth of exploration. Most literature only detect the expression of key proteins (e.g., Bax/Bcl-2 and caspase-3 expression), lacking exploration of upstream regulators (miRNAs, epigenetic modifications) and pathway crosstalk (e.g., PI3K/Akt-NF-κB interactions); animal experiments primarily employ single neurotoxin models that only partially replicate human PD pathology, failing to capture complex etiological factors like α-syn aggregation and neuroinflammatory cascades.

In the future, network pharmacology can be combined with metabolomics, transcriptomics, and neuroimaging to decipher precise neuroprotective mechanisms of polysaccharides and their interactions. Large-scale randomized controlled trials with extended observation periods are required. Standardized extraction/purification protocols are needed to address compositional variation. Advanced pharmaceutical engineering strategies, such as chemical modifications (sulfation/selenization) to enhance bioavailability, nano-delivery systems for improved blood-brain barrier penetration, can be improved to promote the translation of herbal polysaccharides from basic research to clinical practice and accumulate more high - quality clinical data.

While polysaccharides from TCM exhibit promising multi-target anti-PD properties, their clinical translation requires optimized formulation strategies and high-quality evidence generation to advance from basic research to therapeutic applications.

Author contributions

XZ: Writing – original draft, Data curation, Formal Analysis, Software, Visualization. QP: Conceptualization, Writing – review and editing, Methodology, Validation.

Funding

The author(s) declare that no financial support was received for the research and/or publication of this article.

Acknowledgments

We thank Hao Zhang in the Traditional Chinese Medicine Department for his help with interpreting TCM theories of various herbal recipes.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declare that no Generative AI was used in the creation of this manuscript.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

Ben-shlomo, Y., Darweesh, S., Llibre-guerra, J., Marras, C., Luciano, M. S., Tanner, C., et al. (2024). The epidemiology of parkinson's disease. *Lancet* 403 (10423), 283–292. doi:10.1016/s0140-6736(23)01419-8

Bian, Z., Zhang, R., Zhang, X., Zhang, J., Xu, L., Zhu, L., et al. (2023). Extraction, structure and bioactivities of polysaccharides from rehmannia glutinosa: a review. *J. Ethnopharmacol. Apr* 6, 116132. doi:10.1016/j.jep.2022.116132

Cai, J. (2013). Chinese herbal compound preparation for improving neurotransmitter metabolism in parkinson's disease. *China*, 2013–3.

Chen, H., Zhang, H., Shi, L., Li, Y., Ren, Y., Jing, W., et al. (2018). Antioxidative and neuroprotective effects of Lycium barbarum polysaccharide on parkinson's disease mice. *Chin. J. Nerv. Ment. Dis.* 44 (10), 613–618. doi:10.3969/j.issn.1002-0152.2018.10.008

Chen, J., Li, Y., Tian, W., and Cheng, W. (2010). Effect of polygona-polysaccharose (PP) on expression of PPAR-γ in brain tissue of rats with parkinson disease. *Prog. Mod. Biomed.* 10 (05), 814–817+792. doi:10.13241/j.cnki.pmb.2010.05.016

Chen, L., and Zhou, Q. (2015). Astragalus polysaccharide alleviates symptoms of rat with parkinson's disease. *World Sci-technol RD* 37 (02), 168–171. doi:10.16507/j.issn.1006-6055.2015.02.013

Chen, S., Li, Q., Zhang, C., Chen, W., Chen, X., Yin, L., et al. (2024). Effectiveness of congrong shujing granules on expression of substantia nigra metabolites in parkinson's disease patients: a randomized controlled trial. *Chin. J. Integr. Tradit. West. Med.* 44 (03), 296–302. doi:10.7661/j.cjim.20240131.010

Chen, S., Xiao, S., Lin, Y., Yang, S., Xu, Q., Cai, J., et al. (2019). Observation on the effects of congrongjing granules and Western medicine in the treatment of parkinson's disease. *Chin. J. Integr. Med. Cardio-Cerebrovasc. Dis.* 17 (19), 2914–2917. doi:10.12102/j.issn.1672-1349.2019.19.007

Cheng, N., Wang, H., Hao, H., Rahman, F., and Zhang, Y. (2023). Research progress on polysaccharide components of Cistanche deserticola as potential pharmaceutical agents. *Eur. J. Med. Chem.* 245 (Pt1), 114892. doi:10.1016/j.ejmech.2022.114892

Chinese Society of Parkinson's Disease and Movement Disorders (2020). Chinese guidelines for the treatment of Parkinson's disease (4th edition). *Chin. J. Neurol.* 53 (12), 973–986. doi:10.3760/cma.j.cn113694-20200331-00233

Cui, C., Cui, N., Wang, P., Song, S., Liang, H., and Ji, A. (2016). Neuroprotective effect of sulfated polysaccharide isolated from sea cucumber Stichopus japonicus on 6-OHDA-induced death in SH-SY5Y through inhibition of MAPK and NF-κB and activation of PI3K/Akt signaling pathways. *Biochem. Biophys. Res. Commun.* 470 (2), 375–383. doi:10.1016/j.bbrc.2016.01.035

Dai, Z., Yao, X., Xu, X., Xiao, X., Guan, J., Wang, Y., et al. (2022). Study on the neuroprotective effect of Lycium barbarum polysaccharide on neurons of rats with rotenone-induced Parkinson's disease *via* AMPK-mTOR signaling pathway. *Mod. J. Integr. Tradit. Chin.Western Med.* 31 (15), 2080–2083+2158. doi:10.3969/j.issn.1008-8849.2022.15.006

Dong, N., Li, M., Xu, D., Chen, X., Zhan, S., Pan, S., et al. (2021). Immunomodulatory effects of polysaccharides derived from five kinds of edible fungi and their compound polysaccharides on RAW264.7 cells. *Food Res. Dev.* 42 (9). 1–10. doi:10.12161/j.issn.1005-6521.2021.09.001

Dorsey, E. R., and Bloem, B. R. (2018). The parkinson Pandemic-a call to action. *JAMA Neurol.* 75 (1), 9–10. doi:10.1001/jamaneurol.2017.3299

Du, Y., Wan, H., Huang, P., Yang, J., and He, Y. (2022). A critical review of astragalus polysaccharides: from therapeutic mechanisms to pharmaceutics. *Biomed. Pharmacother. Mar.* 147, 112654. doi:10.1016/j.biopha.2022.112654

Fang, L., and Cai, J. (2021). Observation on the curative effect and sleep quality of patients with Parkinson's disease depression treated with congrong shujing granules. *World J. Sleep. Med.* 8 (08), 1346–1348. doi:10.3969/j.issn.2095-7130.2021.08.019

Fu, Y., Zhang, J., Chen, K., Xiao, C., Fan, L., Zhang, B., et al. (2019). An *in vitro* fermentation study on the effects of Dendrobium officinale polysaccharides on human intestinal microbiota from fecal microbiota transplantation donors. *J. Funct. Food.* 53, 44–53. doi:10.1016/j.jff.2018.12.005

Gao, G., Wang, J., Jiang, F., Li, G., Liu, X., Xue, H., et al. (2015a). The neuroprotective effect of sulfated pachymaran on the MPTP-Induced parkinson mice. *Chin. Pharmacol. Bull.* 31 (12), 1699–1704. doi:10.3969/j.issn.1001-1978.2015.12.014

Gao, G., Wu, C., Chen, Z., and Liu, X. (2015b). The protective effect of sulfation pachymaran on the damage of PC12 cells induced by MPP+. *J. Huaibei Norm. Univ. Nat. Scie. Ed.* 36 (04), 52–58. doi:10.3969/j.issn.2095-0691.2015.04.011

Gao, K., Liu, M., Cao, J., Yao, M., Lu, Y., Li, J., et al. (2014). Protective effects of Lycium barbarum polysaccharide on 6-OHDA-induced apoptosis in PC12 cells through the ROS-NO pathway. *Molecules* 20 (1), 293–308. doi:10.3390/molecules20010293

Gao, Z. (2022). *The structure characterization and immunological activity of polysaccharides derived from Cistanche deserticola*. Tianjin, China: Master's Theses Full-text Database.

Geng, X. (2023). Prepared radix rehmanniae research progress in chemical composition changes and pharmacological effects. *Brand Stand.* 3, 106–108. doi:10.3969/j.issn.1674-4977.2023.03.035

Golan, H., Volkov, O., and Shalom, E. (2022). Nuclear imaging in Parkinson's disease: the past, the present, and the future. *J. Neurol. Sci.* 436, 120220. doi:10.1016/j.jns.2022.120220

Guo, D., Zhou, J., Zhang, M., Taximaimaiti, R. Y. S., Wang, X., and Wang, H. (2021). Momordica charantia polysaccharides attenuates MPP+ -Induced injury in parkinson's disease mice and cell models by regulating TLR4/MyD88/NF-κB pathway. *Int. J. Poly. Sci.* 2021, 1–15. doi:10.1155/2021/5575636

Guo, Y., Cao, L., Zhao, Q., Zhang, L., Chen, J., Liu, B., et al. (2016). Preliminary characterizations, antioxidant and hepatoprotective activity of polysaccharide from Cistanche deserticola. *Int. J. Biol. Macromol.* 93 (Pt A), 678–685. doi:10.1016/j.ijbiomac.2016.09.039

Hao, C. ., Xu, Z., Xu, C., and Yao, R. (2024). Anti-herpes simplex virus activities and mechanisms of marine derived compounds. *Front. Cell. Infect. Microbiol.* 13, 1302096. doi:10.3389/fcimb.2023.1302096

He, H., Chen, B., Shi, Q., Zheng, L., Li, X., Li, D., et al. (2022). Advances in molecular mechanisms related to parkinson's disease. *J. Reg. Anat. Oper. Surg.* 31 (5), 456–460. doi:10.11659/jjssx.11E021034

He, L., Fan, H., Li, Y., Jia, L., Qin, S., Wang, L., et al. (2023). Mitochondrial dysfunction and Parkinson's disease. *Chin. J. Pract. Nerv. Dis.* 26 (10), 1305–1310. doi:10.12083/SYSJ.230261

Hu, A., Yin, C., Guo, Y., Wang, B., and Liu, X. (2021). Role of mitochondrial dysfunction and oxidative stress in parkinson's disease. *Med. Recapitul.* (15), 2929–2934. doi:10.3969/j.issn.1006-2084.2021.15.004

Hu, K., Ren, Y., and Jia, D. (2020). Clinical study on chaihu jia longgu muli tang combined with three needles for tremor in treating parkinson disease. *J. New. Chin. Med.* 52 (14), 24–27. doi:10.13457/j.cnki.jncm.2020.14.006

Huang, G., Mei, X., and Hu, J. (2017). The antioxidant activities of natural polysaccharides. *Curr. Drug. Targets* 18 (11), 1296–1300. doi:10.2174/1389450118666170123145357

Huang, L., Zhang, H., Xia, W., Yao, N., Xu, R., He, Y., et al. (2024). Structural characteristics, biological activities and market applications of rehmannia radix polysaccharides: a review. *Int. J. Biol. Macromol.* 282 (Pt 1), 136554. doi:10.1016/j.ijbiomac.2024.136554

Jia, L., and Li, X. (2022). Analysis of the efficacy and safety of the treatment of depression with the addition of chaihu longgu muli decoction. *Chin. Pract. Med.* 17 (10), 30–33. doi:10.14163/j.cnki.11-5547/r.2022.10.009

Jiang, Y., Zeng, X., Dai, H., Luo, S., and Zhang, X. (2024). Polygonatum sibiricum polysaccharide regulation of gut microbiota: a viable approach to alleviate cognitive impairment. *Int. J. Bio. Macromol.* 277, 134494. doi:10.1016/j.ijbiomac.2024.134494

Jin, M., Zhao, K., Huang, Q., Xu, C., and Peng, S. (2012). Isolation, structure and bioactivities of the polysaccharides from Angelica sinensis (oliv.) diels: a review. *Carbohydr. Polym.* 89 (1), 713–722. doi:10.1016/j.carbpol.2012.04.049

Jin, Y., Ge, J., Liu, S., Chen, J., and Li, Y. (2022). Advances in chemical structure,pharmacological action and structure-activity relationship of angelica polysaccharide. *Inf. Tradit. Chin. Med.* 39 (02), 69–77. doi:10.19656/j.cnki.1002-2406.20220213

Joers, V., Tansey, M. G., Mulas, G., and Carta, A. R. (2016). Microglial phenotypes in Parkinson's disease and animal models of the disease. *Prog. Neurobiol.* 207, 57–75. doi:10.1016/j.pneurobio.2016.04.006

Li, H., Shi, R., Ding, F., Wang, H., Han, W., Ma, F., et al. (2016). Astragalus polysaccharide suppresses 6-Hydroxydopamine-Induced neurotoxicity in *Caenorhabditis elegans*. *Oxid. Med. Cell. Longev.* 2016, 4856761. doi:10.1155/2016/4856761

Li, L., Zuo, Z. T., and Wang, Y. (2022). The traditional usages, chemical components and pharmacological activities of wolfiporia cocos: a review. *Am. J. Chin. Med.* 50 (2), 389–440. doi:10.1142/s0192415x22500161

Li, P., Du, Y., Wen, J., Shi, Y., Yan, W., Li, X., et al. (2020). Lycium barbarum polysaccharide regulate MPP+-Induced parkinson's disease model cell apoptosis by activating the PI3K/Akt pathway. *Chin. Remedies. Clin.* 20 (01), 28–30. doi:10.11655/zgywylc2020.01.007

Li, W., Hu, X., Wang, S., Jiao, Z., Sun, T., Liu, T., et al. (2020). Characterization and anti-tumor bioactivity of astragalus polysaccharides by immunomodulation. *Int. J. Biol. Macromol.* 145 (15), 985–997. doi:10.1016/j.ijbiomac.2019.09.189

Li, W., Sun, S., Yuan, H., Wang, H., Zhang, Y., and Sun, B. (2006). Time dependency of astragalus polysaccharides against systemic injury of free radical in astrocyte culture medium. *Chin. J. Clin. Rehabil.* 10 (11), 59–61. doi:10.3321/j.issn:1673-8225.2006.11.023

Li, Y. (2020). A study on the regulation of α-synuclein aggregation by lentinan. *Chin. [Master's thesis]. Master's Theses Full-text Database*. doi:10.27157/d.cnki.ghzku.2020.004324

Liang, Y., Huang, Y., Liu, J., Ou, Y., Li, Y., and Zhang, R. (2024). Autophagy and neurological diseases. *Chin. J. Comp. Med.* 34 (3), 111–119. doi:10.3969/j.issn.1671-7856.2024.03.014

Liang, Y., Liu, G., Xie, L., Su, K., Chang, X., Xu, Y., et al. (2022). Dendrobium candidum polysaccharide reduce atopic dermatitis symptoms and modulate gut microbiota in DNFB-Induced AD-like mice. *Front. Physiol.* 13, 976421. doi:10.3389/fphys.2022.976421

Li, Y. (2020). A study on the regulation of α-synuclein aggregation by lentinan. Chin. [Master's thesis]. Master's Theses Full-text Database. doi:10.27157/d.cnki.ghzku.2020.004324

Lin, Y., Tang, L., Huang, P., Liu, T., Zhong, J., Xu, Q., et al. (2020). Mechanisms of cong rong shu jing compound effects on endoplasmic reticulum stress in a rat model of parkinson's disease. *Altern. Med. 2020 1818307* 2020, 1818307. doi:10.1155/2020/1818307

Liu, W., Cao, J., Jing, Z., Cui, T., Yin, M., Liu, X., et al. (2022). Neurorotective effect of chaihu jia longgu mulitang on parkinson's disease with depression model rats and its mechanism based on AMPK/m TOR signaling pathway. *Chin. J. Exp. Tradit. Med. Form.* 28 (08), 21–29. doi:10.13422/j.cnki.syfjx.20220838

Luo, Q., Cai, Y., Yan, J., Sun, M., and Corke, H. (2004). Hypoglycemic and hypolipidemic effects and antioxidant activity of fruit extracts from Lycium barbarum. *Life Sci.* 76 (2), 137–149. doi:10.1016/j.lfs.2004.04.056

Lv, H., Shen, X., Zhao, F., and Zhu, M. (2024). Effects of Tricholoma matsutake polysaccharides on 1-methy-4-pehnyl-pyridine ion-induced PC12 cell damage. *Acta Anat. Sin.* 55 (01), 49–54. doi:10.16098/j.issn.0529-1356.2024.01.007

Morris, A. D. (1989). *James parkinson his life and times*. Boston (MA): Birkhauser.

Morris, H. R., Spillantini, M. G., Sue, C. M., and Williams-Gray, C. H. (2024). The pathogenesis of parkinson's disease. *Lancet* 403 (10423), 293–304. doi:10.1016/S0140-6736(23)01478-2

Mu, D., ShaRen, G. W., Bao, L., Chen, Y., and Sha, R. (2022). Structure, immunomodulatory and antioxidant activities *in vitro* of ploysaccharides from Radix asparagi. *J. Chin. Inst. Food Sci. Technol.* 22 (08), 51–60. doi:10.16429/j.1009-7848.2022.08.006

Nagatsu, T., Mogi, M., Ichinose, H., and Togari, A. (2000). Changes in cytokines and neurotrophins in parkinson's disease. *J. Neural. Transm. Suppl.* (60), 277–290. doi:10.1007/978-3-7091-6301-6_19

Ni, R. (2023). PET imaging in animal models of Parkinson's disease. *Behav. Brain Res.* 438, 114174. doi:10.1016/j.bbr.2022.114174

Paik, S., Kim, J. K., Silwal, P., Sasakawa, C., and Jo, E. K. (2021). An update on the regulatory mechanisms of NLRP3 inflammasome activation. *Cell. Mol. Immunol.* 18 (5), 1141–1160. doi:10.1038/s41423-021-00670-3

Peng, B., Wen, Y., Yu, R., and Zhu, J. (2018). Advances in extraction,structure characterization and bioactivities of citrus medica polysaccharide. *Food Drug* 20 (03), 236–241. doi:10.3969/j.issn.1672-979X.2018.03.017

Peng, D., Tian, W., An, M., Chen, Y., Zeng, W., Zhu, S., et al. (2023). Characterization of antidiabetic effects of Dendrobium officinale derivatives in a mouse model of type 2 diabetes mellitus. *Food Chem.* 399, 133974. doi:10.1016/j.foodchem.2022.133974

Przedborski, S., and Dawson, T. M. (2001). The role of nitric oxide in parkinson's disease. *Mol. Med.* 62, 113–136. doi:10.1385/1-59259-142-6:113

Ren, H., Li, Z., Gao, R., Zhao, T., Luo, D., Yu, Z., et al. (2022). Structural characteristics of Rehmannia glutinosa polysaccharides treated using different decolorization processes and their antioxidant effects in intestinal epithelial cells. *Foods* 11 (21), 3449. doi:10.3390/foods11213449

Shang, X., Xu, C., Dong, J., Ci, C., Zhang, P., Jin, H., et al. (2023). Mechanism of Dendrobium huoshanense polysaccharide against neuroinflammation in parkinson's disease model: based on NLRP3 inflammasome. *Chin. J. Exp. Tradit. Med. Form.* 29 (11), 97–105. doi:10.13422/j.cnki.syfjx.20230437

Shao, F., Liang, S., and Qin, H. (2020). The role of microglia in Parkinson's disease and its research progress. *Chin. J. Neuroanat.* 36 (5), 565–569. doi:10.16557/j.cnki.1000-7547.2020.05.015

Song, J., Liu, L., Li, Z., Mao, T., Zhang, J., Zhou, L., et al. (2022). Lycium barbarum polysaccharide improves dopamine metabolism and symptoms in an MPTP-Induced model of parkinson's disease. *BMC Med.* 20 (1), 412. doi:10.1186/s12916-022-02621-9

Song, Z., Huang, Y., Wang, Y., Deng, J., Zhen, D., Tan, N., et al. (2024). Antioxidant and anti-aging effects and mechanism of poria cocos polysaccharides in *Caenorhabditis elegans*. *Chin. Tradit. Herb. Drugs.* 55 (04), 1133–1144. doi:10.7501/j.issn.0253-2670.2024.04.008

Steinmetz, J. D., Seeher, K. M., Schiess, N., Nichols, E., Cao, B., Servili, C., et al. (2024). Global, regional, and national burden of disorders affecting the nervous system, 1990–2021: a systematic analysis for the global burden of disease study 2021. *Lancet Neurol.* 23 (4), 344–381. doi:10.1016/s1474-4422(24)00038-3

Sun, S., Xiao, Q., Fan, L., He, L., Li, Y., Fan, H., et al. (2023). Research status of α-synuclein and parkinson's disease. *Chin. J. Clin. Pharmacol.* 39 (8), 1230–1234. doi:10.13699/j.cnki.1001-6821.2023.08

Tan, Y., Yin, L., Sun, Z., Shao, S., Chen, W., Man, X., et al. (2020). Astragalus polysaccharide exerts anti-Parkinson *via* activating the PI3K/AKT/mTOR pathway to increase cellular autophagy level *in vitro*. *Int. J. Biol. Macromol. Jun* 15, 349–356. doi:10.1016/j.ijbiomac.2020.02.282

Tian, X., Liang, T., Liu, Y., Ding, G., Zhang, F., Ma, Z., et al. (2019). Extraction, structural characterization, and biological functions of Lycium barbarum polysaccharides: a review. *Biomolecules* 9 (9), 389. doi:10.3390/biom9090389

Tolosa, E., Garrido, A., Scholz, S. W., and Poewe, W. (2021). Challenges in the diagnosis of parkinson's disease. *Lancet Neurol.* 20 (5), 385–397. doi:10.1016/s1474-4422(21)00030-2

Tu, Y., and Cai, J. G. J. (2017). Chaihu jialonggu muli decoction for parkinson's disease with depression in 34 cases. *Guangming J. Chin. Med.* 32 (04), 520–523. doi:10.3969/j.issn.10038914.2017.04.02

Wang, J., Hu, Y., Wang, D., Liu, J., Zhang, J., Abula, S., et al. (2010). Sulfated modification can enhance the immune-enhancing activity of Lycium barbarum polysaccharides. *Cell. Immunol.* 263 (2), 219–223. doi:10.1016/j.cellimm.2010.04.001

Wang, J., Zhong, Q., Dong, C., Li, H., Wang, H., Xia, F., et al. (2021). Recent progress in the biological activity and mechanism of action of sea cucumber polysaccharides. *Food Sci.* 42 (23), 370–380. doi:10.7506/spkx1002-6630-20200705-055

Wang, L., and Han, J. (2021). Mechanism of Lycium barbarum polysaccharide and UCA1 siRNA single or combined treatment in the MPP+-Induced Parkinson's disease cell model. *Chin. J. Comp. Med.* 31 (11), 55–61. doi:10.3969/j.issn.1671-7856.2021.11.009

Wang, R., Santos, J. M., Dufour, J. M., Stephens, E. R., Miranda, J. M., Washburn, R. L., et al. (2022). Ginger root extract improves GI health in diabetic rats by improving intestinal integrity and mitochondrial function. *Nutrients* 14 (20), 4384. doi:10.3390/nu14204384

Wang, W., Yang, Y., Ying, C., Li, W., Ruan, H., Zhu, X., et al. (2007). Inhibition of glycogen synthase kinase-3beta protects dopaminergic neurons from MPTP toxicity. *Neuropharmacology* 52 (8), 1678–1684. doi:10.1016/j.neuropharm.2007.03.017

Wang, W., Zhao, B., Zhang, Z., Kikuchi, T., Wei, L., Jantrawut, P., et al. (2024). Natural polysaccharides and their derivatives targeting the tumor microenvironment: a review. *Int. J. Biol. Macromol.* 268, 131789. doi:10.1016/j.ijbiomac.2024.131789

Wang, X., Pang, L., Zhang, Y., Xu, J., Ding, D., Yang, T., et al. (2018). Lycium barbarum polysaccharide promotes nigrostriatal dopamine function by modulating PTEN/AKT/mTOR pathway in a Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) murine model of parkinson's disease. *Neurochem. Res. Apr* 43 (4), 938–947. doi:10.1007/s11064-018-2499-6

Wang, Y. (2024). *Isolation and purification of Taiwanofungus camphoratus polysaccharides and its effect on macrophage function*. Nanchang University [Master's thesis]. doi:10.27232/d.cnki.gnchu.2023.000298

Wang, Y., Dong, P., Jin, C., Kang, J., Hu, Y., and Wang, Z. (2019). Analysis on composition of Polygonatum sibiricum polysaccharide and its anti-oxidant activity. *Genomics Appl. Biol.* 38 (05), 2191–2199. doi:10.13417/j.gab.038.002191

Wang, Y., Jin, H., Li, Y., Dong, X., Za, K., and Ma, S. (2021). Immunomodulatory effect of Lycium barbarum polysaccharide of different molecular weights on macrophage RAW264.7. *Chin. J. New Drugs.* 30 (12), 1079–1086. doi:10.3969/j.issn.1003-3734.2021.12.005

Wang, Y., Mo, X., Zhang, W., Li, P., Zhu, X., and Jiang, N. (2023). Research progresses on structure and pharmacological action of Tricholoma matsutake polysaccharides. *Chin. Mod. Med.* 30 (10), 34–38. doi:10.3969/j.issn.1674-4721.2023.10.010

Wirdefeldt, K., Adami, H. O., Cole, P., Trichopoulos, D., and Mandel, J. (2011). Epidemiology and etiology of parkinson's disease: a review of the evidence. *Eur. J. Epidemiol.* 26 (Suppl. 1), S1–S58. doi:10.1007/s10654-011-9581-6

Wu, C. C., and Bratton, S. B. (2013). Regulation of the intrinsic apoptosis pathway by reactive oxygen species. *Antioxid. Redox. Signal.* 19 (6), 546–558. doi:10.1089/ars.2012.4905

Xiao, H., Hong, A., and Cheng, X. (2021). Clinical application of Radix bupleuri keen-oyster soup in the treatment of sleep disorders in patients with parkinson's disease. *World J. Sleep. Med.* 8 (07), 1161–1162. doi:10.3969/j.issn.2095-7130.2021.07.019

Xu, Q., Yang, S., Wu, F., Lin, Y., Zhong, J., Tang, L., et al. (2020). Congrong shujing granule-induced Grp78 expression reduced endoplasmic reticulum stress and neuronal apoptosis in the midbrain in a parkinson's disease rat model. *Evid. Based Complement. Altern. Med.* 30, 4796236. doi:10.1155/2020/4796236

Xu, T., Zhang, H., Wang, S., Xiang, Z., Kong, H., Xue, Q., et al. (2022). A review on the advances in the extraction methods and structure elucidation of poria cocos polysaccharide and its pharmacological activities and drug carrier applications. *Int. J. Biol. Macromol. Sep.* 30, 536–551. doi:10.1016/j.ijbiomac.2022.07.070

Xue, T., Zheng, D., Wen, L., Hou, Q., He, S., Zhang, H., et al. (2024). Advance in Cistanche deserticola Y. C. Ma. Polysaccharides: isolation, structural characterization, bioactivities and application: a review. *Int. J. Biol. Macromol. Oct.* 278 (Pt 2), 134786. doi:10.1016/j.ijbiomac.2024.134786

Yang, L. C., Zhang, Y., Yu, X., Sun, J. W., Li, J. W., Zhang, X. Y., et al. (2018). Research progress of the relationship between autophagy and ULK1 protein. *Med. Recapitul.* 24 (23), 4635–4639. doi:10.3969/j.issn.1006-2084.2018.23.012

Yang, X., Yang, B., Zhang, Y., Liang, Y., Li, K., Xu, C., et al. (2018). The mechanism of programmed cell death of dopaminergic neurons in parkinson's disease. *Neural. Inj. Funct. Reconst.* 13 (6), 296–299. doi:10.16780/j.cnki.sjssgncj.2018.06.008

Yang, Y., Guan, Q., Guo, L., and Han, C. (2018). Antrodia camphorata polysaccharide improved neurobehavioral function in parkinson mice *via* reducing the expression of NLRP3-Caspase1 inflammatory body. *Chin. J. Clin. Pharmacol. Ther.* 23 (04), 406–412. doi:10.12092/j.issn.1009-2501.2018.04.008

Yang, Y., Zhao, X., Wang, Y., Guan, Q., and Han, C. (2017). Antioxidation and anti-inflammatory effects of Antrodia camphorata polysaccharide on parkinsonian mice induced by 6-OHDA. *Chin. J. Mod. Appl. Pharm.* 34 (07), 969–972. doi:10.13748/j.cnki.issn1007-7693.2017.07.008

Yang, Y. N., Sun, L., and Cui, X. (2024). Molecular mechanisms related to parkinson's disease and the intervention effect of traditional Chinese medicine. *Pract. J. Card. Cereb. Pneum. Vasc. Dis.* 32 (09), 25–31. doi:10.12114/j.issn.1008-5971.2024.00.208

Ye, Q., Wang, K., Yuan, X., He, J., Zhou, J., Yuan, C., et al. (2017a). Effects of shudi pingchan granule on behavior and alpha-synuclein expression in rats with parkinson's disease. *Chin. J. Integr. Med. Cardio-Cerebrovasc. Dis.* 15 (04), 419–424. doi:10.3969/j.issn.1672-1349.2017.04.009

Ye, Q., Yuan, X. L., He, J., Zhou, J., Yuan, C. X., Yang, X. M., et al. (2016). Anti-apoptotic effect of shudipingchan granule in the substantia nigra of rat models of parkinson's disease. *Neural Regen. Res.* 11 (10), 1625–1632. doi:10.4103/1673-5374.193242

Ye, Q., Yuan, X. L., Zhang, Y., and Yuan, C. X. (2017b). Clinical study on treatment of parkinson's disease with shudi pingchan granule combined with repetitive transcranial magnetic stimulation. *Tradit. Chin. Med.* 6 (2), 60–67. doi:10.12677/tcm.2017.62011

Yin, S., Wang, H., and Yang, S. (2020). Neuroprotective effects of Cistanche deserticola polysaccharide on Parkinson's rats induced by 6-HODA caused by activating the Wnt/β-catenin signaling pathway. *Chin. J. Integr. Med. Cardio-Cerebrovascu. Dis.* 18 (08), 1227–1230. doi:10.12102/j.issn.1672-1349.2020.08.011

Yu, C., Fang, X., Yu, Y. J., and Yu, Z. (2021). Effect of chaihu jialonggu oyster decoction on Parkinson's dementia and its influence on MMSE score and MoCA score. *Clin. Educ. Gen. Pract.* 19 (11), 985–988. doi:10.13558/j.cnki.issn1672-3686.2021.011.008

Yuan, C., Zhi, H., Chen, S., Chen, Y., Li, J., Shen, W., et al. (2010). Clinical multicenter randomized controlled study on treatment of parkinson's disease with "Shudi Pingchan Decoction" and wWstern medicine. *Shanghai J. Tradit. Chin. Med.* 44 (06), 3–6. doi:10.16305/j.1007-1334.2010.06.041

Yue, H., Zeng, H., and Ding, K. (2020). A review of isolation methods, structure features and bioactivities of polysaccharides from dendrobium species. *Chin. J. Nat. Med. Jan.* 18 (1), 1–27. doi:10.1016/s1875-5364(20)30001-7

Zeng, P., Li, J., Chen, Y., and Zhang, L. (2019). The structures and biological functions of polysaccharides from traditional Chinese herbs. *Prog. Mol. Biol. Transl. Sci.* 163, 423–444. doi:10.1016/bs.pmbts.2019.03.003

Zhang, C., Liu, B., Yuan, Z., Ma, X., and Zeng, F. (2024). Angelica polysaccharide alleviates motor impairment in mice with Parkinson's disease *via* inhibition of astrocyte apoptosis. *Chin. J. Gerontolo.* 44 (13), 3188–3192. doi:10.3969/j.issn.1005-9202.2024.13.030

Zhang, H., Li, F., Zhao, C., Wang, C., Yan, X., Yu, H., et al. (2022). Isolation, purification and characterization of polysaccharide from Schisandra chinensis. *Tianjin J. Tradit. Chin. Med.* 39 (04), 509–515. doi:10.11656/j.issn.1672-1519.2022.04.21

Zhang, L., Hao, J., Zheng, Y., Su, R., Liao, Y., Gong, X., et al. (2018). Fucoidan protects dopaminergic neurons by enhancing the mitochondrial function in a rotenone-induced rat model of parkinson's disease. *Aging Dis.* 9 (4), 590–604. doi:10.14336/AD.2017.0831

Zhang, P., Dong, J., Xu, C., Wang, S., and Cheng, N. (2023). Effects of DHP on dyskinesia, neuronal damage and senescence of astrocytes in mice with parkinson's disease. *Shandong Med. J.* 63 (35), 24–29. doi:10.3969/j.issn.1002-266X.2023.35.006

Zhang, W., Huang, J., Wang, W., Li, Q., Chen, Y., Feng, W., et al. (2016). Extraction, purification, characterization and antioxidant activities of polysaccharides from Cistanche tubulosa. *Int. J. Biol. Macromol.* 93 (PtA), 448–458. doi:10.1016/j.ijbiomac.2016.08.079

Zhang, X., Sun, H., Wang, L., Wang, Y., Liu, C., Bai, Y., et al. (2011). Protective effects of crude polysaccharides from Chroogomphus rutilus on dopaminergic neurons impaired by MPTP in mice. *Mycosystema* 30 (01), 77–84. doi:10.13346/j.mycosystema.2011.01.020

Zhang, X., Wang, Y., Wang, L., and Guan, Z. (2013). Protective effects of crude polysaccharides from Chroogomphus rutilus on SH-SY5Y cells impaired by MPP+. *Acta Physiol. Sin.* 65 (02), 210–216. doi:10.13294/j.aps.2013.02.004

Zhang, Y., Fu, J., Yuan, Z., and Gu, H. (2023). The role of cellular senescence in the development and progression of neurological diseases. *J. China Med. Univ.* 52 (9), 847–851. doi:10.12007/j.issn.0258-4646.2023.09.013

Zhao, B., Zhao, J., Lv, M., Li, X., Wang, J., Yue, Z., et al. (2021). Comparative study of structural properties and biological activities of polysaccharides extracted from Chroogomphus rutilus by four different approaches. *Int. J. Biol. Macromol.* 188, 215–225. doi:10.1016/j.ijbiomac.2021.08.025

Zhao, D., Niu, J., Du, Z., Zhou, C., Ding, S., Du, M., et al. (2023). Exploration of antidepressant mechanism of chaihu and longgu mulitang based on cAMP/PKA/CREB/BDNF signaling pathway. *Chin. J. Exp. Tradit. Med. Formul.* 29 (03), 17–25. doi:10.13422/j.cnki.syfjx.20221709

Zhao, L., Wang, Y., Shen, H., Shen, X., Nie, Y., Wang, Y., et al. (2012). Structural characterization and radioprotection of bone marrow hematopoiesis of two novel polysaccharides from the root of Angelica sinensis (oliv.) diels. *Fitoterapia* 83 (8), 1712–1720. doi:10.1016/j.fitote.2012.09.029

Zhao, X., Zhao, W., Duan, C., Zheng, J., Sun, N., Yang, J., et al. (2024). Research progress on relationship between chemical characterization and immune regulation of astragalus polysaccharides. *Chin. Arch. Tradit. Chin. Med.* 42 (03), 108–112. doi:10.13193/j.issn.1673-7717.2024.03.020

Zheng, Y., Ren, W., Zhang, L., Zhang, Y., Liu, D., and Liu, Y. (2020). A review of the pharmacological action of astragalus polysaccharide. *Front. Pharmacol.* 11, 349. doi:10.3389/fphar.2020.00349

Zhou, G., Li, Y., Peng, Y., Yang, Y., Cui, J., and Lv, Q. (2021). Effects of astragalus polysaccharide injection on the levels of urine CXCL10, E-cadherin and MMP-9 in patients with diabetic nephropathy. *Med. Pharm. J. Chin. PLA.* 33 (1), 94–97. doi:10.3969/j.issn.2095-140X.2021.01.021

Zhou, N., Zhao, S., and Liang, X. (2021). Study on the anti-oxidative damage and neuroprotective effects of pholidota chinensis lindl. Polysaccharides on a rat model of parkinson's disease. China med. *Pharm* 11 (21), 32–36. doi:10.3969/j.issn.2095-0616.2021.21.009

Zhou, Y., Wang, S., Feng, W., Zhang, Z., and Li, H. (2021). Structural characterization and immunomodulatory activities of two polysaccharides from rehmanniae radix praeparata. *Int. J. Biol. Macromol. Sep.* 1 (186), 385–395. doi:10.1016/j.ijbiomac.2021.06.100

Zhu, Y., Zhu, X., Zhou, Y., and Zhang, D. (2019). Oxidative stress and parkinson's disease. *Chin. J. Pract. Nerv. Dis.* . (11), 1271–1276. doi:10.12083/SYSJ.2019.11.176

Frontiers in Pharmacology

Explores the interactions between chemicals and living beings

The most cited journal in its field, which advances access to pharmacological discoveries to prevent and treat human disease.

Discover the latest Research Topics

See more →

Frontiers

Avenue du Tribunal-Fédéral 34
1005 Lausanne, Switzerland
frontiersin.org

Contact us

+41 (0)21 510 17 00
frontiersin.org/about/contact