frontiers | Research Topics

Diabetic kidney disease: routes to drug development, pharmacology and underlying molecular mechanisms, volume II

Edited by
Swayam Prakash Srivastava and Divya Bhatia

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 978-2-8325-6316-8
DOI 10.3389/978-2-8325-6316-8

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

Diabetic kidney disease: routes to drug development, pharmacology and underlying molecular mechanisms, volume II

Topic editors

Swayam Prakash Srivastava — University of Michigan, United States

Divya Bhatia — Weill Cornell Medical Center, NewYork-Presbyterian, United States

Citation

Srivastava, S. P., Bhatia, D., eds. (2025). *Diabetic kidney disease: routes to drug development, pharmacology and underlying molecular mechanisms, volume II*. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-8325-6316-8

Table of contents

05 Editorial: Diabetic kidney disease: routes to drug development, pharmacology and underlying molecular mechanisms, volume II
Divya Bhatia and Swayam Prakash Srivastava

10 Influence of the combination of SGLT2 inhibitors and GLP-1 receptor agonists on eGFR decline in type 2 diabetes: post-hoc analysis of RECAP study
Yoshimi Muta, Kazuo Kobayashi, Masao Toyoda, Atsuhito Tone, Daisuke Suzuki, Daisuke Tsuriya, Hideo Machimura, Hidetoshi Shimura, Hiroshi Takeda, Hisashi Yokomizo, Kei Takeshita, Keiichi Chin, Keizo Kanasaki, Kouichi Tamura, Masaaki Miyauchi, Masuo Saburi, Miwa Morita, Miwako Yomota, Moritsugu Kimura, Nobuo Hatori, Shinichi Nakajima, Shun Ito, Shunichiro Tsukamoto, Takashi Murata, Takaya Matsushita, Takayuki Furuki, Takuya Hashimoto, Tomoya Umezono, Yuichi Takashi and Daiji Kawanami

22 Improving glycemic control: transitioning from dulaglutide to tirzepatide in patients with type 2 diabetes undergoing hemodialysis
Emiko Otsuka, Mineaki Kitamura, Satoshi Funakoshi, Hiroshi Mukae and Tomoya Nishino

32 Comparative efficacy and safety of SGLT2is and ns-MRAs in patients with diabetic kidney disease: a systematic review and network meta-analysis
Si-Qi Yang, Xi Zhao, Jing Zhang, Huan Liu, Yu-Han Wang and Yao-Guang Wang

46 The role of metabolic memory in diabetic kidney disease: identification of key genes and therapeutic targets
Tongyue Yang, Qi Feng, Mingwei Shao, Mengxing Pan, Feng Guo, Yi Song, Fengjuan Huang, Zhao Linlin, Jiao Wang, Lina Wu, Guijun Qin and Yanyan Zhao

62 Assessment of the effect of the SLC5A2 gene on eGFR: a Mendelian randomization study of drug targets for the nephroprotective effect of sodium-glucose cotransporter protein 2 inhibition
Gailing Liu

70 Exploring potential targets for natural product therapy of DN: the role of SUMOylation
Jingjing Wang, Rui Zhang, Chenguang Wu, Lifan Wang, Peng Liu and Ping Li

87 *Sphagnum cuspidatulum* extract prevents acute kidney injury induced by high-fat diet and streptozotocin via alleviation of oxidative stress and apoptosis in pre-diabetic rats
Pongrapee Laorodphun, Sutheera Chaisen, Sarocha Amattat, Pornchita Maphet, Narin Printrakul, Hataichanok Pandith, Aussara Panya, Burit Kongmali, Myat Theingi Swe and Phatchawan Arjinajarn

101 Effects of *Abelmoschus manihot* (L.) and its combination with irbesartan in the treatment of diabetic nephropathy via the gut–kidney axis
Hongmei Yu, Haitao Tang, Rengui Saxu, Yuhui Song, Xu Cui, Jingjing Xu, Nan Li, Siyuan Cui, Haitao Ge, Wei Tang and Harvest F. Gu

115 β-cryptoxanthin suppresses oxidative stress via activation of the Nrf2/HO-1 signaling pathway in diabetic kidney disease
Jingjing Ke, Hualong Zang, Yang Liu, Qiuping Teng, Jiao Hua, Dan Peng and Ping Wang

131 FGF21 ameliorates diabetic nephropathy through CDK1-dependently regulating the cell cycle
Yudie Zhang, Fan Wang, Chongyang Zhang, Fan Yao, Bin Zhang, Yongping Zhang and Xiaobo Sun

146 Shenshuaikang enema restores the intestinal barrier and microbiota-gut-kidney axis balance to alleviate chronic kidney disease via NF-κB pathway
Yan Ye, Xiaopeng Huang, Xueying Li, Fei Gao, Wenzhen Zhong, Anqi Tang, Liangbin Zhao, Dengpiao Xie and Naijing Ye

158 Baicalin: a potential therapeutic agent for acute kidney injury and renal fibrosis
Xiaoming Li, Rui Xu, Dan Zhang, Ji Cai, He Zhou, Tao Song, Xianyao Wang, Qinghong Kong, Liujin Li, Zhaohui Liu, Zhixu He, Zhengzhen Tang, Jun Tan and Jidong Zhang

178 Incidence of hyperkalemia RAASi and SGLT-2i treatment in individuals with diabetic kidney disease: a systematic review and network meta-analysis
Yahui Yuan, Chun Chen, Yuping Lin, Yehao Luo, Zhaojun Yang, Jingyi Guo, Qiaoyun Liu, Lu Sun and Guanjie Fan

188 Clinical efficacy and safety of sodium-glucose cotransporter protein-2 (SGLT-2) inhibitor, glucagon-like peptide-1 (GLP-1) receptor agonist, and Finerenone in type 2 diabetes mellitus with non-dialysis chronic kidney disease: a network meta-analysis of randomized clinical trials
Jingyi Guo, Maoying Wei, Wenhua Zhang, Yijia Jiang, Aijing Li, Churan Wang, Dan Yin, Anning Sun and Yanbing Gong

202 Landscape analysis of m6A modification regulators reveals LRPPRC as a key modulator in tubule cells for DKD: a multi-omics study
Li Jiang, Hongda Yu, Jie Jian, Xulin Sai, Yudian Wang, Yufei Zhang and Xiai Wu

216 Renal protective effects of vitamin E for drug-induced kidney injury: a meta-analysis
Lingfei Meng, Shengmao Liu and Wenpeng Cui

frontiers | Frontiers in Pharmacology

TYPE Editorial
PUBLISHED 25 April 2025
DOI 10.3389/fphar.2025.1609100

Check for updates

OPEN ACCESS

EDITED AND REVIEWED BY
Giuseppe Remuzzi,
Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Italy

*CORRESPONDENCE
Swayam Prakash Srivastava,
swayam.cdri@gmail.com
spsr@umich.edu

RECEIVED 09 April 2025
ACCEPTED 17 April 2025
PUBLISHED 25 April 2025

CITATION
Bhatia D and Srivastava SP (2025) Editorial: Diabetic kidney disease: routes to drug development, pharmacology and underlying molecular mechanisms, volume II.
Front. Pharmacol. 16:1609100.
doi: 10.3389/fphar.2025.1609100

COPYRIGHT
© 2025 Bhatia and Srivastava. 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: Diabetic kidney disease: routes to drug development, pharmacology and underlying molecular mechanisms, volume II

Divya Bhatia[1] and Swayam Prakash Srivastava[2]*

[1]Division of Nephrology and Hypertension, Joan and Sanford I. Weill Department of Medicine, Weill Cornell Medicine, New York, NY, United States, [2]Life Sciences Institute, University of Michigan, Ann Arbor, MI, United States

KEYWORDS

diabetic kidney disease, sirtuin 3, glucocorticoid receptor, fibroblasts growth factor receptor 1, angiopoeitin like protein 4, kidney failure, endothelial-to-mesenchymal transition, kidney fibrosis

Editorial on the Research Topic
Diabetic kidney disease: routes to drug development, pharmacology and underlying molecular mechanisms, volume II

Introduction

Diabetic kidney disease (DKD) is the leading cause of chronic kidney disease (CKD), driven primarily by metabolic dysfunction, immune cell infiltration, and inflammation in the kidney. These changes lead to nephron loss, mesenchymal activation, and finally kidney fibrosis (Li and Susztak, 2025). DKD accounts for almost half of all CKD cases, with around 40% of individuals with diabetes developing DKD (Li and Susztak, 2025; Cooper and Warren, 2019). Despite its prevalence, DKD progression varies among patients, and its underlying mechanism remains poorly understood. DKD often remains asymptomatic until it advances to kidney failure. Kidney fibrosis is the final pathological outcome of CKD in the diabetic kidney. Several theories have been proposed regarding the origin of myofibroblasts, which play crucial roles in the progression of kidney fibrosis. Myofibroblasts can derive from tubular epithelial cells via partial epithelial-to-mesenchymal transition (EMT), endothelial cells through endothelial-to-mesenchymal transition (EndMT), or profibrotic (M2-like)-macrophages via macrophage-to-mesenchymal transition (MMT) (Lovisa et al., 2020; Bhatia et al., 2019). Additionally, myofibroblasts have also been proposed to originate from activated resident fibroblasts or bone marrow-derived cells (Srivastava et al., 2019; Srivastava et al., 2020a). In DKD, an increase in endothelial cell permeability, and a decrease in pericytes coverage contribute to tissue damage and the release of chemokines further exaggerate the infiltration of immune cells and inflammation. The excessive inflammation-mediated tissue injury results in the deposition of the components of extracellular matrix (ECM), such as collagens, fibronectin, desmin, and integrins. Ultimately, these pathological alterations disrupt kidney structure, function, and metabolism, resulting in kidney fibrosis and failure (Rayego-Mateos et al.,

2023). A recent study highlighted a comprehensive multimodal atlas of the human kidney (Li et al., 2024). Using single-cell assay for transposase-accessible chromatin and RNA sequencing (scATAC-seq) and spatially resolved metabolomics, this study has characterized region-specific metabolic signatures of different nephron segments, including endothelial cells and proximal tubular epithelial cells (Li et al., 2024). Spatial metabolomics further demonstrated suppressed fatty acid oxidation (FAO) along with an accumulation of long-chain acylcarnitines, a type of lipids that are key intermediates in FAO, in proximal tubular cells from patients with kidney disease (Kang et al., 2015; Mukhi et al., 2023). Related research in type I diabetic (T1DM) patients identified that despite preserved kidney function and the absence of albuminuria at an early stage, metabolic disturbances can be present (Choi et al., 2024). Utilizing single-cell RNA sequencing, spatial metabolomics, and positron emission tomography imaging to assess tricarboxylic acid cycle (TCA) cycle activity, they reported suppressed oxidative metabolism in the renal cortex and medulla of T1DM patients (Choi et al., 2024). Overall, this study highlights that early metabolic impairments could serve as preclinical markers for DKD, suggesting a critical role of mitochondrial dysfunction in the progression of DKD. Elevated urinary lactate levels have emerged as a biomarker of mitochondrial dysfunction and correlate with a decline in kidney function in diabetic patients (Darshi et al., 2024). Mitophagy, the mitochondrial quality control program that recycles defective mitochondria is also impaired during DKD (Bhatia et al., 2019; Bhatia et al., 2020; Bhatia and Srivastava, 2023). Mitochondrial proteome data profiling in healthy podocytes revealed that NADH:ubiquinone oxidoreductase subunit S4 (NDUFS4) stabilizes respiratory supercomplexes. In diabetic podocytes, suppressed NDUFS4 levels were also associated with reduced mitochondrial function, contributing to DKD progression (Mise et al., 2023).

Here, we discussed two sections:

New cellular and molecular mechanisms

Suppressed FAO in diabetic renal tubules, endothelial cells, and podocytes is concomitantly associated with aberrant glycolysis and elevated lactate levels (Srivastava et al., 2018). This metabolic reprogramming is believed to generate metabolites that sustain myofibroblasts' survival, contributing to fibrosis progression (Srivastava et al., 2018). This mesenchymal-metabolic shift, characterized by a gain of mesenchymal activity due to altered metabolism, is likely driven by mitochondrial dysfunction (Srivastava et al., 2021a). Targeting mesenchymal metabolic shift represents a potential therapeutic strategy for DKD (Srivastava et al., 2021a). In recent years, our group has demonstrated the anti-EndMT, anti-inflammatory, and anti-fibrotic effects of endothelial SIRT3, endothelial glucocorticoid receptors, and endothelial fibroblasts growth factor receptor 1 in diabetes (Srivastava and Goodwin, 2023; Srivastava et al., 2021b; Srivastava et al., 2021c; Li et al., 2020a). These anti-fibrotic effects are accompanied by improved mitochondrial function and structure, reduced inflammation, attenuation of mesenchymal metabolic shifts and partial EMT in the kidney tubules. Moreover, we reported that mitochondrial fusion, biogenesis, mitophagy, and antioxidant defense in the kidney are suppressed while fission is induced during kidney fibrosis (Bhatia et al., 2019; Bhatia et al., 2022). Mitochondrial quality control program helps in attenuating kidney macrophage-derived inflammation and progression of kidney fibrosis (Bhatia et al., 2019; Bhatia et al., 2022). Apart from the suppressed fatty acid oxidation, *de novo* lipogenesis (DNL) plays a critical role in kidney fibrogenesis (Mukhi et al., 2023; Srivastava et al., 2024). In diabetic tubules and podocytes, key DNL enzymes, including fatty acid synthase (FASN), acetyl-CoA carboxylase (ACC), perilipin and sterol regulatory element-binding protein 1 (SREBF1) are upregulated. The inhibition of FASN using a small molecule inhibitor FASNALL suppresses DNL and mitigates renal fibrogenesis in diabetic mice (Mukhi et al., 2023; Srivastava et al., 2024). Angiopoietin like-4 (ANGPTL4), a secretory protein, regulates lipid metabolism, FAO, and DNL by inhibiting lipoprotein lipase (LPL) in the kidney (Srivastava et al., 2024). While ANGPTL4 is chiefly secreted by adipose tissue and liver and mostly in a sialylated form (Clement et al., 2014; Clement et al., 2011). Kidney podocytes and tubules also secrete a hyposialylated form of ANGPTL4, which plays pro-proteinuric and fibrogenic roles in the diabetic kidney (Clement et al., 2011). The fibrogenic form of the ANGPTL4 functions independently of LPL (Srivastava et al., 2024). Excessive lipid flux suppressed FAO and increased DNL that contribute to lipid accumulation, and finally mitochondrial dysfunction in the tubular epithelial cells. Mitochondrial injury leads to the release of mitochondrial DNA (mtDNA) into the cytosol, where it activates the c-GAS-STING pathway and triggers tubulointerstitial inflammation (Bhatia et al., 2020). The inflamed tubules undergo mesenchymal activation, ultimately leading to tubulointerstitial fibrosis during DKD (Srivastava et al., 2024; Chung et al., 2019). Figure 1 demonstrate that healthy endothelial cells secrete healthy angiocrine factors that nourishes the other neighbouring cells and are important for the podocyte and tubule cell health. However, during inflammation which is associated to induce the pro-EndMT signals disrupts the endothelial cell metabolism in diabetes. Disruption of the endothelial cell metabolism leads to alterations in the angiocrine factors or secretion of unhealthy angiocrine factors which not only worsen the cells itself but the cell organelles, such as mitochondria. Therefore EndMT-derived angiocrine factors, disrupt the tubules cells and podocytes possibly through inflammation and partial EMT processes. However, the EMT in the podocyte is still needs further investigation for the researchers. Accumulative effects disrupt the essential crosstalk among endothelial cell, tubular and podocyte cell, finally leading to proteinuria, and severe fibrosis which is phenomenal feature of diabetic kidney disease.

In this Research Topic, a manuscript by Zhang et al. demonstrated that fibroblast growth factor (FGF)-21 by reducing glomerular damage, inflammation, and thrombosis while regulating the cell cycle through CDK1, exerts protective functions in diabetic nephropathy (DN). Additionally, Wang et al. described the role of SUMOylation in DKD and highlighted that natural product-mediated modulation of SUMOylation represents a potential therapeutic strategy for the treatment of DN. Yang et al. reported that N-myc downstream regulated gene 1 (*NDRG1*) modulates the progression of kidney fibrosis in DKD. Finally, Liu et al. studied a

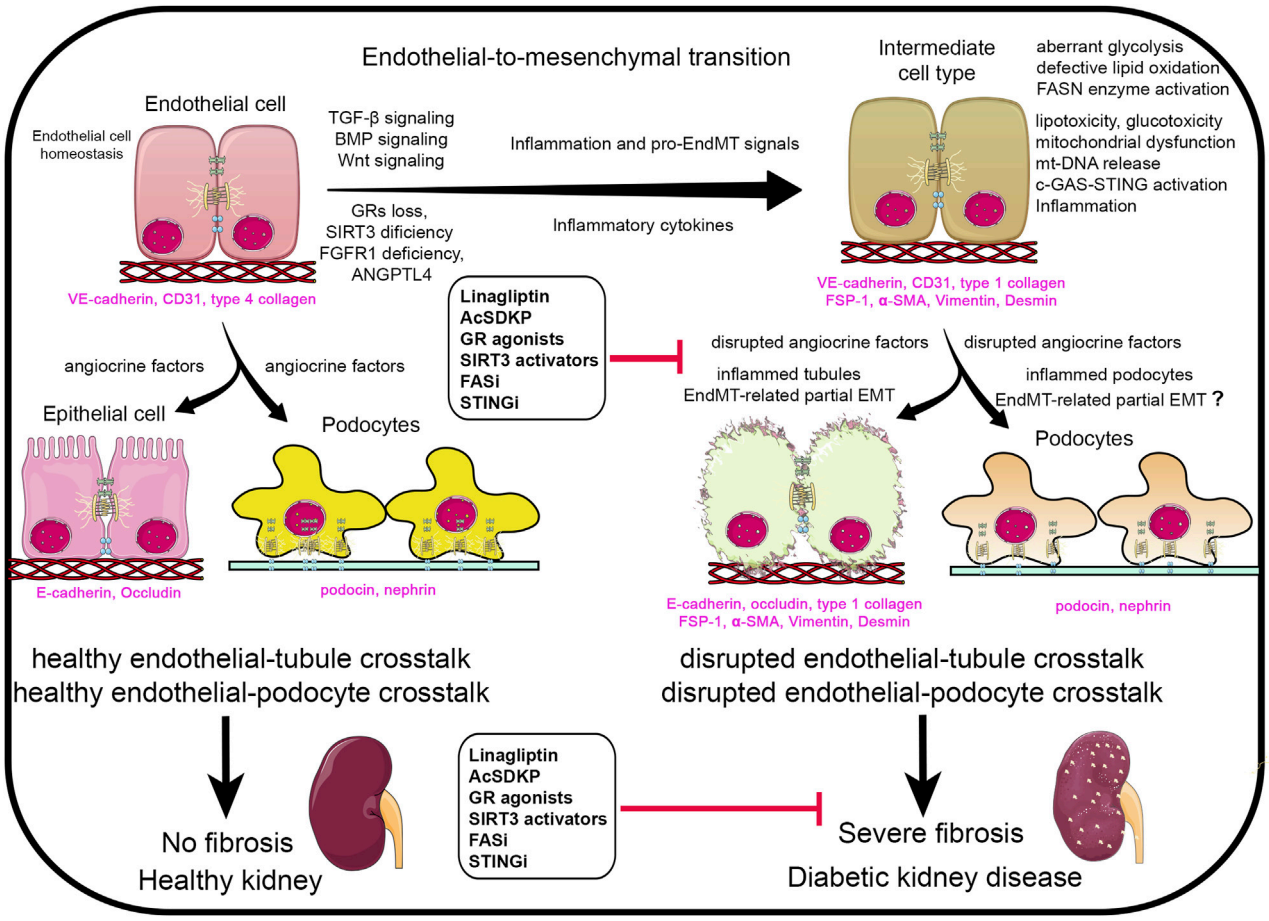


FIGURE 1
Renal angiocrine factors regulating pro-endothelial-to-mesenchymal transition (EndMT), pro-epithelial-to-mesenchymal transition (EMT) and pro-inflammatory signals. Abbreviations- TGF-β: Transforming growth factor-β.; GRs: glucocorticoid receptors.; BMP: Bone morphogenetic protein.; FGFR1: fibroblasts growth factor receptor 1.; ANGPLT4: angiopoietin like-4 protein.; AcSDKP: N-seryl-acetyl-lysyl proline.; FASi: fatty acid synthetase inhibitor.; STINGi: STING inhibitor.; mt-DNA: mitochondrial DNA.; FSP-1: fibroblasts specific protein 1.; α-SMA: α-smooth muscle actin. Components of this figure were created using Servier Medical Art templates, which are licensed under a Creative Commons Attribution 3.0 Unported License; https://smart.servier.com.

relationship between *SLC5A2* gene expression and estimated glomerular filtration rate (eGFR), wherein inhibition of *SLC5A2* was associated with higher eGFR.

New therapeutics

A significant knowledge gap exists between basic biology and drug development, which contributes to the suboptimal treatment options for DN. A deeper understanding of DKD is urgently needed to develop novel therapeutics that can effectively suppress DKD progression at early stages. The currently available therapeutic regimens can retard, but not stop the progression towards kidney failure, and are often associated with side effects and intolerance. Thus, there is a pressing need for new therapeutic strategies to improve kidney function in diabetic patients. Additionally, standard clinical practices for managing DKD progression often fail to accurately prevent kidney function decline. Despite the critical importance of early detection and management of DKD, reliable diagnostic biomarkers and effective therapeutic targets remain lacking, presenting a major challenge for both clinicians and researchers. Therefore, there is an unmet need to explore new strategies for the diagnosis and management of patients with CKD. Though several therapeutic agents have been evaluated for their efficacy in preclinical models, preventing kidney fibrosis progression remains a significant challenge. Sodium-glucose cotransporter-2 (SGLT-2) inhibitor empagliflozin has been shown to attenuate kidney fibrosis via mitigating EMT and aberrant glycolysis, enhancing lipid oxidation and improving mitochondrial function by inducing SIRT3 in the mouse model of DKD (Li et al., 2020b). Another class of drugs, DPP-4 inhibitors such as linagliptin, has been reported to reduce kidney fibrosis via targeting profibrotic DPP-4-β1 integrin-mediated pathway in tubules and endothelial cells during DKD (Shi et al., 2015; Kanasaki et al., 2014). Additionally, WNT inhibition (via LGK-974) and mineralocorticoid receptor antagonism have shown impressive results in reducing DKD progression (Srivastava et al., 2021b). The conventional therapy of DKD involves reno-protective drugs, such as angiotensin-converting enzyme inhibitors (ACEi) and angiotensin receptor blockers (ARBs). The efficacy of ACEi and

ARBs to the delay event or halt progession to kidney failure of proteinuric CKD is well established. An endogenous peptide, AcSDKP exerts significant antifibrotic effects by improving mitochondrial function, enhancing FAO, suppressing aberrant glycolysis-lactate pathway, inflammation, and partial EMT and EndMT processes in the diabetic kidney (Srivastava et al., 2020b; Nagai et al., 2014).

In this Research Topic, Li et al. demonstrated the antifibrotic effects of the phytochemical baicalin in a model of acute kidney injury (AKI). A study from Ke et al. showed that β-cryptoxanthin improved renal oxidative stress by activating Nrf2/HO-1 signaling pathway in diabetes. Muta et al. suggested that the addition of GLP-1 receptor agonists (GLP-1Ra) to patients already receiving SGLT2 inhibitors (SGLT2i) was associated with a slower annual decline in eGFR. A retrospective study by Otsuka et al. reported that transitioning from dulaglutide to tirzepatide improved glycemic control without elevating the risk of hypoglycemia in patients with type 2 diabetes undergoing hemodialysis. A study by Yang et al. suggested that SGLT2 inhibitors may be recommended as a therapeutic strategy to maximize renal and cardiovascular protection in DKD while posing a minimal risk of hyperkalemia than non-steroid mineralocorticoid receptor antagonists (ns-MRAs).

Conclusion

Our first Research Topic provided foundational insights into the design of potential therapeutic agents for patients with DKD (Bhatia and Srivastava, 2023). This Research Topic offers a comprehensive overview of emerging pathophysiologic mechanisms and therapeutic targets that can be used for diverse phenotypes of DKD. We addressed key regulatory pathways involved in modulating the progression of kidney fibrosis and discussed their potential implications for future therapeutic strategies. Additionally, we highlighted novel cell-specific biological pathways and clinical datasets that could be further investigated in larger cohorts to improve DKD management. Our insights aim to bridge the gap between mechanistic understanding and the development of targeted therapies for DKD.

Author contributions

DB: Funding acquisition, Writing – review and editing, Software, Resources, Visualization, Investigation. SS: Investigation, Formal Analysis, Supervision, Writing – review and editing, Funding acquisition, Software, Writing – original draft, Validation, Resources, Visualization, Project administration, Conceptualization.

Funding

The author(s) declare that financial support was received for the research and/or publication of this article. DB is currently supported by T32HL134629. Additional support for future work has been awarded to DB through 5U24DK137318, which will commence upon the completion of T32HL134629.

Acknowledgments

DB acknowledges support fr om the National Institutes of Health grants T32HL134629 and 5U24DK137318.

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.

References

Bhatia, D., Capili, A., and Choi, M. E. (2020). Mitochondrial dysfunction in kidney injury, inflammation, and disease: potential therapeutic approaches. *Kidney Res. Clin. Pract.* 39, 244–258. doi:10.23876/j.krcp.20.082

Bhatia, D., Capili, A., Nakahira, K., Muthukumar, T., Torres, L. K., Choi, A. M. K., et al. (2022). Conditional deletion of myeloid-specific mitofusin 2 but not mitofusin 1 promotes kidney fibrosis. *Kidney Int.* 101, 963–986. doi:10.1016/j.kint.2022.01.030

Bhatia, D., Chung, K. P., Nakahira, K., Patino, E., Rice, M. C., Torres, L. K., et al. (2019). Mitophagy-dependent macrophage reprogramming protects against kidney fibrosis. *JCI Insight* 4, e132826. doi:10.1172/jci.insight.132826

Bhatia, D., and Srivastava, S. P. (2023). Editorial: diabetic kidney disease: routes to drug development, pharmacology and underlying molecular mechanisms. *Front. Pharmacol.* 14, 1252315. doi:10.3389/fphar.2023.1252315

Choi, Y. J., Richard, G., Zhang, G., Hodgin, J. B., Demeke, D. S., Yang, Y., et al. (2024). Attenuated kidney oxidative metabolism in young adults with type 1 diabetes. *J. Clin. Invest.* 134, e183984. doi:10.1172/JCI183984

Chung, K. W., Dhillon, P., Huang, S., Sheng, X., Shrestha, R., Qiu, C., et al. (2019). Mitochondrial damage and activation of the STING pathway lead to renal inflammation and fibrosis. *Cell Metab.* 30, 784–799. doi:10.1016/j.cmet.2019.08.003

Clement, L. C., Avila-Casado, C., Macé, C., Soria, E., Bakker, W. W., Kersten, S., et al. (2011). Podocyte-secreted angiopoietin-like-4 mediates proteinuria in glucocorticoid-sensitive nephrotic syndrome. *Nat. Med.* 17, 117–122. doi:10.1038/nm.2261

Clement, L. C., Macé, C., Avila-Casado, C., Joles, J. A., Kersten, S., and Chugh, S. S. (2014). Circulating angiopoietin-like 4 links proteinuria with hypertriglyceridemia in nephrotic syndrome. *Nat. Med.* 20, 37–46. doi:10.1038/nm.3396

Cooper, M., and Warren, A. M. (2019). A promising outlook for diabetic kidney disease. *Nat. Rev. Nephrol.* 15, 68–70. doi:10.1038/s41581-018-0092-5

Darshi, M., Kugathasan, L., Maity, S., Sridhar, V. S., Fernandez, R., Limonte, C. P., et al. (2024). Glycolytic lactate in diabetic kidney disease. *JCI Insight* 9, e168825. doi:10.1172/jci.insight.168825

Kanasaki, K., Shi, S., Kanasaki, M., He, J., Nagai, T., Nakamura, Y., et al. (2014). Linagliptin-mediated DPP-4 inhibition ameliorates kidney fibrosis in streptozotocin-induced diabetic mice by inhibiting endothelial-to-mesenchymal transition in a therapeutic regimen. *Diabetes* 63, 2120–2131. doi:10.2337/db13-1029

Kang, H. M., Ahn, S. H., Choi, P., Ko, Y. A., Han, S. H., Chinga, F., et al. (2015). Defective fatty acid oxidation in renal tubular epithelial cells has a key role in kidney fibrosis development. *Nat. Med.* 21, 37–46. doi:10.1038/nm.3762

Li, H., Li, D., Ledru, N., Xuanyuan, Q., Wu, H., Asthana, A., et al. (2024). Transcriptomic, epigenomic, and spatial metabolomic cell profiling redefines regional human kidney anatomy. *Cell Metab.* 36, 1105–1125.e10. doi:10.1016/j.cmet.2024.02.015

Li, J., Liu, H., Srivastava, S. P., Hu, Q., Gao, R., Li, S., et al. (2020a). Endothelial FGFR1 (fibroblast growth factor receptor 1) deficiency contributes differential fibrogenic effects in kidney and heart of diabetic mice. *Hypertension* 76, 1935–1944. doi:10.1161/HYPERTENSIONAHA.120.15587

Li, J., Liu, H., Takagi, S., Nitta, K., Kitada, M., Srivastava, S. P., et al. (2020b). Renal protective effects of empagliflozin via inhibition of EMT and aberrant glycolysis in proximal tubules. *JCI Insight* 5, e129034. doi:10.1172/jci.insight.129034

Li, S., and Susztak, K. (2025). Mitochondrial dysfunction has a central role in diabetic kidney disease. *Nat. Rev. Nephrol.* 21, 77–78. doi:10.1038/s41581-024-00919-w

Lovisa, S., Fletcher-Sananikone, E., Sugimoto, H., Hensel, J., Lahiri, S., Hertig, A., et al. (2020). Endothelial-to-mesenchymal transition compromises vascular integrity to induce Myc-mediated metabolic reprogramming in kidney fibrosis. *Sci. Signal* 13, eaaz2597. doi:10.1126/scisignal.aaz2597

Mise, K., Long, J., Galvan, D. L., Ye, Z., Fan, G., Serysheva, I. I., et al. (2023). NDUFS4 regulates cristae remodeling in diabetic kidney disease. *Res. Sq.* 34, 39. doi:10.1681/asn.20233411s139a

Mukhi, D., Li, L., Liu, H., Doke, T., Kolligundla, L. P., Ha, E., et al. (2023). ACSS2 gene variants determine kidney disease risk by controlling *de novo* lipogenesis in kidney tubules. *J. Clin. Invest.* 134, e172963. doi:10.1172/JCI172963

Nagai, T., Kanasaki, M., Srivastava, S. P., Nakamura, Y., Ishigaki, Y., Kitada, M., et al. (2014). N-acetyl-seryl-aspartyl-lysyl-proline inhibits diabetes-associated kidney fibrosis and endothelial-mesenchymal transition. *Biomed. Res. Int.* 2014, 696475. doi:10.1155/2014/696475

Rayego-Mateos, S., Rodrigues-Diez, R. R., Fernandez-Fernandez, B., Mora-Fernández, C., Marchant, V., Donate-Correa, J., et al. (2023). Targeting inflammation to treat diabetic kidney disease: the road to 2030. *Kidney Int.* 103, 282–296. doi:10.1016/j.kint.2022.10.030

Shi, S., Srivastava, S. P., Kanasaki, M., He, J., Kitada, M., Nagai, T., et al. (2015). Interactions of DPP-4 and integrin β1 influences endothelial-to-mesenchymal transition. *Kidney Int.* 88, 479–489. doi:10.1038/ki.2015.103

Srivastava, S. P., and Goodwin, J. E. (2023). Loss of endothelial glucocorticoid receptor accelerates organ fibrosis in db/db mice. *Am. J. Physiol. Ren. Physiol.* 325, F519–F526. doi:10.1152/ajprenal.00105.2023

Srivastava, S. P., Goodwin, J. E., Kanasaki, K., and Koya, D. (2020b). Metabolic reprogramming by N-acetyl-seryl-aspartyl-lysyl-proline protects against diabetic kidney disease. *Br. J. Pharmacol.* 177, 3691–3711. doi:10.1111/bph.15087

Srivastava, S. P., Hedayat, F. A., Kanasaki, K., and Goodwin, J. E. (2019). microRNA crosstalk influences epithelial-to-mesenchymal, endothelial-to-mesenchymal, and macrophage-to-mesenchymal transitions in the kidney. *Front. Pharmacol.* 10, 904. doi:10.3389/fphar.2019.00904

Srivastava, S. P., Kanasaki, K., and Goodwin, J. E. (2020a). Loss of mitochondrial control impacts renal health. *Front. Pharmacol.* 11, 543973. doi:10.3389/fphar.2020.543973

Srivastava, S. P., Li, J., Kitada, M., Fujita, H., Yamada, Y., Goodwin, J. E., et al. (2018). SIRT3 deficiency leads to induction of abnormal glycolysis in diabetic kidney with fibrosis. *Cell Death Dis.* 9, 997. doi:10.1038/s41419-018-1057-0

Srivastava, S. P., Li, J., Takagaki, Y., Kitada, M., Goodwin, J. E., Kanasaki, K., et al. (2021a). Endothelial SIRT3 regulates myofibroblast metabolic shifts in diabetic kidneys. *iScience* 24, 102390. doi:10.1016/j.isci.2021.102390

Srivastava, S. P., Zhou, H., Setia, O., Dardik, A., Fernandez-Hernando, C., and Goodwin, J. (2021c). Podocyte glucocorticoid receptors are essential for glomerular endothelial cell homeostasis in diabetes mellitus. *J. Am. Heart Assoc.* 10, e019437. doi:10.1161/JAHA.120.019437

Srivastava, S. P., Zhou, H., Setia, O., Liu, B., Kanasaki, K., Koya, D., et al. (2021b). Loss of endothelial glucocorticoid receptor accelerates diabetic nephropathy. *Nat. Commun.* 12, 2368. doi:10.1038/s41467-021-22617-y

Srivastava, S. P., Zhou, H., Shenoi, R., Morris, M., Lainez-Mas, B., Goedeke, L., et al. (2024). Renal Angptl4 is a key fibrogenic molecule in progressive diabetic kidney disease. *Sci. Adv.* 10, eadn6068. doi:10.1126/sciadv.adn6068

frontiers | Frontiers in Pharmacology

TYPE Original Research
PUBLISHED 27 March 2024
DOI 10.3389/fphar.2024.1358573

Check for updates

OPEN ACCESS

EDITED BY
Divya Bhatia,
Weill Cornell Medical Center, NewYork-Presbyterian, United States

REVIEWED BY
Richard Pratley,
AdventHealth, United States
Oscar Lorenzo,
Health Research Institute Foundation Jimenez Diaz (IIS-FJD), Spain
Keiichiro Matoba,
Jikei University School of Medicine, Japan

*CORRESPONDENCE
Daiji Kawanami,
kawanami@fukuoka-u.ac.jp

RECEIVED 20 December 2023
ACCEPTED 11 March 2024
PUBLISHED 27 March 2024

CITATION
Muta Y, Kobayashi K, Toyoda M, Tone A, Suzuki D, Tsuriya D, Machimura H, Shimura H, Takeda H, Yokomizo H, Takeshita K, Chin K, Kanasaki K, Tamura K, Miyauchi M, Saburi M, Morita M, Yomota M, Kimura M, Hatori N, Nakajima S, Ito S, Tsukamoto S, Murata T, Matsushita T, Furuki T, Hashimoto T, Umezono T, Takashi Y and Kawanami D (2024), Influence of the combination of SGLT2 inhibitors and GLP-1 receptor agonists on eGFR decline in type 2 diabetes: post-hoc analysis of RECAP study.
Front. Pharmacol. 15:1358573.
doi: 10.3389/fphar.2024.1358573

COPYRIGHT
© 2024 Muta, Kobayashi, Toyoda, Tone, Suzuki, Tsuriya, Machimura, Shimura, Takeda, Yokomizo, Takeshita, Chin, Kanasaki, Tamura, Miyauchi, Saburi, Morita, Yomota, Kimura, Hatori, Nakajima, Ito, Tsukamoto, Murata, Matsushita, Furuki, Hashimoto, Umezono, Takashi and Kawanami. 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.

Influence of the combination of SGLT2 inhibitors and GLP-1 receptor agonists on eGFR decline in type 2 diabetes: post-hoc analysis of RECAP study

Yoshimi Muta[1], Kazuo Kobayashi[2], Masao Toyoda[3], Atsuhito Tone[4], Daisuke Suzuki[5], Daisuke Tsuriya[6], Hideo Machimura[7], Hidetoshi Shimura[8], Hiroshi Takeda[9], Hisashi Yokomizo[1], Kei Takeshita[6], Keiichi Chin[10], Keizo Kanasaki[11], Kouichi Tamura[2], Masaaki Miyauchi[12], Masuo Saburi[13], Miwa Morita[11], Miwako Yomota[11], Moritsugu Kimura[3], Nobuo Hatori[14], Shinichi Nakajima[15], Shun Ito[16], Shunichiro Tsukamoto[2], Takashi Murata[17,18], Takaya Matsushita[13], Takayuki Furuki[19], Takuya Hashimoto[6], Tomoya Umezono[20], Yuichi Takashi[1] and Daiji Kawanami[1]*

[1]Department of Endocrinology and Diabetes, Fukuoka University School of Medicine, Fukuoka, Japan, [2]Department of Medical Science and Cardiorenal Medicine, Yokohama City University Graduate School of Medicine, Kanagawa, Japan, [3]Division of Nephrology, Endocrinology and Metabolism, Department of Internal Medicine, Tokai University School of Medicine, Kanagawa, Japan, [4]Department of Internal Medicine, Diabetes Center, Okayama Saiseikai General Hospital, Okayama, Japan, [5]Suzuki Diabetes Clinic, Kanagawa, Japan, [6]Division of Endocrinology and Metabolism, 2nd Department of Internal Medicine, Hamamatsu University School of Medicine, Shizuoka, Japan, [7]Machimura Internal Medicine Clinic, Kanagawa, Japan, [8]Shimura Clinic, Kanagawa, Japan, [9]Takeda Clinic, Kanagawa, Japan, [10]Hakuai Clinic, Kanagawa, Japan, [11]Department of Internal Medicine 1, Endocrinology and Metabolism, Shimane University Faculty of Medicine, Shimane, Japan, [12]Miyauchi Diabetes Clinic, Kanagawa, Japan, [13]Department of Diabetology, Endocrinology and Metabolism, Tokyo Medical University Hachioji Medical Center, Tokyo, Japan, [14]Kobayashi Hospital, Kanagawa, Japan, [15]Sagami Junkanki Clinic, Kanagawa, Japan, [16]Department of Internal Medicine, Sagamihara Red Cross Hospital, Kanagawa, Japan, [17]Department of Clinical Nutrition, National Hospital Organization Kyoto Medical Center, Kyoto, Japan, [18]Diabetes Center, National Hospital Organization Kyoto Medical Center, Kyoto, Japan, [19]Hadano Station South Gate Clinic, Kanagawa, Japan, [20]Umezono Internal Medicine Clinic, Kanagawa, Japan

Accumulating evidence has demonstrated that both SGLT2 inhibitors (SGLT2i) and GLP-1 receptor agonists (GLP1Ra) have protective effects in patients with diabetic kidney disease. Combination therapy with SGLT2i and GLP1Ra is commonly used in patients with type 2 diabetes (T2D). We previously reported that in combination therapy of SGLT2i and GLP1Ra, the effect on the renal composite outcome did not differ according to the preceding drug. However, it remains unclear how the initiation of combination therapy is associated with the renal function depending on the preceding drug. In this *post hoc* analysis, we analyzed a total of 643 T2D patients (GLP1Ra-preceding group, n = 331; SGLT2i-preceding group, n = 312) and investigated the differences in annual eGFR decline. Multiple imputation and propensity score matching were performed to compare the annual eGFR decline. The reduction in annual eGFR decline in the SGLT2i-preceding group (pre: −3.5 ± 9.4 mL/min/1.73 m^2/year, post: −0.4 ± 6.3 mL/min/1.73 m^2/year, p < 0.001), was significantly smaller after the

initiation of GLP1Ra, whereas the GLP1Ra-preceding group tended to slow the eGFR decline but not to a statistically significant extent (pre: −2.0 ± 10.9 mL/min/1.73 m^2/year, post: −1.8 ± 5.4 mL/min/1.73 m^2/year, p = 0.83) after the initiation of SGLT2i. After the addition of GLP1Ra to SGLT2i-treated patients, slower annual eGFR decline was observed. Our data raise the possibility that the renal benefits—especially annual eGFR decline—of combination therapy with SGLT2i and GLP1Ra may be affected by the preceding drug.

KEYWORDS

sodium-glucose cotransporter 2 inhibitors, glucagon-like peptide 1 receptor agonists, renal outcome, combination therapy, preceding drug, diabetic kidney disease

1 Introduction

Diabetic kidney disease (DKD) is the leading cause of end-stage kidney disease (ESKD) worldwide (Kim and Kim, 2022). Preventing the onset and progression of DKD is important for preventing ESKD and cardiovascular disease (CVD) (Jankowski et al., 2021). DKD is characterized by albuminuria and estimated glomerular filtration rate (eGFR) decline (Norris et al., 2018). However, recent studies have shown that the prevalence of impaired kidney function with normo-albuminuria is increasing among patients with type 2 diabetes (T2D) (Kume et al., 2019), and those individuals are at increased risk for ESKD and all-cause mortality (Yamamoto et al., 2022). These data suggest that the pathophysiology of DKD is complex.

SGLT2 inhibitors (SGLT2i) and GLP-1 receptor antagonists (GLP1Ra) are widely used for the treatment of T2D and have been shown to have potent glucose-lowering and weight loss effects. Accumulating evidence has demonstrated that SGLT2i and GLP1Ra both have beneficial effects on DKD.

Approximately 40% of T2D patients develop chronic kidney disease (CKD) (Norris et al., 2018). In Japan, 9.9% of patients with type 1 diabetes (T1D) and 15.3% of patients with T2D have a reduced kidney function (eGFR <60) (Shikata et al., 2020). Therefore, it is important to establish an efficient therapeutic strategy against DKD using currently available drugs such as SGLT2i and GLP1Ra.

The International Diabetes Practice Guidelines for CKD, Kidney Disease Improving Global Outcomes (KDIGO) 2022, SGLT2i and metformin are listed as first-line drug therapies for diabetes-associated CKD, and GLP1Ra is preferred as second-line drug therapy (Rossing et al., 2022). Although the renoprotective effects of SGLT2i and GLP1Ra have been reported, the efficacy of their combination therapy has not been fully investigated. Because of side effects, excessive hypoglycemic effects, and medical costs, the initiation of both drugs at the same time is not clinically practiced, and one drug is added after the initiation of treatment with the initial drug. It has not yet been established which drug should be administered first from the viewpoint of renal protection. To clarify this point, we performed the RECAP study (the renoprotective effects of combination treatment with SGLT2i and GLP1Ra in patients with T2D according to their preceding medication). However, in the SGLT2i and GLP1Ra combination therapy, the preceding drug did not affect the renal composite outcome (Kobayashi et al., 2023).

Recently, eGFR decline was implicated as a surrogate endpoint for ESKD (Grams et al., 2019). Therefore, we performed a *post hoc* analysis of the RECAP study to examine 1) whether the combination therapy of SGLT2i and GLP1Ra is favorable for eGFR decline and 2) how the initiation of combination therapy influences eGFR decline depending on the preceding drug. Evaluations were performed before and after combination therapy with SGLT2i and GLP1Ra in T2D patients.

2 Materials and methods

2.1 Study design

The design of the RECAP study is shown in Supplementary Figure S1. T2D patients who received both SGLT2i and GLP1Ra from April 2010 to December 2021 who had received their preceding medication for at least 6 months, who had received concomitant medication for at least 12 months, and for whom clinical data were available from baseline, the time of drug addition, and the final observation were eligible for inclusion. The following data were collected: sex, age, height, body weight (BW), systolic blood pressure (SBP), diastolic blood pressure (DBP), eGFR, glycated hemoglobin A_{1c} (HbA_{1c}), urinalysis results (urine albumin-to-creatinine ratio (ACR) [mg/g Cr] or qualitative proteinuria), alanine aminotransferase (ALT) level, aspartate aminotransferase (AST) level, platelet count, and concomitant medications (including hypoglycemic drugs, antihypertensive drugs, and statins). eGFR was determined as follows: eGFR (mL/min/1.73 m^2) = 194 × $age^{-0.287}$ × serum $creatinine^{-1.094}$ × (0.739 for women) (Matsuo et al., 2009). Qualitative proteinuria values were converted to albuminuria values using the following formula: predicted ACR = exp (5.2659 + 0.2934 × log (min (PCR/50, 1)) + 1.5643 × log (max(min(PCR/500, 1), 0.1)) + 1.1109 × log (max (PCR/500, 1))-0.0773×(if female) + 0.0797×(if diabetic) + 0.1265×(if hypertensive))) (Sumida et al., 2020). Patients with any of the following conditions were excluded from the study: T1D; chronic dialysis; severe liver dysfunction (e.g., liver cirrhosis or severe infection), terminal-stage malignancy, pregnancy, treatment discontinuation, and those who opted out during the course of the study.

The details of the study participants and the dataset used in this study are shown in Supplementary Figure S2. Based on the inclusion criteria, we collected data of 688 patients from 18 medical facilities. Because of the exclusion criteria, 45 patients were excluded and 643 patients (331 patients were previously treated with SGLT2i and later treated with GLP1Ra [SGLT2i-preceding group], and

312 patients were previously treated with GLP1Ra and later treated with SGLT2i [GLP1Ra-preceding group]) were analyzed in this study. Because some data were missing from 643 patients, we performed a multiple imputation (MI) and this dataset complemented by MI was used for the main statistical analysis in this study as full analysis set (FAS). In contrast, after excluding patients with any missing data, 418 patients (227 in the SGLT2i-preceding group, and 191 in the GLP1Ra group) remained. We used this dataset without missing data, that is called complete case analysis set (CCA), to conduct a sensitivity analysis.

As shown in Supplementary Figure S1, patients were first treated with either SGLT2i or GLP1Ra, after more than 6 months, another drug was added and the combination therapy of SGLT2i and GLP1Ra was administered. Therefore, we collected data from three points; at baseline, at the time of drug addition, and at the final observation as shown in Supplementary Figure S1. The median (range) period from baseline to the time of drug addition (monotherapy period) was 23 months (6–114 months). The median (range) period from drug addition to the final observation (combination therapy time) was 31 months (12–85 months). The median (range) period from baseline to the final observation (total observation period) was 59 months (19–134 months) in the total study participants.

The present study was approved by the Institutional Review Board for Clinical Research of Tokai University, Japan on 6 December 2021.

2.2 Office BP measurement

The BP was measured as previously described (Kobayashi et al., 2019). Office BP measurements were performed at each institution using their validated cuff oscillometric devices. According to the JSH 2014 guidelines (Shimamoto et al., 2014), office BP was measured in a quiet environment after resting for a few minutes in a seated position on a chair with legs uncrossed. When two consecutive measurements were taken 1–2 min apart, the average value was defined as the office BP.

2.3 Outcomes

The annual eGFR decline (annual ΔeGFR) during monotherapy (SGLT2i or GLP1Ra) and after combination therapy was evaluated in the SGLT2i-preceding and GLP1Ra-preceding groups. We also evaluated the change in the logarithmic value of ACR (LnACR) in this *post hoc* analysis.

2.4 Statistical method

2.4.1 Missing value analysis

MI was performed to account for missing values (Rubin, 1987). MI is used to replace missing values with other plausible values by creating multiple filling-in patterns to avoid bias caused by missing data. This is recognized as an alternative approach to the analysis of incomplete data (Rubin and Schenker, 1991). In the RECAP study, each missing value was replaced with a set of substituted plausible values by creating 100 complete datasets using MI with the chained equations method (Hershberger and Fisher, 2003; Enders, 2010; Aloisio et al., 2014). For imputation, 100 complete datasets were created using the following covariates: age, sex, height, BW, SBP, DBP, HbA_{1c}, eGFR, LnACR, types of SGLT2i and GLP1Ra, use of concomitant medications (hypoglycemic drugs, antihypertensive drugs, and statins, and period of treatment with either or both SGLT2i or GLP1Ra). The clinical data at baseline, at the time of drug addition, and at the final observation that were associated with the outcome were used for MI (Supplementary Figure S2) (Furukawa et al., 2017).

2.4.2 Statistical analysis for the description of data and comparison

Normally distributed data are presented as the mean ± standard deviation (SD). Data that showed a skewed distribution were reported as the median [25th percentile, 75th percentile]. For parametric variables, comparisons of clinical characteristics between the two groups were performed using an unpaired *t*-test, The chi-square test was used for nonparametric variables and for categorical data, while a general linear mixed model (GLMM) with Bonferroni correction was used to compare the clinical findings between the three points (at baseline, at the time of drug addition, and at the final observation) as described previously.

Adjusted eGFR decline was calculated by a multiple linear regression analysis using eGFR at baseline, sex, age, BW, BMI, SBP, DBP, HbA1c, and treatment periods in the CCA set.

Multiple regression analysis was performed to identify the independent factors related to change in eGFR decline before and after the combination treatment of SGLT2i and GLP1Ra. A stepwise method was performed using covariates as follows; sex, the duration of T2D, preceding drug, periods of the combination treatment, age, BMI, HbA1c, MAP, eGFR, LnACR at the time of drug addition, changes in BMI, HbA1c, MAP, and LnACR from at the time of drug addition to at the final observation. All statistical analyses were performed using IBM SPSS Statistics 28.0 (IBM Inc., Armonk, NY, USA). Statistical significance was set at $p < 0.05$.

2.4.3 Propensity score matching

Differences were observed in the baseline characteristics of the SGLT2i-preceding and GLP1Ra-preceding groups. Adjustments were needed for comparison between the groups. Propensity score (PS) matching was conducted to compare the annual eGFR decline and change in albuminuria.

In each dataset built by MI, the PS for the SGLT2i-preceding group was calculated by a logistic analysis that included the following covariates: sex, age, height, BW, body mass index (BMI), SBP, DBP, HbA_{1c}, eGFR, LnACR at baseline, history of T2D, concomitant medications at baseline (hypoglycemic drugs, antihypertensive drugs, and statins), and treatment periods for monotherapy and combination therapy. Because individual PSs were calculated using datasets built by MI, the average PS was used as the representative value. PS matching was performed using representative PS values with the following algorithm: 1:1 nearest neighbor match (caliper value = 0.047, calculated as 0.2 × the SD of PS (Austin, 2011)) with no replacement. In the PS-matching model, the paired *t*-test was used for parametric variables, and the McNemar test was used for categorical data.

TABLE 1 Clinical characteristics at baseline.

	Unadjusted model			PS-matching model		
	GLP1Ra-preceding group, *n* = 331	SGLT2i-preceding group, *n* = 312	*p*-value	GLP1Ra-preceding group, *n* = 203	SGLT2i-preceding group, *n* = 203	Standardized difference
Age (years)	55.7 ± 13.5	56.5 ± 12.7	0.10	57.1 ± 13.6	57.0 ± 13.2	0.007
Sex (female [%])	152 (46%)	130 (42%)	0.27*	89 (44%)	87 (43%)	0.02
History of type 2 diabetes >10 years (%)	281 (85%)	237 (76%)	0.006*	165 (81%)	159 (78%)	0.07
BW (kg)	79.5 ± 20.1	79.4 ± 18.1	0.95	78.7 ± 18.5	78.8 ± 17.0	0.006
BMI	29.8 ± 6.3	29.5 ± 5.6	0.51	29.4 ± 5.5	29.2 ± 5.3	0.04
SBP (mmHg)	132.0 ± 18.4	135.4 ± 18.9	0.02	133.1 ± 19.1	134.7 ± 19.4	0.08
DBP (mmHg)	76.6 ± 12.3	78.7 ± 13.6	0.04	76.7 ± 12.4	78.2 ± 13.5	0.12
HbA_{1c} (mmol/mol [%])	73.6 ± 18.6 (8.9 ± 1.7)	71.0 ± 17.3 (8.6 ± 1.6)	0.07	72.8 ± 17.8 (8.7 ± 11.6)	71.9 ± 18.2 (8.7 ± 1.7)	0.05
eGFR (mL/min/1.73 m^2)	78.8 ± 28.7	78.2 ± 26.0	0.79	76.6 ± 26.7	77.7 ± 26.9	0.04
LnACR	3.75 ± 1.91	3.76 ± 1.97	0.91	3.72 ± 1.90	3.77 ± 1.95	0.003
Periods of the monotherapy (month)	31.8 ± 23.1	23.9 ± 14.0	<0.001	25.1 ± 18.3	24.7 ± 14.5	0.03
Periods of the combination therapy (month)	38.8 ± 18.6	28.5 ± 13.5	<0.001	31.6 ± 15.0	31.9 ± 14.0	0.02
Total periods of the study (month)	70.6 ± 27.0	52.4 ± 15.7	<0.001	56.7 ± 19.4	56.6 ± 14.7	0.006
Concomitant medications						
Sulphonylurea	108 (33%)	91 (29%)	0.34*	58 (29%)	64 (32%)	0.06
Metformin	169 (51%)	190 (61%)	0.01*	115 (57%)	114 (56%)	0.01
Insulin	141 (43%)	140 (45%)	0.56*	95 (47%)	90 (44%)	0.05
Pioglitazone	35 (11%)	51 (16%)	0.03*	29 (14%)	29 (14%)	0
αGI	40 (12%)	48 (15%)	0.22*	30 (15%)	29 (14%)	0.01
Glinide	14 (4.2%)	14 (4.5%)	0.87*	11 (5%)	11 (5%)	0
RAS inhibitor	166 (50%)	160 (51%)	0.77*	108 (53%)	96 (47%)	0.12
CCB	128 (39%)	110 (35%)	0.37*	83 (41%)	83 (41%)	0
b blocker	53 (16%)	49 (16%)	0.92*	33 (16%)	33 (16%)	0
MRB	14 (4%)	12 (%)	0.81*	10 (5%)	9 (4%)	0.02
Diuretics	53 (16%)	30 (10%)	0.10*	23 (11%)	25 (12%)	0.03
Statin	160 (48%)	160 (51%)	0.46*	109 (54%)	98 (45%)	0.11

The left panel shows the unadjusted model of the full analysis set (FAS) with multiple imputation (MI). The right panel shows the PS-matching model. Values represent the mean±SD or n/total n (%). *p*-values were determined by an unpaired t test or *chi-square test. Abbreviations: αGI, alpha glucosidase inhibitor; BMI, body mass index; BW, body weight; DBP, diastolic blood pressure; CCB, calcium channel blocker; eGFR, estimated glomerular filtration; FAS, full analysis set; GLP1Ra, glucagon-like peptide 1 receptor agonists; HbA_{1c}, glycated hemoglobin A_{1c}; LnACR, logarithmic value of urine albumin-to-creatinine ratio; MAP, mean arterial pressure; MI, multiple imputation; MRB, mineral corticoid receptor blocker; PS, propensity score; RAS, renin-angiotensin system inhibitor; SBP, systolic blood pressure; SGLT2i, sodium-glucose cotransporter inhibitors.

3 Results

3.1 Baseline characteristics

The clinical characteristics at baseline of FAS with MI ($n = 643$) before and after PS matching in the RECAP study are shown in Table 1. Before PS matching, the SGLT2i-preceding and GLP1Ra-preceding groups showed significant differences in the history of T2D >10 years (76% vs. 85%, $p = 0.006$), SBP (mmHg) (135.4 ± 18.9 vs. 132.0 ± 18.4, $p = 0.02$), DBP (mmHg) (78.7 ± 13.6 vs. 76.6 ± 12.3, $p = 0.04$), period of monotherapy (month) (23.9 ± 14.0 vs. 31.8 ± 23.1, $p < 0.001$), period of combination therapy (month)

TABLE 2 The renal function and eGFR decline during the study periods on unadjusted model (FAS with MI).

A) Changes in eGFR (mL/min/1.73m^2)												
	FAS						CCA					
	At baseline	At the time of drug addition	At the final observation	*p*-value*			At baseline	At the time of drug addition	At the final observation	*p*-value*		
				Baseline vs. addition	Addition vs. final observation	Baseline vs. final observation				Baseline vs. addition	Addition vs. final observation	Baseline vs. final observation
Total (n = 643)	78.5 ± 27.4	74.0 ± 26.5	70.8 ± 26.8	<0.001	<0.001	<0.001	78.8 ± 28.6	74.0 ± 27.6	70.1 ± 27.8	<0.001	<0.001	<0.001
SGLT2i-preceding group (n = 331 in FAS n = 277 in CCA)	78.2 ± 26.0	73.2 ± 26.0	71.4 ± 26.1	<0.001	0.06	<0.001	79.1 ± 26.0	74.0 ± 26.4	72.2 ± 26.6	<0.001	0.22	<0.001
GLP1Ra-preceding group (n = 312 in FAS n = 191 in CCA)	78.8 ± 28.7	74.7 ± 27.0	70.1 ± 27.5	<0.001	0.06	<0.001	78.5 ± 30.6	73.9 ± 28.7	68.3 ± 28.9	<0.001	<0.001	<0.001

B) Annual eGFR decline (mL/min/1.73 m^2/year)							
		FAS			CCA		
		From at baseline to at the time of drug addition	From the time of drug addition to the final observation	*p*-value*	From at baseline to at the time of drug addition	From the time of drug addition to the final observation	*p*-value*
Total (n = 643)	Treatment periods (months)	28.0 ± 19.6	33.8 ± 17.1		28.3 ± 20.3	34.3 ± 17.3	
	eGFR decline (mL/min/1.73 m^2/year)	−2.6 ± 9.9	−1.2 ± 6.0	0.005	−2.5 ± 10.1	−1.4 ± 6.2	<0.001
	Adjusted eGFR decline (mL/min/1.73 m^2/year)*				−2.1 ± 2.7	−1.6 ± 2.4	0.002

(Continued on following page)

10.3389/fphar.2024.1358573

TABLE 2 (*Continued*) The renal function and eGFR decline during the study periods on unadjusted model (FAS with MI).

B) Annual eGFR decline (mL/min/1.73 m^2/year)							
		FAS			CCA		
		From at baseline to at the time of drug addition	From the time of drug addition to the final observation	*p*-value*	From at baseline to at the time of drug addition	From the time of drug addition to the final observation	*p*-value*
SGLT2i-preceding group (*n* = 331 in FAS *n* = 277 in CCA)	Treatment periods (months)	23.9 ± 14.0	28.5 ± 13.5		24.0 ± 14.6	28.7 ± 13.8	
	eGFR decline (mL/min/1.73 m^2/year)	−3.4 ± 9.5	−0.9 ± 6.5	<0.001	−3.3 ± 9.0	−0.8 ± 6.8	0.004
	Adjusted eGFR decline (mL/min/1.73 m^2/year)*				−2.3 ± 2.5	−1.7 ± 2.3	0.01
GLP1Ra-preceding group (*n* = 312 in FAS *n* = 191 in CCA)	Treatment periods (months)	31.8 ± 23.1	38.9 ± 23.1		31.9 ± 23.5	39.1 ± 18.6	
	eGFR decline (mL/min/1.73 m^2/year)	−1.9 ± 10.3	−1.6 ± 5.3	0.69	−1.8 ± 11.0	−2.0 ± 5.7	0.78
	Adjusted eGFR decline (mL/min/1.73 m^2/year)*				−2.0 ± 2.9	−1.5 ± 2.4	0.06

Changes in eGFR (A) and Annual eGFR decline (B) are shown respectively. In complete case analysis (CCA) set, values of adjusted eGFR decline were shown. Adjusted eGFR decline = 14.48 – eGFR at baseline × 0.087 + 1.504 × (if female) – 0.069 × Age + 0.052 × BW - 0.097 × BMI - 0.018 × SBP - 0.032 × DBP - 0.048 × HbA1c (mol/l) + Treatment periods(months) × 0.022. Values are mean±SD *p-values were analyzed by paired t test among two groups or by the general linear mixed model with Bonferroni correction among three groups.

10.3389/fphar.2024.1358573

(28.5 ± 13.5 vs. 38.8 ± 18.6, $p < 0.001$), total study period (month) (52.4 ± 15.7 vs. 70.6 ± 27.0, $p < 0.001$), use of metformin (61% vs. 51%, $p = 0.01$) and pioglitazone (16% vs. 11%, $p = 0.03$) at baseline. In the PS-matching model, the range of standardized differences in covariates was 0.0–0.12. Therefore, the PS-matching model was considered to be well-balanced between the groups.

3.2 Annual eGFR decline on FAS

Table 2A shows that, in the analysis of 643 patients, the eGFR was significantly lower by passage of time, with values (mL/min/1.73 m^2) of 78.5 ± 27.4 at baseline, 74.0 ± 26.5 at the time of drug addition, and 70.8 ± 26.8 at the final observation ($p < 0.001$ between each period). Similar results of eGFR decline were observed in CCA set analysis. Table 2B shows the annual eGFR decline (mL/min/1.73 m^2/year) was −2.6 ± 9.9 in monotherapy with a period of 28.0 ± 19.6 months and −1.2 ± 6.0 in combination therapy with a period of 33.8 ± 17.1 months. The difference was statistically significant ($p = 0.005$). Table 2 also shows the changes in eGFR and annual eGFR decline depending on the preceding drug in the unadjusted model. A significant difference in annual eGFR decline was observed in patients in the SGLT2i-preceding group before and after the initiation of combination therapy ($p < 0.001$).

3.3 Adjusted eGFR decline on CCA

The calculated formula is as follows; adjusted eGFR decline = 14.48 – eGFR at baseline × 0.087 + 1.504 × (if female) – 0.069 × Age + 0.052 × BW - 0.097 × BMI - 0.018 × SBP - 0.032 × DBP - 0.048 × HbA1c (mol/L) + Treatment periods(months) × 0.022.

Although this adjustment could not eliminate all the effects of confounding factors, annual eGFR decline (mL/min/1.73 m^2/year) significantly changed from −2.1 ± 2.7 to −1.6 ± 2.4 ($p = 0.002$) among all patients. In the analysis depending on the preceding drugs, annual eGFR decline (mL/min/1.73 m^2/year) significantly changed from −2.3 ± 2.5 to −1.7 ± 2.3 in SGLT2i-preceding group ($p = 0.001$), whereas that changed from −2.0 ± 2.9 to −1.5 ± 2.4 in GLP1Ra-preceding group ($p = 0.06$) (Table 2B).

Multiple regression analysis identified that 1) eGFR at the time of drug addition, 2) change in HbA1c from at the time of drug addition to the final observation, and 3) the preceding of SGLT2i were independent and significant determinants of eGFR decline. These regression coefficient values were −0.10 (95% CI, −0.14, −0.05, $p < 0.001$), 0.08 (95% CI, 0.01, 0.16, $p = 0.02$), and 2.68 (95% CI, 0.27, 5.01, $p = 0.03$), respectively.

3.4 Results of PS-matching model

Using the PS matching method, a matching model of 203 patients in each group was constructed (Supplementary Figure S2).

3.4.1 eGFR decline on PS-matching model

For the evaluation of the eGFR decline before and after combination therapy, we analyzed the eGFR decline separately for the SGLT2i-preceding and GLP1Ra-preceding groups in the PS-matching model. In the SGLT2i-preceding group, the annual eGFR decline (mL/min/1.73 m^2/year) was −3.5 ± 9.4 and −0.4 ± 6.3 in the monotherapy and combination therapy periods, respectively, ($p < 0.001$). On the other hand, in the GLP1Ra-preceding group, the annual eGFR decline (ml/min/1.73 m^2/year) was −2.0 ± 10.9 and −1.8 ± 5.4 in the monotherapy and combination therapy periods, respectively. They did not differ to a statistically significant extent ($p = 0.83$) (Figure 1).

3.4.2 LnACR on PS-matching model

The LnACR values at baseline, at the time drug addition, and at the final observation were 3.77 ± 1.95, 3.79 ± 1.99, and 3.94 ± 2.00, respectively, in the SGLT2i-preceding group. Those values were 3.72 ± 1.90, 3.85 ± 1.95, and 3.78 ± 1.78, respectively, in the GLP1Ra-preceding group. None of the groups showed a significant difference in LnACR at any of the three time points.

3.4.3 Changes in other clinical findings on PS-matching model

In comparison to baseline, lower HbA_{1c}, BW, SBP and DBP were observed after combination therapy with SGLT2i and GLP1Ra. Table 3 shows the changes in clinical characteristics before and after combination therapy in the PS-matching model. In the monotherapy period, the HbA_{1c} value did not differ between the groups; however, lower HbA1c levels were observed after combination therapy in both the SGLT2i-preceding and GLP1Ra-preceding groups (both $p < 0.001$). A lighter BW was observed in the monotherapy period ($p = 0.03$ in the SGLT2i-preceding group and $p = 0.007$ in the GLP1Ra-preceding group), and further lighter BW was observed in both groups in the combination therapy period (both $p < 0.001$) (Table 3). There was no difference in SBP or DBP. However, in the GLP1Ra-preceding group, a further decrease in SBP was observed ($p = 0.03$).

4 Discussion

We performed this *post hoc* study to analyze the relationship between the use of SGLT2i and GLP1Ra combination therapy and renal function in T2D patients. We observed that the annual eGFR decline in the monotherapy period of SGLT2i or GLP1Ra (−2.6 ± 9.9 mL/min/1.73 m^2/year) was significantly lower after combination therapy (−1.2 ± 6.0 mL/min/1.73 m^2/year) ($p = 0.005$). In the PS matching model that adjusted the baseline characteristics of the two groups, in the SGLT2i-preceding group, the eGFR decline was significantly lower after the addition of GLP1Ras in comparison to SGLT2i alone (−3.5 ± 9.4 mL/min/1.73 m^2/year to −0.4 ± 6.3 mL/min/1.73 m^2/year, $p < 0.001$). On the other hand, the annual eGFR decline was not significantly changed by the addition of SGLT2i to the GLP1Ra-preceding group (−2.0 ± 10.9 mL/min/1.73 m^2/year to −1.8 ± 5.4 mL/min/1.73 m^2/year, $p = 0.83$).

In our previous study, the decrease in eGFR was slower in patients treated with SGLT2i than in those treated with GLP1Ra during monotherapy (Kobayashi et al., 2022). We hypothesize that this occurred because SGLT2i was administered for a shorter period (24.7 ± 14.5 months) in the present study, and the effect of the initial dip was greater. The mechanisms underlying this discrepancy

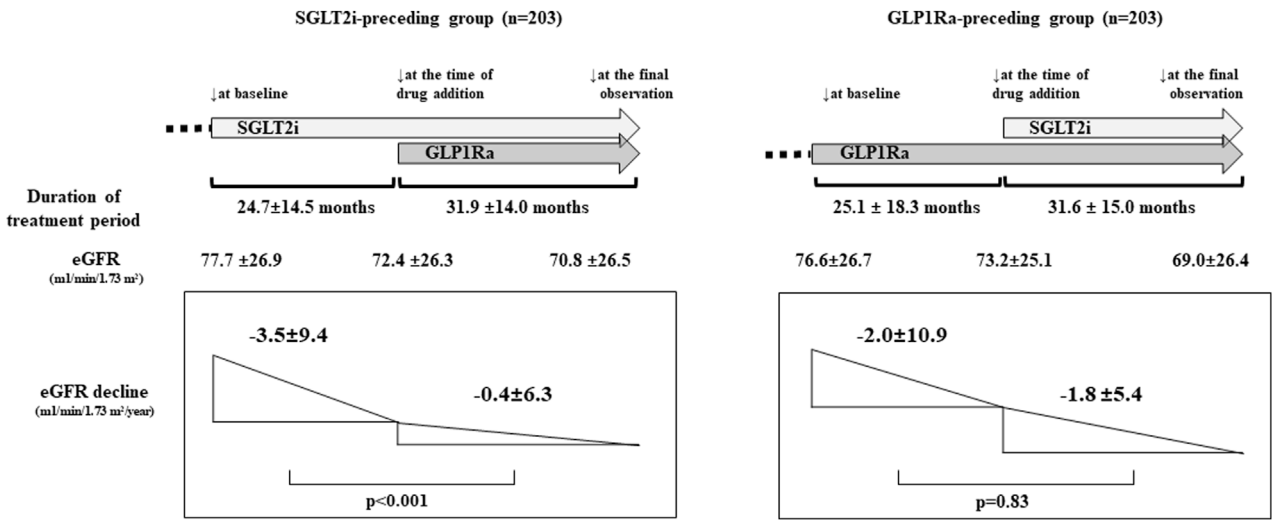


FIGURE 1
The renal function and estimated glomerular filtration rate (eGFR) decline before and after sodium-glucose cotransporter inhibitors (SGLT2i) and GLP-1 receptor agonists (GLP1Ra) combination therapy (propensity score [PS]-matching model). The eGFR at baseline, at the time of addition of drug, and the final observation were shown. ($p < 0.001$; at baseline vs. at the time of drug addition, and at baseline vs. at the final observation. $p = 0.33$; at the time of drug addition vs. the final observation in the SGLT2i-preceding group. $p < 0.001$ between each point in the GLP1Ra-preceding group. p-values were analyzed by general linear mixed model). The administration of SGLT2i prior to GLP1Ra significantly reduced the annual eGFR decline. p-values were analyzed by a paired t-test.

remain unclear, but the lack of a significant difference in the annual eGFR decline in the GLP1Ra-preceding group may be due to the initial dip that occurs in the early phase of SGLT2i treatment. However, with an observation period of approximately 2 years, this effect was considered to have disappeared.

Another possible mechanism may be the difference in the mean BP at the time of drug treatment. It is well known that BP control influences the renal function decline (Soejima et al., 2022). We previously reported a lower BP in SGLT2i than in GLP1Ra (Kobayashi et al., 2019). Consistent with this observation, both SBP and DBP were significantly lower during the SGLT2i monotherapy period in the SGLT2i-preceding group. In contrast, GLP1Ra monotherapy showed no change in BP in the GLP1Ra-preceding group. The lower BP during SGLT2i monotherapy may have contributed to the subsequent reduction in annual eGFR decline with combination therapy. These findings suggest that reducing BP before the initiation of combination therapy may be related to the efficient induction of renoprotection.

In this study, no significant changes were observed in the ACR between the two groups. Considering these results, the degree of ACR was relatively low; therefore, it was difficult to determine the effect of combination therapy.

A series of large-scale randomized controlled trials (RCTs) demonstrated that SGLT2i use reduced the risk of kidney disease progression in patients with and without diabetes (Nuffield Department of Population Health Renal Studies and Consortium, 2022). The beneficial effects of SGLT2i on albuminuria and eGFR decline in DKD have been demonstrated by EMPA-REG OUTCOME (Wanner et al., 2016), CANVAS (Perkovic et al., 2018), DECLARE-TIMI58 (Mosenzon et al., 2019), and CREDENCE (Perkovic et al., 2019). These effects were consistent in DAPA-CKD (Heerspink et al., 2020; Heerspink et al., 2021) and EMPA-KIDNEY (The EMPA-KIDNEY Collaborative Group et al., 2023), which included CKD patients with and without diabetes. Recently, a *post hoc* analysis of SUSTAIN6 and PIONEER6 demonstrated that semaglutide slowed the eGFR decline in T2D patients with a high risk of CVD (Tuttle et al., 2023), suggesting that GLP1Ra has potentially favorable effects on the renal function.

With combination therapy of SGLT2i and GLP1Ra, the preceding drug did not affect the renal outcome in the main analysis of RECAP study (Kobayashi et al., 2023). In this *post hoc* analysis, the SGLT2i-preceding group had a slower rate of eGFR decline in comparison to the GLP1Ra-preceding group, and multiple regression analysis identified the preceding of SGLT2i as an independent and significant factor of change in eGFR decline. Interestingly, we also demonstrated a combination of both drugs had additive renoprotection. Furthermore, the addition of GLP1Ra to SGLT2i showed an advantage in the decrease in BW. These findings suggest that there is no problem in starting SGLT2i first with the expectation of renoprotection, however, if a stronger protective effect is desired, a combination of GLP1Ra is preferable. In addition, GLP1Ra may be used first in patients with obesity who should prioritize BW reduction. Recent studies demonstrated that SGLT2i had cardioprotective effects in HFpEF (heart failure with preserved ejection fraction) and HFrEF (heart failure with reduced ejection fraction) with or without diabetes (Solomon et al., 2022; Cortes et al., 2023). On the other hand, GLP1Ra can be prescribed only for patients with T2D. Therefore, it remains unclear whether similar results can be observed in patients without T2D.

5 Limitations

The present study was associated with several limitations. First, the data were analyzed as a *post hoc* analysis from a retrospective study and

TABLE 3 Clinical characteristics before and after combination therapy with SGLT2i and GLP1Ra depending on the preceding drug in the PS-matching model.

	The SGLT2i-preceding group (*n* = 203)						The GLP1R-preceding group (*n* = 203)					
	At baseline	At the time of drug addition	At the final observation	*p*-value*			At baseline	At the time of drug addition	At the final observation	*p*-value*		
				Baseline vs. addition	Addition vs. final observation	Baseline vs. final observation				Baseline vs. addition	Addition vs. final observation	Baseline vs. final observation
HbA_{1c} (mol/mol [%])	71.9 ± 18.2 (8.7 ± 1.7)	69.3 ± 17.0 (8.5 ± 1.6)	62.4 ± 15.0 (7.9 ± 1.4)	0.21	<0.001	<0.001	72.8 ± 17.8 (8.8 ± 1.6)	70.9 ± 16.2 (8.6 ± 1.5)	92.9 ± 15.2 (7.9 ± 1.4)	0.46	<0.001	<0.001
BW (kg)	78.8 ± 17.0	77.8 ± 16.9	75.5 ± 17.2	0.03	<0.001	<0.001	78.7 ± 18.5	77.3 ± 18.4	73.6 ± 18.3	0.007	<0.001	<0.001
SBP (mmHg)	134.7 ± 19.4	130.4 ± 18.6	128.9 ± 17.4	0.003	1.0	<0.001	133.1 ± 19.1	132.5 ± 17.7	129.3 ± 16.1	1.0	0.03	0.01
DBP (mmHg)	78. ± 13.5	75.8 ± 12.4	74.6 ± 12.5	0.01	0.50	<0.001	76.7 ± 12.4	75.8 ± 11.8	74.6 ± 12.3	0.61	0.63	0.03

Treatment periods (months); The SGLT2i-preceding group; from baseline to the time of addition of drug (24.7±14.5), from the time of drug addition to the final observation (31.9±14.0). The GLP1Ra-preceding group; from baseline to at the time of drug addition (25.1±18.3), from the time of drug addition to the final observation (31.6±15.0). Values present the mean±SD. *p-values were analyzed between two groups by a paired t test, among three groups by a general linear mixed model with Bonferroni correction. Abbreviations: BW, body weight; SBP, systolic blood pressure; DBP, diastolic blood pressure.

10.3389/fphar.2024.1358573

may have been insufficient to evaluate eGFR decline. Second, the relatively low numbers of participants may have resulted in insufficient power to detect differences in the effect of treatment on eGFR decline. Third, it remains unclear whether our results can be generalized to T2D patients. The analysis target was patients who required combination therapy with SGLT2i and GLP1Ra for the treatment of T2D. Combination therapy has been used for glycemic management and/or BW reduction but not for renoprotection. Indeed, obese patients were enrolled (mean BMI: 29.2 ± 5.3 kg/m^2 in the SGLT2i-preceding group and 29.4 ± 5.5 kg/m^2 in the GLP1Ra-preceding group) in this study, and the influence of combination therapy on obesity-related glomerulopathy was not evaluated sufficiently. Fourth, we could not exclude the possibility that clinicians selected SGLT2i first because of renoprotective properties of SGLT2i. Supplementary Figure S3 shows the distribution of the years at the initiation of treatment depending on the drug. Approximately one-third of patients in GLP1Ra preceding group started GLP1Ra before 2014 when SGLT2i were not commercially available in Japan. In contrast, some patients in the SGLT2i-preceding group may have started SGLT2i based on expectation of renoprotective effects. It is possible that patients at high risk for renal outcomes started SGLT2i first, which may have influenced the eGFR decline in this study. Fifth, the population included in this study was only Japanese. Therefore, the generalizability of these results to other races requires further validation. Finally, the PS-matching could balance known confounding variables as much as possible, including gender, duration of diabetes, HbA1c, BMI and BP to eliminate baseline differences between groups, but it could not ensure that all measured baseline characteristics were matched and consider the influence of unknown variables.

6 Conclusion

In conclusion, this retrospective study demonstrated that SGLT2i and GLP1Ra combination therapy could be beneficial in reducing the annual eGFR decline in patients with T2D. Among the combination therapies, the SGLT2i-preceding group showed a significant reduction in annual eGFR decline, while the GLP1Ra-preceding group did not.

Further studies are required to clarify the additive effect of SGLT2i and GLP1Ra combination therapy on renal protection. In particular, the effects of preceding drugs on the rate of annual eGFR decline should be investigated in a prospective RCT.

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 Institutional Review Board for Clinical Research of Tokai 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 because Since the research targets patients who have received treatment in the past, it is difficult to obtain consent from individual subjects of the research. Therefore, we publicized information on "the fact that the research is being conducted, its contents, methods, the opportunity to refuse participation in the research, and contact information" by publicly announcing the information disclosure document. The information disclosure document was posted on the website of the Institutional Review Board for Clinical Research of Tokai University and in the outpatient of the Division of Nephrology, Endocrinology and Metabolism, Department of Internal Medicine, Tokai University School of Medicine.

Author contributions

YM: Writing–original draft, Writing–review and editing.
KaK: Writing–original draft, Writing–review and editing.
MT: Writing–original draft, Writing–review and editing.
AT: Writing–original draft, Writing–review and editing.
DS: Writing–original draft, Writing–review and editing.
DT: Writing–original draft, Writing–review and editing.
HM: Writing–original draft, Writing–review and editing.
HS: Writing–original draft, Writing–review and editing.
HT: Writing–original draft, Writing–review and editing.
HY: Writing–original draft, Writing–review and editing.
KeT: Writing–original draft, Writing–review and editing.
KC: Writing–original draft, Writing–review and editing.
KeK: Writing–original draft, Writing–review and editing.
KoT: Writing–original draft, Writing–review and editing.
MaM: Writing–original draft, Writing–review and editing.
MS: Writing–original draft, Writing–review and editing.
MiM: Writing–original draft, Writing–review and editing.
MY: Writing–original draft, Writing–review and editing.
MK: Writing–original draft, Writing–review and editing.
NH: Writing–original draft, Writing–review and editing.
SN: Writing–original draft, Writing–review and editing.
SI: Writing–original draft, Writing–review and editing.
ST: Writing–original draft, Writing–review and editing.
TMu: Writing–original draft, Writing–review and editing.
TMa: Writing–original draft, Writing–review and editing.
TF: Writing–original draft, Writing–review and editing.
TH: Writing–original draft, Writing–review and editing.
TU: Writing–original draft, Writing–review and editing.
YT: Writing–original draft, Writing–review and editing.
DK: Writing–original draft, Writing–review and editing.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Acknowledgments

We are grateful to all participants and acknowledge the support of the members of the RECAP study who contributed considerably to data collection.

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.

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.2024.1358573/full#supplementary-material

SUPPLEMENTARY FIGURE S1
Patients with T2D who were treated with both SGLT2i and GLP1Ra from April 2010 to December 2021. Patients who had been on monotherapy for at least 6 months and on combination therapy for at least 12 months were registered in the study.

SUPPLEMENTARY FIGURE S2
A total of 688 patients were registered, and 45 patients were excluded. The data of 643 patients (SGLT2i-preceding group, *n* = 312; GLP1Ra-preceding group, *n* = 331) were analyzed as the FAS. The multiple imputation method was applied to FAS data. Propensity score matching was conducted to compare the annual ΔeGFR and LnACR.

SUPPLEMENTARY FIGURE S3
Distribution of years at the initiation of treatment for each type of drug. Approximately one-third of patients in GLP1Ra preceding group started GLP1Ra before 2014 when SGLT2i were not commercially available in Japan. The vertical axis shows years and the horizontal axis shows numbers of patients.

References

Aloisio, K. M., Swanson, S. A., Micali, N., Field, A., and Horton, N. J. (2014). Analysis of partially observed clustered data using generalized estimating equations and multiple imputation. *Stata J.* 14, 863–883. doi:10.1177/1536867x1401400410

Austin, P. C. (2011). Optimal caliper widths for propensity-score matching when estimating differences in means and differences in proportions in observational studies. *Pharm. Stat.* 10, 150–161. doi:10.1002/pst.433

Cortes, M., Lorenzo, O., Lumpuy-Castillo, J., Martinez-Albaladejo, S., Taibo-Urquia, M., Pello, A. M., et al. (2023). Dapagliflozin improved cardiac function and structure in diabetic patients with preserved ejection fraction: results of a single centre, observational prospective study. *J. Clin. Med.* 12, 6698. doi:10.3390/jcm12206698

Enders, C. K. (2010). *Applied missing data analysis*. New York: Applied missing data.

Furukawa, K., Preston, D. L., Misumi, M., and Cullings, H. M. (2017). Handling incomplete smoking history data in survival analysis. *Stat. Methods Med. Res.* 26, 707–723. doi:10.1177/0962280214556794

Grams, M. E., Sang, Y., Ballew, S. H., Matsushita, K., Astor, B. C., Carrero, J. J., et al. (2019). Evaluating glomerular filtration rate slope as a surrogate end point for ESKD in clinical trials: an individual participant meta-analysis of observational data. *J. Am. Soc. Nephrol.* 30, 1746–1755. doi:10.1681/ASN.2019010008

Heerspink, H. J. L., Jongs, N., Chertow, G. M., Langkilde, A. M., Mcmurray, J. J. V., Correa-Rotter, R., et al. (2021). Effect of dapagliflozin on the rate of decline in kidney function in patients with chronic kidney disease with and without type 2 diabetes: a prespecified analysis from the DAPA-CKD trial. *Lancet Diabetes Endocrinol.* 9, 743–754. doi:10.1016/S2213-8587(21)00242-4

Heerspink, H. J. L., Stefansson, B. V., Correa-Rotter, R., Chertow, G. M., Greene, T., Hou, F. F., et al. (2020). Dapagliflozin in patients with chronic kidney disease. *N. Engl. J. Med.* 383, 1436–1446. doi:10.1056/NEJMoa2024816

Hershberger, S. L., and Fisher, D. G. (2003). A note on determining the number of imputations for missing data. *Struct. Equ. Model. A Multidiscip. J.* 10, 648–650. doi:10.1207/s15328007sem1004_9

Jankowski, J., Floege, J., Fliser, D., Bohm, M., and Marx, N. (2021). Cardiovascular disease in chronic kidney disease: pathophysiological insights and therapeutic options. *Circulation* 143, 1157–1172. doi:10.1161/CIRCULATIONAHA.120.050686

Kim, M. K., and Kim, D. M. (2022). Current status of diabetic kidney disease and latest trends in management. *J. Diabetes Investig.* 13, 1961–1962. doi:10.1111/jdi.13895

Kobayashi, K., Toyoda, M., Hatori, N., Sakai, H., Furuki, T., Sato, K., et al. (2022). Comparison of the blood pressure management between sodium-glucose cotransporter 2 inhibitors and glucagon-like peptide 1 receptor agonists. *Sci. Rep.* 12, 16106. doi:10.1038/s41598-022-20313-5

Kobayashi, K., Toyoda, M., Kimura, M., Hatori, N., Furuki, T., Sakai, H., et al. (2019). Renal effects of sodium glucose co-transporter 2 inhibitors in Japanese type 2 diabetes mellitus patients with home blood pressure monitoring. *Clin. Exp. Hypertens.* 41, 637–644. doi:10.1080/10641963.2018.1529778

Kobayashi, K., Toyoda, M., Tone, A., Kawanami, D., Suzuki, D., Tsuriya, D., et al. (2023). Renoprotective effects of combination treatment with sodium-glucose cotransporter inhibitors and GLP-1 receptor agonists in patients with type 2 diabetes mellitus according to preceding medication. *Diab Vasc. Dis. Res.* 20, 14791641231222837. doi:10.1177/14791641231222837

Kume, S., Araki, S. I., Ugi, S., Morino, K., Koya, D., Nishio, Y., et al. (2019). Secular changes in clinical manifestations of kidney disease among Japanese adults with type 2 diabetes from 1996 to 2014. *J. Diabetes Investig.* 10, 1032–1040. doi:10.1111/jdi.12977

Matsuo, S., Imai, E., Horio, M., Yasuda, Y., Tomita, K., Nitta, K., et al. (2009). Revised equations for estimated GFR from serum creatinine in Japan. *Am. J. Kidney Dis.* 53, 982–992. doi:10.1053/j.ajkd.2008.12.034

Mosenzon, O., Wiviott, S. D., Cahn, A., Rozenberg, A., Yanuv, I., Goodrich, E. L., et al. (2019). Effects of dapagliflozin on development and progression of kidney disease in patients with type 2 diabetes: an analysis from the DECLARE-TIMI 58 randomised trial. *Lancet Diabetes Endocrinol.* 7, 606–617. doi:10.1016/S2213-8587(19)30180-9

Norris, K. C., Smoyer, K. E., Rolland, C., Van Der Vaart, J., and Grubb, E. B. (2018). Albuminuria, serum creatinine, and estimated glomerular filtration rate as predictors of cardio-renal outcomes in patients with type 2 diabetes mellitus and kidney disease: a systematic literature review. *BMC Nephrol.* 19, 36. doi:10.1186/s12882-018-0821-9

Nuffield Department of Population Health Renal Studies Group, SGLT2 inhibitor Meta-Analysis Cardio-Renal Trialists' ConsortiumSGLT2 inhibitor Meta-Analysis Cardio-Renal Trialists' Consortium (2022). Impact of diabetes on the effects of sodium glucose co-transporter-2 inhibitors on kidney outcomes: collaborative meta-analysis of large placebo-controlled trials. *Lancet* 400, 1788–1801. doi:10.1016/S0140-6736(22)02074-8

Perkovic, V., De Zeeuw, D., Mahaffey, K. W., Fulcher, G., Erondu, N., Shaw, W., et al. (2018). Canagliflozin and renal outcomes in type 2 diabetes: results from the CANVAS Program randomised clinical trials. *Lancet Diabetes Endocrinol.* 6, 691–704. doi:10.1016/S2213-8587(18)30141-4

Perkovic, V., Jardine, M. J., Neal, B., Bompoint, S., Heerspink, H. J. L., Charytan, D. M., et al. (2019). Canagliflozin and renal outcomes in type 2 diabetes and nephropathy. *N. Engl. J. Med.* 380, 2295–2306. doi:10.1056/NEJMoa1811744

Rossing, P., Caramori, M. L., Chan, J. C. N., Heerspink, H. J. L., Hurst, C., Khunti, K., et al. (2022). Executive summary of the KDIGO 2022 clinical Practice guideline for diabetes management in chronic kidney disease: an update based on rapidly emerging new evidence. *Kidney Int.* 102, 990–999. doi:10.1016/j.kint.2022.06.013

Rubin, D. B. (1987). *Multiple imputation for nonresponse in surveys*. John Wiley and Sons.

Rubin, D. B., and Schenker, N. (1991). Multiple imputation in health-care databases: an overview and some applications. *Stat. Med.* 10, 585–598. doi:10.1002/sim.4780100410

Shikata, K., Kodera, R., Utsunomiya, K., Koya, D., Nishimura, R., Miyamoto, S., et al. (2020). Prevalence of albuminuria and renal dysfunction, and related clinical factors in

Japanese patients with diabetes: the Japan Diabetes Complication and its Prevention prospective study 5. *J. Diabetes Investig.* 11, 325–332. doi:10.1111/jdi.13116

Shimamoto, K., Ando, K., Fujita, T., Hasebe, N., Higaki, J., Horiuchi, M., et al. (2014). The Japanese society of hypertension guidelines for the management of hypertension (JSH 2014). *Hypertens. Res.* 37, 253–390. doi:10.1038/hr.2014.20

Soejima, H., Ogawa, H., Morimoto, T., Okada, S., Matsumoto, C., Nakayama, M., et al. (2022). Kidney function deterioration is dependent on blood pressure levels: 11.2 year follow-up in diabetic patients. *Heart Vessels* 37, 1873–1881. doi:10.1007/s00380-022-02085-0

Solomon, S. D., Mcmurray, J. J. V., Claggett, B., De Boer, R. A., Demets, D., Hernandez, A. F., et al. (2022). Dapagliflozin in heart failure with mildly reduced or preserved ejection fraction. *N. Engl. J. Med.* 387, 1089–1098. doi:10.1056/NEJMoa2206286

Sumida, K., Nadkarni, G. N., Grams, M. E., Sang, Y., Ballew, S. H., Coresh, J., et al. (2020). Conversion of urine protein-creatinine ratio or urine dipstick protein to urine albumin-creatinine ratio for use in chronic kidney disease screening and prognosis: an individual participant-based meta-analysis. *Ann. Intern Med.* 173, 426–435. doi:10.7326/M20-0529

The EMPA-KIDNEY Collaborative Group, Herrington, W. G., Staplin, N., Wanner, C., Green, J. B., Hauske, S. J., Emberson, J. R., et al. (2023). Empagliflozin in patients with chronic kidney disease. *N. Engl. J. Med.* 388, 117–127. doi:10.1056/NEJMoa2204233

Tuttle, K. R., Bosch-Traberg, H., Cherney, D. Z. I., Hadjadj, S., Lawson, J., Mosenzon, O., et al. (2023). *Post hoc* analysis of SUSTAIN 6 and PIONEER 6 trials suggests that people with type 2 diabetes at high cardiovascular risk treated with semaglutide experience more stable kidney function compared with placebo. *Kidney Int.* 103, 772–781. doi:10.1016/j.kint.2022.12.028

Wanner, C., Inzucchi, S. E., Lachin, J. M., Fitchett, D., Von Eynatten, M., Mattheus, M., et al. (2016). Empagliflozin and progression of kidney disease in type 2 diabetes. *N. Engl. J. Med.* 375, 1801–1802. doi:10.1056/NEJMc1611290

Yamamoto, Y., Hanai, K., Mori, T., Yokoyama, Y., Yoshida, N., Murata, H., et al. (2022). Kidney outcomes and all-cause mortality in people with type 2 diabetes exhibiting non-albuminuric kidney insufficiency. *Diabetologia* 65, 234–245. doi:10.1007/s00125-021-05590-5

frontiers | Frontiers in Pharmacology

TYPE Original Research
PUBLISHED 30 May 2024
DOI 10.3389/fphar.2024.1362242

Check for updates

OPEN ACCESS

EDITED BY
Divya Bhatia,
NewYork-Presbyterian, United States

REVIEWED BY
Hong-Ping Guan,
Rezubio Pharmaceuticals Co., Ltd., China
Lina Naseralallah,
Hamad Medical Corporation, Qatar
Michihiro Hosojima,
Niigata University, Japan

*CORRESPONDENCE
Mineaki Kitamura,
mineaki82@yahoo.co.jp

RECEIVED 27 December 2023
ACCEPTED 06 May 2024
PUBLISHED 30 May 2024

CITATION
Otsuka E, Kitamura M, Funakoshi S, Mukae H and Nishino T (2024), Improving glycemic control: transitioning from dulaglutide to tirzepatide in patients with type 2 diabetes undergoing hemodialysis.
Front. Pharmacol. 15:1362242.
doi: 10.3389/fphar.2024.1362242

COPYRIGHT
© 2024 Otsuka, Kitamura, Funakoshi, Mukae and Nishino. 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.

Improving glycemic control: transitioning from dulaglutide to tirzepatide in patients with type 2 diabetes undergoing hemodialysis

Emiko Otsuka[1,2], Mineaki Kitamura[1,2]*, Satoshi Funakoshi[2], Hiroshi Mukae[3] and Tomoya Nishino[1]

[1]Department of Nephrology, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan, [2]Nagasaki Renal Center, Nagasaki, Japan, [3]Department of Respiratory Medicine, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan

Background: Tirzepatide—a dual glucose-dependent insulinotropic peptide and glucagon-like peptide-1 receptor agonist—is used to treat type 2 diabetes. However, the efficacy and safety of tirzepatide in patients undergoing hemodialysis remain unclear.

Methods: We conducted a single-center retrospective study of patients with type 2 diabetes undergoing hemodialysis who were transitioned from dulaglutide to tirzepatide. We continuously monitored glucose levels in patients undergoing hemodialysis before and after switching from dulaglutide to tirzepatide.

Results: Fourteen patients (mean age: 61.9 ± 9.9 years, male: female = 11:3) were included in this study. After switching to tirzepatide, time in range increased to 50.8% from 42.7% (p = 0.02), time above range decreased to 37.8% from 48.4% (p = 0.02), and mean glucose levels decreased to 137.4 mg/dL from 156.6 mg/dL (p = 0.006). In contrast, there was no significant difference in time below range before and after tirzepatide administration (11.3% and 8.9%) (p = 0.75). Three patients experienced dyspepsia (21.4%), and one patient experienced nausea (7.1%); however, no critical adverse events were reported.

Conclusion: Transitioning from dulaglutide to tirzepatide improved glycemic control without increasing hypoglycemia in patients undergoing hemodialysis for type 2 diabetes.

KEYWORDS

tirzepatide, dulaglutide, hemodialysis, continuous glucose monitoring, GLP-1, GIP, CGM, glycemic control

1 Introduction

Diabetes is the most common cause of end-stage renal disease (ESRD). Patients with type 2 diabetes have a high mortality rate, mainly owing to cardiovascular diseases. This is particularly evident in patients undergoing hemodialysis (Saran et al., 2017; Ahmadmehrabi and Tang, 2018). Glycemic control is crucial to improve clinical outcomes and significantly reduce cardiovascular risk and mortality. However, anti-diabetic drugs such as sodium-glucose cotransporter 2 (SGLT2) inhibitors may be of

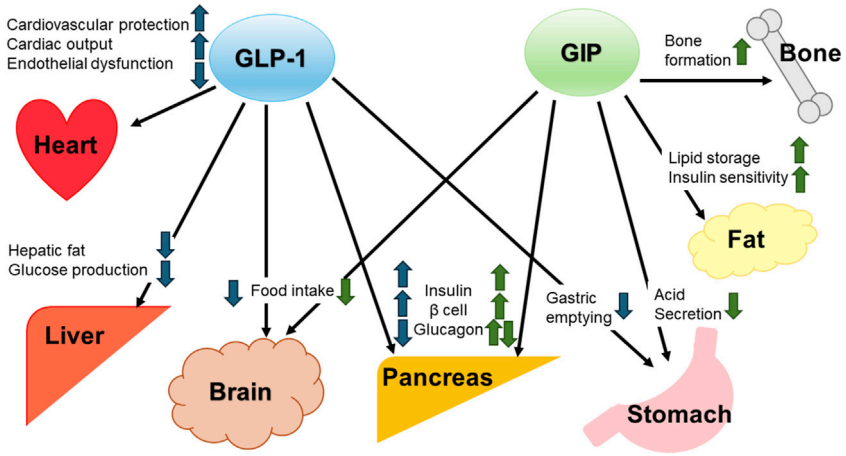


FIGURE 1
Complementary effects of GIP and GLP-1 GIP, glucose-dependent insulinotropic peptide. GLP-1, glucagon-like peptide-1.

limited use or contraindicated in ESRD (Rocco and Berns, 2012; Williams and Garg, 2014); thus, they are often treated with insulin. Glycemic control with insulin is often difficult, especially in patients undergoing hemodialysis. Insulin is partially metabolized in the kidneys, and its effects are prolonged in patients with renal insufficiency (Rave et al., 2001). Blood insulin and glucose levels fluctuate during hemodialysis and even after dialysis (Abe et al., 2007). Therefore, patients undergoing hemodialysis frequently experience large glycemic excursions that put them at a greater risk of hyperglycemia and hypoglycemia (Galindo et al., 2023).

Incretin hormones are currently commonly used for the treatment of diabetes. The main incretin hormones are glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP) (Nauck and Meier, 2018).

Dulaglutide, a weekly formulation of the GLP-1 receptor agonists (GLP-1 RA) launched in Japan in 2015, is effective in reducing body weight, controlling fasting and postprandial glycemia, and has a positive effect on atherosclerotic cardiovascular outcomes (Ruda et al., 2023). The current guidelines for managing type 2 diabetes recommend GLP-1 RAs as first-line injectable therapy even before insulin initiation (American Diabetes Association Professional Practice Committee, 2022). Moreover, dulaglutide has also been reported to be useful in glycemic control in patients undergoing hemodialysis (Yajima et al., 2018; Ugamura et al., 2022). However, some patients treated with dulaglutide have inadequate glycemic control and need more effective drugs.

Tirzepatide is a novel dual GLP-1 and GIP agonist (Coskun et al., 2018). A combination of GLP-1 and GIP can act on pancreatic beta cells synergistically and complementarily through distinct metabolic effects (Bastin and Andreelli, 2019). Moreover, GIP exerts therapeutic benefits beyond its primary incretin role by improving insulin sensitivity and lipid homeostasis in adipose tissue (Asmar et al., 2016). GLP-1 and GIP delay gastrointestinal secretion and suppress appetite, resulting in more effective glycemic control and body weight reduction (Holst, 2021). In addition, GLP-1 is reported to be cardioprotective (Mosenzon et al., 2021) and hepatoprotective (Muzurović et al., 2022), and GIP is reported to inhibit bone resorption (Nissen et al., 2014). The complementary effects of GIP and GLP-1 are shown in Figure 1. Tirzepatide significantly improves glycemic control in patients with type 2 diabetes with or without basal insulin regimens (Dahl et al., 2022; Karagiannis et al., 2022), and a clinical trial demonstrated the superiority of tirzepatide compared with dulaglutide in terms of glycemic control (Inagaki et al., 2022).

However, the safety and efficacy of tirzepatide in patients undergoing hemodialysis remain unclear. Therefore, we aimed to compare the glycemic control between dulaglutide and tirzepatide in patients undergoing hemodialysis. We analyzed the transition in glucose levels using continuous glucose monitoring (CGM) before and after switching from dulaglutide to tirzepatide in patients undergoing hemodialysis.

2 Materials and methods

2.1 Study design and patients

We included patients with type 2 diabetes undergoing hemodialysis whose prescriptions for diabetes were transitioned from dulaglutide to tirzepatide at the Nagasaki Renal Center between June 2023 and August 2023. Blood glucose levels were analyzed using CGM. Patients who changed their insulin dose or other diabetic drugs during this period were excluded.

2.2 Blood glucose measurement

Blood glucose levels were evaluated using CGM for 7 days in patients on dulaglutide and tirzepatide. Dulaglutide was dosed at 0.75 mg once a week for at least 3 months and CGM was started during the hemodialysis sessions and recorded for 7 days. After the discontinuation of CGM, tirzepatide (2.5 mg) was administered 1 week after the last dose of dulaglutide. The second CGM

period started 7–14 days after initiating tirzepatide treatment and was recorded for 7 days. All patients undergoing hemodialysis at our facility did not eat during the dialysis sessions to avoid hypotension. However, the included patients were asked to eat as usual outside dialysis hours. Consequently, each CGM period included 3 days of hemodialysis days and 4 days of non-hemodialysis days. Mean blood glucose levels and time in range (TIR), time above range (TAR), and time below range (TBR) were analyzed using FreeStyle Libre Pro (Abbott Japan Tokyo, Japan).

We set the TIR at 80–140 mg/dL, TAR at >140 mg/dL, and TBR at <80 mg/dL. An international expert panel published a consensus on the glycemic target range of 70–180 mg/dL and a percentage of reading time per day of over 70% (Danne et al., 2017; Battelino et al., 2019). According to the Internal Consensus on the Use of CGM 2017, 70–140 mg/dL is a secondary target range (Danne et al., 2017), and some studies show that this tight range improves the survival rate in critically ill patients (Krinsley and Preiser, 2015; Lanspa et al., 2019). Moreover, a target range of 70–140 mg/dL seems to have advantages over a 70–180 mg/dL range for assessing glycemic status and progress toward stricter glycemic control, particularly when approaching normal glucose levels (Dunn et al., 2024). However, it is crucial to prevent excessive hypoglycemia in patients with severe complications such as renal failure. Therefore, we set the threshold at 80 mg/dL.

2.3 Statistical analysis

Values are shown as the mean ± standard deviation. Differences in TIR, TAR, TBR, and mean blood glucose levels before and after switching to tirzepatide were analyzed using the Wilcoxon signed-rank test. A p-value of <0.05 was considered statistically significant. Statistical analyses were conducted using JMP Pro 17.0.0 (SAS Institute Inc., Cary, NC, United States).

2.4 Ethical statements

This study was approved by the Clinical Research Ethics Committee of the Nagasaki Renal Center (Nagasaki, Japan) (approval number: 23025) and was conducted in accordance with the 1964 Declaration of Helsinki and its subsequent amendments. The requirement for informed consent was waived by the Clinical Research Ethics Committee of the Nagasaki Renal Center (Nagasaki, Japan) owing to the retrospective study design.

3 Results

Sixteen patients with type 2 diabetes undergoing hemodialysis were transitioned from dulaglutide to tirzepatide at the Nagasaki Renal Center from June 2023 to August 2023. All the participants were prescribed 0.75 mg of dulaglutide once a week for at least 3 months and transitioned to 2.5 mg of tirzepatide once a week. Two patients were excluded because their insulin dose or other anti-diabetic drugs were changed during the study period. One patient was hospitalized owing to an infection, and his insulin dose decreased due to appetite loss during hospitalization. Another patient was prescribed liraglutide outside the facility.

Fourteen patients without other diabetes medications or insulin changes during the observation period were included in this study. All patients underwent hemodialysis three times a week for 4 h in each dialysis session. Their mean age was 61.9 ± 9.9 years; 11 were male and 3 were female. The cause of ESRD was type 2 diabetes in 13 patients and chronic glomerulonephritis in one patient. The duration of diabetes was unknown for four patients, while the mean disease duration for the other 10 patients was 25.7 ± 7.5 years, with 45.4 ± 32.2 (months) under dulaglutide. The baseline patient characteristics and data after transitioning from dulaglutide to tirzepatide are shown in Table 1. Interdialytic weight gain and total protein declined after transitioning from dulaglutide to tirzepatide; however, albumin and Geriatric Nutritional Risk Index did not show significant changes.

For each of the 14 patients, CGM was carried out during 3 dialysis days and 4 non-dialysis days before and after switching to tirzepatide, resulting in CGM data being collected for a total of 42 dialysis days and 56 non-dialysis days. Before switching to tirzepatide, TIR, TAR, and TBR values were 42.7%, 48.4%, and 8.9%, respectively. The mean glucose level was 156.6 mg/dL. After switching to tirzepatide, the TIR increased to 50.8% (p = 0.02) (Figure 2), while the TAR and mean glucose level decreased to 37.8% (p = 0.02) (Figure 3) and 137.4 mg/dL (p = 0.006) (Figure 4), respectively. In contrast, the TBR was 11.3%, and there was no significant difference in the TBR between the baseline and after tirzepatide administration (p = 0.75) (Figure 5). TIR, TAR, TBR, and mean glucose levels were also analyzed separately on hemodialysis days and non-hemodialysis days. On hemodialysis days, before and after switching to tirzepatide, TIR were 44.8% and 52.2% (p = 0.02), TAR were 40.9% and 32.6% (0.07), TBR were 13.7% and 14.3% (p = 0.76), and mean glucose levels were 144.0 mg/dL and 130.5 mg/dL (p = 0.02), respectively. (Supplementary Figure S1) On non-hemodialysis days, TIR were 47.9% and 51.7% (p = 0.25), TAR were 46.9% and 35.4% (p = 0.03), TBR were 11.6% and 8.4% (p = 0.37), and mean glucose levels were 157.9 mg/dL and 141.3 mg/dL (p = 0.08), respectively. (Supplementary Figure S2) The median and the average of the continuous blood glucose levels plots of all the included patients on hemodialysis days and non-dialysis days are shown in Figures 6A, B, 7A, B, respectively. The differences in blood glucose levels from the baseline on hemodialysis days and non-hemodialysis days are shown in Supplementary Figures S3A, B, S4A, B, respectively.

Three months after switching to tirzepatide, glycemic albumin did not show significant changes compared to that before switching (21.4 ± 3.6% to 20.6 ± 5.1%), and dry weight decreased from 71.6 kg to 70.1 kg (−1.5 ± 0.3 kg, p = 0.001). Dyspepsia was observed in three patients and nausea was observed in one patient shortly after switching to tirzepatide, however; there were no critical adverse events reported for 3 months (Table 2).

4 Discussion

This retrospective study analyzed the serum glucose levels in switching from dulaglutide to tirzepatide in patients with type 2 diabetes undergoing hemodialysis using CGM.

A previous study on patients with type 2 diabetes who did not undergo hemodialysis revealed robust improvements in glycemic control and body weight without an increased risk of hypoglycemia.

TABLE 1 Baseline patient characteristics and data after transitioning from dulaglutide to tirzepatide.

Characteristic	Baseline	After transitioning	*p*-value
Age (years)	61.9 ± 9.9	—	—
Sex (male) number (%)	11 (78.6)	—	—
Dialysis vintage (years)	5.6 ± 2.5	—	—
Diabetes vintage (years)	25.7 ± 7.5	—	—
Dosing dulaglutide (months)	45.4 ± 32.2	—	—
Dry weight (kg)	71.6 ± 17.1	71.5 ± 16.9	0.75
BMI (kg/m^2)	25.7 ± 6.7	25.1 ± 6.5	0.73
IDWG (kg)	3.5 ± 1.2	2.7 ± 1.6	0.02
Glycated Albumin (%)	21.4 ± 3.6	21 ± 4.7	0.45
Hemoglobin (g/dL)	11.0 ± 1.06	10.8 ± 0.9	0.55
Creatinine (mg/dL)	10.4 ± 1.6	10.8 ± 2.1	0.17
BUN (mg/dL)	56.7 ± 14.7	53.6 ± 15.5	0.22
Total protein (g/dL)	6.8 ± 0.6	6.6 ± 0.5	0.04
Albumin (g/dL)	3.6 ± 0.4	3.5 ± 0.4	0.19
Potassium (mEq/L)	5.1 ± 0.6	5.3 ± 1.4	0.35
Calcium (mg/dL)	8.7 ± 0.4	8.7 ± 0.5	0.98
Phosphate (mg/dL)	5.5 ± 1.6	5.3 ± 1.4	0.35
GNRI	94.0 ± 6.1	92.6 ± 6.5	0.12
Insulin treatment number (%)	5 (35.7)	5 (35.7)	—
Oral anti-diabetic agent treatment			
Glinide number (%)	3 (21.4)	3 (21.4)	—
Alpha-glucosidase inhibitor number (%)	3 (21.4)	3 (21.4)	—

Data are expressed as the median ±SD. Insulin treatment and oral anti-diabetic agent treatment data are expressed as the number of patients (%).
BMI, body mass index; IDWG, interdialytic weight gain; BUN, blood urea nitrogen; GNRI, geriatric nutritional risk index.

Participants were randomly assigned to four groups (1:1:1:1): one group received tirzepatide once a week at 5 mg, another group at 10 mg, a third group at 15 mg, and the fourth group received a placebo. They were then monitored for 40 weeks. The average reduction in hemoglobin A1c (HbA1c) levels from the starting point was 1.87% for those on tirzepatide 5 mg, 1.89% for those on tirzepatide 10 mg, and 2.07% for those on tirzepatide 15 mg, in contrast to an increase of 0.04% for those on placebo. This led to estimated differences in treatment effectiveness compared with placebo of −1.91% for tirzepatide 5 mg, −1.93% for tirzepatide 10 mg, and −2.11% for tirzepatide 15 mg (all $p < 0.001$). Tirzepatide induced a dose-dependent reduction in body weight of 7.0–9.5 kg (Rosenstock et al., 2021). Similarly, tirzepatide improved glycemic control compared with semaglutide and insulin degludec (Frías et al., 2021; Ludvik et al., 2021). The SURPASS-4 study found that tirzepatide was more effective than glargine in reducing HbA1c levels in patients with type 2 diabetes and high cardiovascular risk. Additionally, it resulted in fewer cases of hypoglycemia, which can raise the risk of cardiovascular events (Del Prato et al., 2021). However, there is limited information on how well tirzepatide works in type 2 diabetes patients who are on hemodialysis.

Dulaglutide improved glycemic control without inducing hypoglycemia in type 2 diabetes undergoing hemodialysis (Yajima et al., 2018; Ugamura et al., 2022). Moreover, glycoalbumin levels (median −1.8%; $p = 0.026$) and the daily total insulin dose (−15.0 U/day; $p = 0.002$) significantly decrease (Ugamura et al., 2022). The mean and % CV of glucose levels significantly decrease after dulaglutide administration according to CGM (Yajima et al., 2018). Although the GLP-1RA dulaglutide is used in patients undergoing hemodialysis, some patients experienced inadequate glycemic control with dulaglutide.

In the general population, tirzepatide is superior to dulaglutide in terms of glycemic control (Inagaki et al., 2022; Nicholls et al., 2024). The use of tirzepatide in patients with chronic kidney disease (CKD) depends on three aspects. The first is the safety of GLP-1 in patients with CKD as well as the general population. A previous study showed that renal impairment did not have any clinically relevant effect on tirzepatide pharmacokinetics (Urva et al., 2020). The second is the efficacy of GLP-1 in patients with CKD as well as

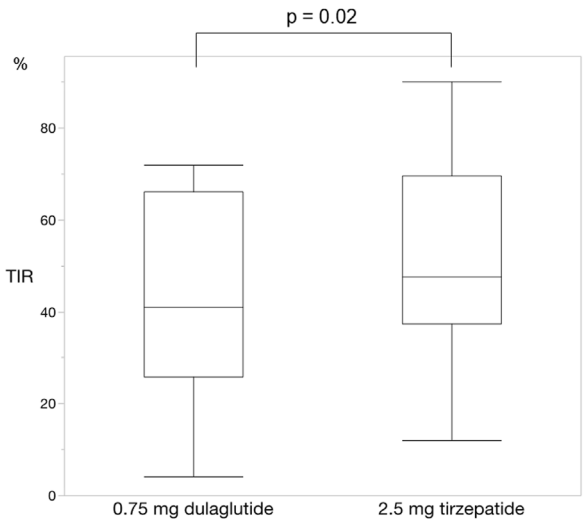


FIGURE 2
Change in time in range. The time in range significantly improved changing from dulaglutide to tirzepatide. Wilcoxon rank-sum test was used in the analysis. TIR, time in range.

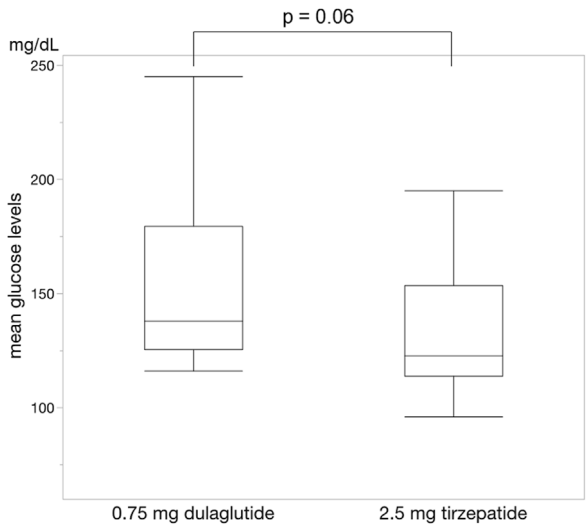


FIGURE 4
Change in mean blood glucose levels. The mean blood glucose levels were significantly lower during administrating tirzepatide than that of dulaglutide. Wilcoxon rank-sum test was used in the analysis.

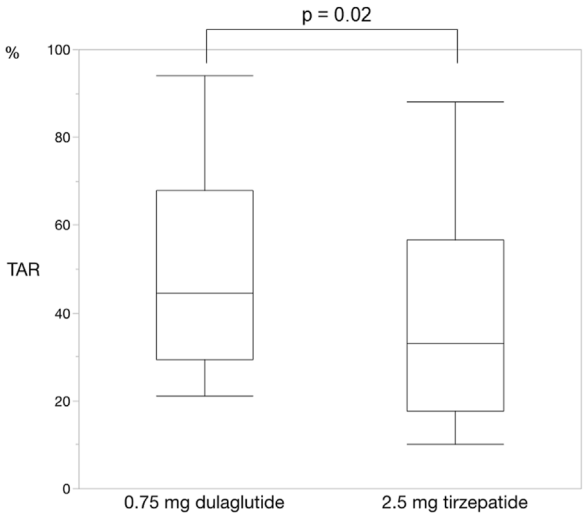


FIGURE 3
Change in time above range. The time above range also improved changing from dulaglutide to tirzepatide. Wilcoxon rank-sum test was used in the analysis. TAR, time above range.

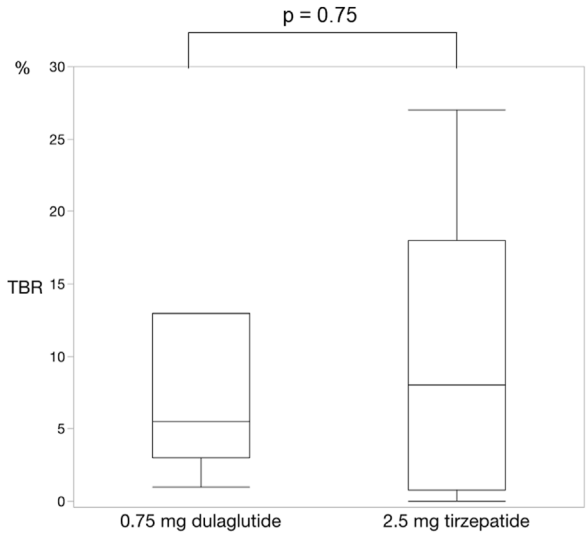


FIGURE 5
Change in time below range. There was no significant difference in the time below range between dulaglutide and tirzepatide. Wilcoxon rank-sum test was used in the analysis. TBR, time below range.

the general population. A previous study reported that tirzepatide is also effective in glycemic control in patients including those with CKD (Del Prato et al., 2021; Nicholls et al., 2024). The third is the protection of renal function. Tirzepatide treatment reduced albuminuria and the estimated glomerular filtration rate (eGFR) slope in patients with type 2 diabetes having CKD (Heerspink et al., 2022; Bosch et al., 2023). Recently, the renoprotective effects of tirzepatide were demonstrated using cystatin C-derived eGFR (Heerspink et al., 2023). In patients with type 2 diabetes and at least moderately increased albuminuria, a combination treatment of SGLT2 inhibitors, GLP-1 RA, and a nonsteroidal mineralocorticoid receptor antagonist resulted in observed gains in cardiovascular and kidney event-free and overall survival (Neuen et al., 2024). Thus, tirzepatide use seems to be reasonable in patients with CKD.

However, the efficacy of tirzepatide compared to dulaglutide in patients undergoing hemodialysis remain unclear. Thus, using CGM, this retrospective study analyzed the effect of switching from dulaglutide to tirzepatide on serum glucose levels in patients with type 2 diabetes undergoing hemodialysis.

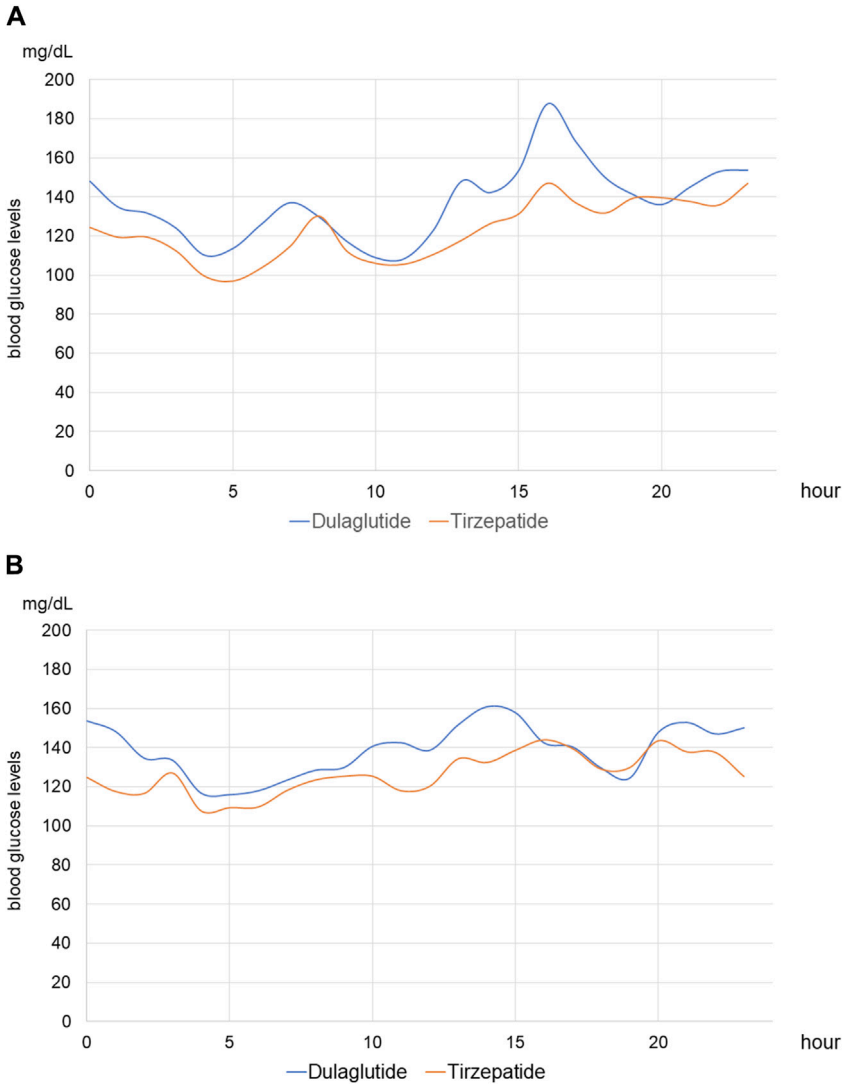


FIGURE 6
Median continuous blood glucose levels on hemodialysis and non-hemodialysis days **(A)** Median continuous blood glucose levels of all patients on hemodialysis days. The blue line shows those in periods of treatment with dulaglutide, and the red line shows those in periods of treatment with tirzepatide. **(B)** Median continuous blood glucose levels of all patients on non-hemodialysis days.

Recommendations from the International Consensus on Time In Range state that the target range of TIR is over 70% per day (Battelino et al., 2019). However, the TIR during the period of once-weekly dulaglutide was only 42.7% in our study, indicating that additional anti-diabetic agents should be considered to achieve the target range of TIR. Furthermore, international consensus guidelines specify a target range for TBR of <4% and <1% in patients aged 60 years or those at high risk (Battelino et al., 2019). This study observed a TBR of 8.9% during the dulaglutide administration period, even in the absence of symptomatic hypoglycemia. This TBR level was significantly higher in high-risk patients, and alternative anti-diabetic agents are preferable to dulaglutide to decrease hyperglycemia and hypoglycemia.

Tirzepatide is a dual GLP-1 and GIP agonist, not solely a GLP-1RA. It may achieve more favorable glycemic control than dulaglutide through three possible mechanisms. First, GLP-1 and GIP interact with each other to improve blood glucose levels more effectively compared with GLP-1 single agonists. GLP-1RA acts synergistically with GIP activation to gain a broad improvement in metabolic health with the hypothesis that enhancing insulin secretion by dual actions on pancreatic β cells improves glycemia, restores sensitivity to GIP, and involves additional mechanisms of action (Zhou et al., 2023). Second, tirzepatide could decrease blood glucose levels more effectively in the hyperglycemic state, while not affecting glucose levels in normal and/or hypoglycemic states. This is because glucagon levels are based on blood glucose levels. In contrast to GLP-1, GIP exhibits glucagonotropic effects in normal and/or hypoglycemic states; conversely, it suppresses glucagon secretion in the hyperglycemic state (Christensen et al., 2011). Moreover, GLP-1 receptor expression decreases in a hyperglycemic state, but GIP receptor expression increases under the effect of acute hyperglycemia (Willard et al., 2020). Our CGM plots also showed reduced fluctuations in blood glucose levels after switching from dulaglutide to tirzepatide. In particular, post-dialysis blood glucose levels were elevated when

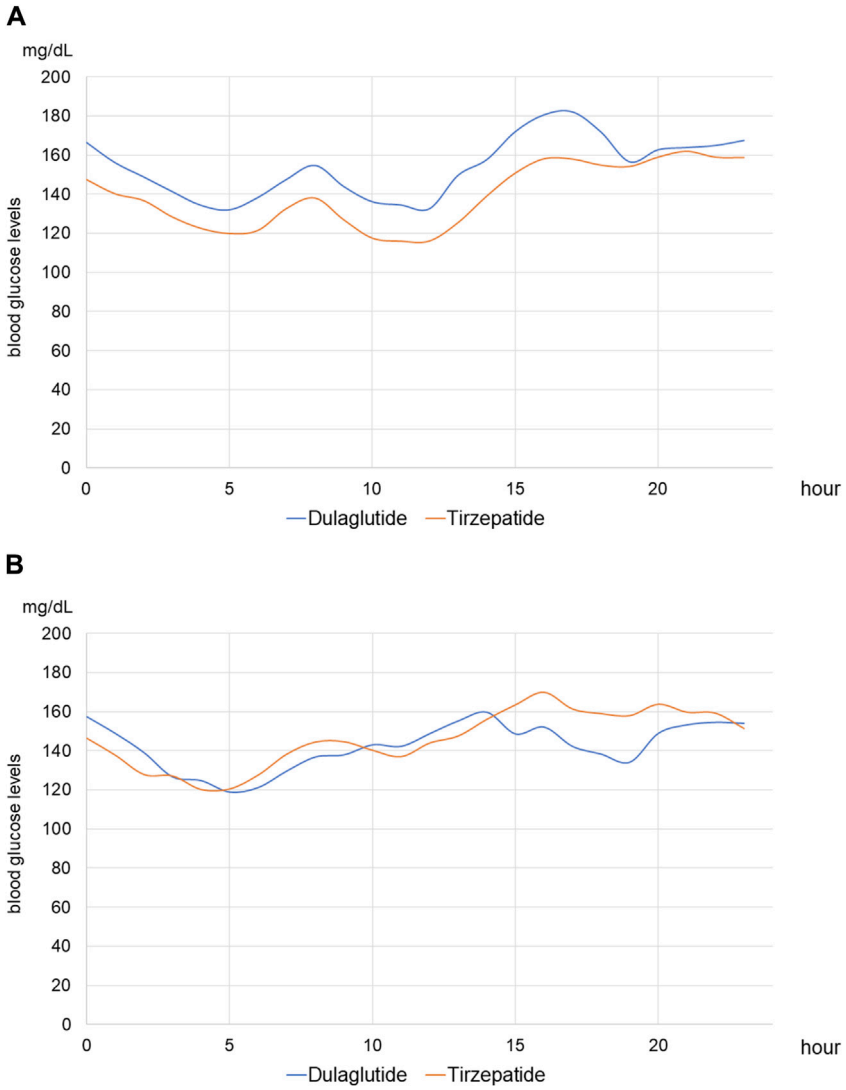


FIGURE 7
Average blood glucose level on hemodialysis and non-hemodialysis days of continuous glucose monitoring **(A)** Average continuous blood glucose levels of all patients on hemodialysis days. **(B)** Average continuous blood glucose levels of all patients on non-hemodialysis days.

TABLE 2 Adverse events after transitioning from dulaglutide to tirzepatide.

Event	No. of patients (%)
Death	0 (0)
Nausea	1 (7.1)
Vomiting	0 (0)
Diarrhea	0 (0)
Dyspepsia	3 (21.4)

Data expressed as the number of patients (%).

treated with dulaglutide, whereas this elevation was suppressed after switching to tirzepatide. On the other hand, CGM plots on non-dialysis days did not show an obvious improvement. The improvement of glycemic control might be due to not on the non-hemodialysis days but on the hemodialysis days, and a longer-term study is needed to elucidate the efficacy of tirzepatide. Third, tirzepatide is an imbalanced dual agonist that favors the GIP receptor over GLP-1, and it is preferable to maximize glycemic control while suppressing gastrointestinal disorders (Finan et al., 2013). GLP-1 has multiple glucose-lowering actions, one of which is delaying gastric emptying. This effect induces gastrointestinal disorders, such as nausea and vomiting, which makes it difficult to increase the dose of GLP-1RA. However, this effect was not described for GIP. Thus, an imbalanced dual agonist favoring the GIP receptor over GLP-1 can achieve better glycemic control than GLP-1RA alone.

The most common adverse events in the patients administered tirzepatide or dulaglutide were nasopharyngitis (tirzepatide range 13.8–18.2% vs. dulaglutide 16.4%), nausea (11.9–20.0% vs. 7.5%), and constipation (13.8–17.7% vs. 10.7%) (Inagaki et al., 2022). In our study, dyspepsia and nausea were observed at 21.4% and 7.1%, respectively, which were slightly higher than the prior observation; however, nasopharyngitis and constipation were not observed, and no

patient needed to stop the treatment owing to adverse events, with no critical adverse events reported.

In our study, dry weight decreased by approximately 1.5 kg after 3 months of transitioning to tirzepatide. All tirzepatide doses are superior to all comparators in terms of body weight reduction (Jastreboff et al., 2022; Karagiannis et al., 2022). This is probably because tirzepatide has the same effect on gastric emptying delay as selective GLP-1RA (Urva et al., 2020). Moreover, GIP increases lipogenesis, and enhances the lipid-buffering capacity of white adipose tissue (Samms et al., 2020). These mechanisms seem to improve obesity. In patients with early-stage CKD, a body mass index ≥35 kg/m^2 is associated with poorer outcomes in terms of renal function (Lu et al., 2014). However, the relationship between obesity and CKD progression is still controversial. Moreover, some reports show that obesity is associated with improved survival in patients undergoing hemodialysis (Schmidt and Salahudeen, 2007). Recently, the presence of both sarcopenia and obesity, termed sarcopenic obesity, has been considered to be a risk factor for mortality and cardiovascular diseases in patients undergoing hemodialysis (de Oliveira Matos et al., 2022). Tirzepatide will improve obesity, but whether it also reduces the mortality in patients undergoing hemodialysis remains controversial.

Our facility routinely examines glycoalbumin instead of HbA1cas a glycemic control parameter. The standard method of monitoring glycemic control has been the periodic measurement of the level of HbA1c (Davies et al., 2018). However, HbA1c is influenced by various factors in patients undergoing hemodialysis, such as shortened erythrocyte lifespan, administration of erythropoietin as a stimulating agent for the treatment of renal anemia, the administration of iron preparations, uremia, and blood transfusion, all of which have the potential for rendering HbA1c measurements inaccurate (Abe et al., 2022). Glycoalbumin is more strongly correlated with plasma glucose levels than HbA1c in patients undergoing hemodialysis (Chen et al., 2017; Hoshino et al., 2018; Kohzuma et al., 2021). Nonetheless, glycoalbumin reflects average glucose levels and not fluctuating blood glucose levels. In patients undergoing hemodialysis, blood glucose levels tend to fluctuate, with frequent large glycemic excursions; therefore, it is crucial to use parameters that reflect fluctuations in blood glucose levels. CGM sensors continuously measure glucose concentrations in the interstitial fluid using a glucose oxidase reaction. Thus, CGM can track the fluctuation in blood glucose levels, thereby helping to provide accurate glycemic control and prevent hypoglycemia (Galindo et al., 2023). We believe that CGM is the most reliable method for effective glycemic control in patients undergoing hemodialysis.

This study has several limitations. First, the sample size was relatively small. Second, we used CGM and measured glycoalbumin, although we did not measure HbA1c. Continuous glucose monitoring reflects glycemic variability more accurately; however, it cannot be compared with other studies using HbA1c. Third, according to CGM data separated into hemodialysis and non-dialysis days, blood glucose levels improved substantially on hemodialysis days; however, on non-dialysis days there was no obvious improvement. Finally, the observation period was short, and a prolonged observation period is needed to clarify the long-term effectiveness and adverse events of tirzepatide in patients undergoing hemodialysis.

5 Conclusion

In our study, TIR increased without an increase in hypoglycemic episodes after switching from dulaglutide to tirzepatide in patients with type 2 diabetes undergoing hemodialysis. Transitioning from dulaglutide to tirzepatide can improve glycemic control without increasing hypoglycemia in patients undergoing hemodialysis for type 2 diabetes. A large-scale study is required to verify these results.

Data availability statement

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

Ethics statement

The studies involving humans were approved by the Clinical Research Ethics Committee of the Nagasaki Renal Center. The studies were conducted in accordance with the local legislation and institutional requirements. The ethics committee/institutional review board waived the requirement of written informed consent for participation from the participants or the participants' legal guardians/next of kin because of the retrospective study design.

Author contributions

EO: Conceptualization, Methodology, Project administration, Writing–original draft, Writing–review and editing. MK: Conceptualization, Methodology, Project administration, Writing–original draft, Writing–review and editing. SF: Conceptualization, Methodology, Project administration, Writing–original draft, Writing–review and editing. HM: Writing–original draft, Writing–review and editing. TN: Writing–original draft, Writing–review and editing.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Acknowledgments

We special thank to Masatoshi Hayashida for his special assistance.

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.

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.2024.1362242/full#supplementary-material

SUPPLEMENTARY FIGURE S1
Change of time in range, time above range, time below range, and mean blood glucose levels on hemodialysis days. The time in range and the mean blood glucose levels improved upon changing from dulaglutide to tirzepatide on hemodialysis days. Wilcoxon rank-sum test was used in the analysis. **(A)** Change of time in range on hemodialysis days **(B)** Change of time above range on hemodialysis days **(C)** Change of time below range on hemodialysis days **(D)** Change of mean blood glucose levels on hemodialysis days.

SUPPLEMENTARY FIGURE S2
Change of time in range, time above range, time below range, and mean blood glucose levels on non-hemodialysis days. The time in range did not show significant improvement upon changing from dulaglutide to tirzepatide on non-hemodialysis days. The time above range improved changing from dulaglutide to tirzepatide on hemodialysis days. Wilcoxon rank-sum test was used in the analysis. **(A)** Change of time in range on non-hemodialysis days **(B)** Change of time above range on non-hemodialysis days **(C)** Change of time below range on non-hemodialysis days **(D)** Change of mean blood glucose levels on non-hemodialysis days.

SUPPLEMENTARY FIGURE S3
The differences in median blood glucose levels from the baseline on hemodialysis and non-hemodialysis days of continuous glucose monitoring. The differences in median blood glucose levels of all patients from the baseline on hemodialysis are shown, and the baselines are the time of starting CGM. The blue line shows those in periods of dulaglutide treatment, and the red line shows those in periods of tirzepatide treatment. **(A)** The difference in median blood glucose levels from the baseline on hemodialysis **(B)** The difference in median blood glucose levels from the baseline on non-hemodialysis.

SUPPLEMENTAL FIGURE S4
The differences in average blood glucose levels from the baseline on hemodialysis and non-hemodialysis days of continuous glucose monitoring. The differences in average blood glucose levels of all patients from the baseline on hemodialysis are shown, and the baselines are the time of starting CGM. The blue line shows the average blood glucose levels during dulaglutide treatment, and the red line shows those during tirzepatide treatment. **(A)** The difference in average blood glucose levels from the baseline on hemodialysis **(B)** The difference in average blood glucose levels from the baseline on non-hemodialysis.

References

Abe, M., Kaizu, K., and Matsumoto, K. (2007). Evaluation of the hemodialysis-induced changes in plasma glucose and insulin concentrations in diabetic patients: comparison between the hemodialysis and non-hemodialysis days. *Ther. Apher. Dial.* 11 (4), 288–295. doi:10.1111/J.1744-9987.2007.00492.X

Abe, M., Matsuoka, T., Kawamoto, S., Miyasato, K., and Kobayashi, H. (2022). Toward revision of the 'best practice for diabetic patients on hemodialysis 2012.' *Kidney Dial.* 2 (4), 495–511. doi:10.3390/kidneydial2040045

Ahmadmehrabi, S., and Tang, W. H. W. (2018). Hemodialysis-induced cardiovascular disease. *Semin. Dial.* 31 (3), 258–267. doi:10.1111/sdi.12694

American Diabetes Association Professional Practice Committee (2022). 9. Pharmacologic approaches to glycemic treatment: standards of medical care in diabetes-2022. *Diabetes Care* 45 (Suppl. 1), 125–143. doi:10.2337/dc22-S009

Asmar, M., Simonsen, L., Asmar, A., Holst, J. J., Dela, F., and Bülow, J. (2016). Insulin plays a permissive role for the vasoactive effect of GIP regulating adipose tissue metabolism in humans. *J. Clin. Endocrinol. Metab.* 101 (8), 3155–3162. doi:10.1210/jc.2016-1933

Bastin, M., and Andreelli, F. (2019). Dual GIP–GLP1-receptor agonists in the treatment of type 2 diabetes: a short review on emerging data and therapeutic potential. *Diabetes Metab. Syndr. Obes.* 12, 1973–1985. doi:10.2147/DMSO.S191438

Battelino, T., Danne, T., Bergenstal, R. M., Amiel, S. A., Beck, R., Biester, T., et al. (2019). Clinical targets for continuous glucose monitoring data interpretation: recommendations from the international consensus on time in range. *Diabetes Care* 42 (8), 1593–1603. doi:10.2337/dci19-0028

Bosch, C., Carriazo, S., Soler, M. J., Ortiz, A., and Fernandez-Fernandez, B. (2023). Tirzepatide and prevention of chronic kidney disease. *Clin. Kidney J.* 16 (5), 797–808. doi:10.1093/ckj/sfac274

Chen, C. W., Drechsler, C., Suntharalingam, P., Karumanchi, S. A., Wanner, C., and Berg, A. H. (2017). High glycated albumin and mortality in persons with diabetes mellitus on hemodialysis. *Clin. Chem.* 63 (2), 477–485. doi:10.1373/clinchem.2016.258319

Christensen, M., Vedtofte, L., Holst, J. J., Vilsbøll, T., and Knop, F. K. (2011). Glucose-dependent insulinotropic polypeptide: a bifunctional glucose-dependent regulator of glucagon and insulin secretion in humans. *Diabetes* 60 (12), 3103–3109. doi:10.2337/db11-0979

Coskun, T., Sloop, K. W., Loghin, C., Alsina-Fernandex, J., Urva, S., Bokvist, K. B., et al. (2018). LY3298176, a novel dual GIP and GLP-1 receptor agonist for the treatment of type 2 diabetes mellitus: from discovery to clinical proof of concept. *Mol. Metab.* 18 (October), 3–14. doi:10.1016/j.molmet.2018.09.009

Dahl, D., Onishi, Y., Norwood, P., Huh, R., Bray, R., Patel, H., et al. (2022). Effect of subcutaneous tirzepatide vs placebo added to titrated insulin glargine on glycemic control in patients with Type 2 diabetes: the SURPASS-5 Randomized Clinical Trial. *JAMA* 327 (6), 534–545. doi:10.1001/JAMA.2022.0078

Danne, T., Nimri, R., Battelino, T., Bergenstal, R. M., Close, K. L., DeVries, J. H., et al. (2017). International consensus on use of continuous glucose monitoring. *Diabetes Care* 40 (12), 1631–1640. doi:10.2337/dc17-1600

Davies, M. J., D'Alessio, D. A., Fradkin, J., Kernan, W. N., Mathieu, C., Mingrone, G., et al. (2018). Management of hyperglycemia in type 2 diabetes, 2018. A consensus report by the American diabetes association (ada) and the European association for the study of diabetes (easd). *Diabetes Care* 41 (12), 2669–2701. doi:10.2337/dci18-0033

Del Prato, S., Kahn, S. E., Pavo, I., Weerakkody, G. J., Yang, Z., Doupis, J., et al. (2021). Tirzepatide versus insulin glargine in type 2 diabetes and increased cardiovascular risk (SURPASS-4): a randomised, open-label, parallel-group, multicentre, phase 3 trial. *Lancet* 398 (10313), 1811–1824. doi:10.1016/S0140-6736(21)02188-7

de Oliveira Matos, B., da Costa Rosa, C. S., Ribeiro, H. S., Marcos, N. M., Losilla, M. P. R., Monteiro, H. L., et al. (2022). Obesity phenotypes are, in part, associated with physical activity in diabetic hemodialysis patients. *Int. Urol. Nephrol.* 54 (7), 1751–1759. doi:10.1007/s11255-021-03060-w

Dunn, T. C., Ajjan, R. A., Bergenstal, R. M., and Xu, Y. (2024). Is it time to move beyond TIR to TITR? Real-world data from over 20,000 users of continuous glucose monitoring in patients with type 1 and type 2 diabetes. *Diabetes Technol. Ther.* 26 (3), 203–210. doi:10.1089/dia.2023.0565

Finan, B., Ma, T., Ottaway, N., Müller, T. D., Habegger, K. M., Heppner, K. M., et al. (2013). Unimolecular dual incretins maximize metabolic benefits in rodents, monkeys, and humans. *Sci. Transl. Med.* 5 (209), 209ra151. doi:10.1126/scitranslmed.3007218

Frías, J. P., Davies, M. J., Rosenstock, J., Pérez Manghi, F. C., Fernández Landó, L., Bergman, B. K., et al. (2021). Tirzepatide versus semaglutide once weekly in patients with type 2 diabetes. *N. Engl. J. Med.* 385 (6), 503–515. doi:10.1056/nejmoa2107519

Galindo, R. J., de Boer, I. H., Neumiller, J. J., and Tuttle, K. R. (2023). Continuous glucose monitoring to optimize management of diabetes in patients with advanced CKD. *Clin. J. Am. Soc. Nephrol.* 18 (1), 130–145. doi:10.2215/CJN.04510422

Heerspink, H. J. L., Sattar, N., Pavo, I., Haupt, A., Duffin, K. L., Yang, Z., et al. (2022). Effects of tirzepatide versus insulin glargine on kidney outcomes in type 2 diabetes in the SURPASS-4 trial: post-hoc analysis of an open-label, randomised, phase 3 trial. *Lancet Diabetes Endocrinol.* 10 (11), 774–785. doi:10.1016/S2213-8587(22)00243-1

Heerspink, H. J. L., Sattar, N., Pavo, I., Haupt, A., Duffin, K. L., Yang, Z., et al. (2023). Effects of tirzepatide versus insulin glargine on cystatin C-based kidney function: a SURPASS-4 *post hoc* analysis. *Diabetes Care* 46 (8), 1501–1506. doi:10.2337/dc23-0261

Holst, J. J. (2021). Treatment of type 2 diabetes and obesity on the basis of the incretin system: the 2021 banting medal for scientific achievement award lecture. *Diabetes* 70 (11), 2468–2475. doi:10.2337/DBI21-0026

Hoshino, J., Hamano, T., Abe, M., Hasegawa, T., Wada, A., Ubara, Y., et al. (2018). Glycated albumin versus hemoglobin A1c and mortality in diabetic hemodialysis

patients: a cohort study. *Nephrol. Dial. Transpl.* 33 (7), 1150–1158. doi:10.1093/ndt/gfy014

Inagaki, N., Takeuchi, M., Oura, T., Imaoka, T., and Seino, Y. (2022). Efficacy and safety of tirzepatide monotherapy compared with dulaglutide in Japanese patients with type 2 diabetes (SURPASS J-mono): a double-blind, multicentre, randomised, phase 3 trial. *Lancet Diabetes Endocrinol.* 10, 623–633. doi:10.1016/S2213-8587(22)00188-7

Jastreboff, A. M., Aronne, L. J., Ahmad, N. N., Wharton, S., Connery, L., Alves, B., et al. (2022). Tirzepatide once weekly for the treatment of obesity. *N. Engl. J. Med.* 387, 205–216. doi:10.1056/NEJMoa2206038

Karagiannis, T., Avgerinos, I., Liakos, A., Del Prato, S., Matthews, D. R., Tsapas, A., et al. (2022). Management of type 2 diabetes with the dual GIP/GLP-1 receptor agonist tirzepatide: a systematic review and meta-analysis. *Diabetologia* 65 (8), 1251–1261. doi:10.1007/s00125-022-05715-4

Kohzuma, T., Tao, X., and Koga, M. (2021). Glycated albumin as biomarker: evidence and its outcomes. *J. Diabetes Complicat.* 35 (11), 108040. doi:10.1016/J.JDIACOMP.2021.108040

Krinsley, J. S., and Preiser, J. C. (2015). Time in blood glucose range 70 to 140 mg/dl >80% is strongly associated with increased survival in non-diabetic critically ill adults. *Crit. Care* 19 (1), 179. doi:10.1186/s13054-015-0908-7

Lanspa, M. J., Krinsley, J. S., Hersh, A. M., Wilson, E. L., Holmen, J. R., Orme, J. F., et al. (2019). Percentage of time in range 70 to 139 mg/dL is associated with reduced mortality among critically ill patients receiving IV insulin infusion. *Chest* 156 (5), 878–886. doi:10.1016/j.chest.2019.05.016

Lu, J. L., Kalantar-Zadeh, K., Ma, J. Z., Quarles, L. D., and Kovesdy, C. P. (2014). Association of body mass index with outcomes in patients with CKD. *J. Am. Soc. Nephrol.* 25 (9), 2088–2096. doi:10.1681/ASN.2013070754

Ludvik, B., Giorgino, F., Jódar, E., Frias, J. P., Fernández Landó, L., Brown, K., et al. (2021). Once-weekly tirzepatide versus once-daily insulin degludec as add-on to metformin with or without SGLT2 inhibitors in patients with type 2 diabetes (SURPASS-3): a randomised, open-label, parallel-group, phase 3 trial. *Lancet* 398 (10300), 583–598. doi:10.1016/S0140-6736(21)01443-4

Mosenzon, O., Alguwaihes, A., Leon, J. L. A., Bayram, F., Darmon, P., Davis, T. M. E., et al. (2021). CAPTURE: a multinational, cross-sectional study of cardiovascular disease prevalence in adults with type 2 diabetes across 13 countries. *Cardiovasc. Diabetol.* 20 (1), 154. doi:10.1186/s12933-021-01344-0

Muzurović, E. M., Volčanšek, Š., Tomšić, K. Z., Janež, A., Mikhailidis, D. P., Rizzo, M., et al. (2022). Glucagon-like peptide-1 receptor agonists and dual glucose-dependent insulinotropic polypeptide/glucagon-like peptide-1 receptor agonists in the treatment of obesity/metabolic syndrome, prediabetes/diabetes and non-alcoholic fatty liver disease—current evidence. *J. Cardiovasc. Pharmacol. Ther.* 27, 10742484221146371. doi:10.1177/10742484221146371

Nauck, M. A., and Meier, J. J. (2018). Incretin hormones: their role in health and disease. *Diabetes Obes. Metab.* 20 (Suppl. 1), 5–21. doi:10.1111/DOM.13129

Neuen, B. L., Heerspink, H. J. L., Vart, P., Claggett, B. L., Fletcher, R. A., Arnott, C., et al. (2024). Estimated lifetime cardiovascular, kidney, and mortality benefits of combination treatment with SGLT2 inhibitors, GLP-1 receptor agonists, and nonsteroidal MRA compared with conventional care in patients with type 2 diabetes and albuminuria. *Circulation* 149 (6), 450–462. doi:10.1161/CIRCULATIONAHA.123.067584

Nicholls, S. J., Bhatt, D. L., Buse, J. B., Prato, S. D., Kahn, S. E., Lincoff, A. M., et al. (2024). Comparison of tirzepatide and dulaglutide on major adverse cardiovascular events in participants with type 2 diabetes and atherosclerotic cardiovascular disease: SURPASS-CVOT design and baseline characteristics. *Am. Heart J.* 267, 1–11. doi:10.1016/j.ahj.2023.09.007

Nissen, A., Christensen, M., Knop, F. K., Vilsbøll, T., Holst, J. J., and Hartmann, B. (2014). Glucose-dependent insulinotropic polypeptide inhibits bone resorption in humans. *J. Clin. Endocrinol. Metab.* 99 (11), E2325–E2329. doi:10.1210/jc.2014-2547

Rave, K., Heise, T., Pfützner, A., Heinemann, L., and Sawicki, P. T. (2001). Impact of diabetic nephropathy on pharmacodynamic and pharmacokinetic properties of insulin in type 1 diabetic patients. *Diabetes Care* 24 (5), 886–890. doi:10.2337/diacare.24.5.886

Rocco, M. V., and Berns, J. S. (2012). KDOQI clinical practice guideline for diabetes and CKD: 2012 update. *Am. J. Kidney Dis.* 60 (5), 850–886. doi:10.1053/j.ajkd.2012.07.005

Rosenstock, J., Wysham, C., Frías, J. P., Kaneko, S., Lee, C. J., Fernández Landó, L., et al. (2021). Efficacy and safety of a novel dual GIP and GLP-1 receptor agonist tirzepatide in patients with type 2 diabetes (SURPASS-1): a double-blind, randomised, phase 3 trial. *Lancet* 398 (10295), 143–155. doi:10.1016/S0140-6736(21)01324-6

Ruda, A. I., Ciobanu, D. M., Inceu, G., Rusu, A., and Roman, G. (2023). The effect of Dulaglutide on glycemic and weight control in patients with type 2 diabetes. *Med. Pharm. Rep.* 96 (1), 52–57. doi:10.15386/mpr-2425

Samms, R. J., Coghlan, M. P., and Sloop, K. W. (2020). How may GIP enhance the therapeutic efficacy of GLP-1? *Trends Endocrinol. Metab.* 31, 410–421. doi:10.1016/j.tem.2020.02.006

Saran, R., Robinson, B., Abbott, K. C., Agodoa, L. Y. C., Albertus, P., Ayanian, J., et al. (2017). US renal data system 2016 annual data report: epidemiology of kidney disease in the United States. *Am. J. Kidney Dis.* 69 (3), A7–A8. doi:10.1053/j.ajkd.2016.12.004

Schmidt, D., and Salahudeen, A. (2007). The obesity-survival paradox in hemodialysis patients: why do overweight hemodialysis patients live longer? *Nutr. Clin. Pract.* 22 (1), 11–15. doi:10.1177/011542650702200111

Ugamura, D., Hosojima, M., Kabasawa, H., Tanabe, N., Yoshizawa, Y., Suzuki, Y., et al. (2022). An exploratory clinical trial on the efficacy and safety of glucagon-like peptide-1 receptor agonist dulaglutide in patients with type 2 diabetes on maintenance hemodialysis. *Ren. Replace. Ther.* 8 (1), 26. doi:10.1186/s41100-022-00409-4

Urva, S., Coskun, T., Loghin, C., Cui, X., Beebe, E., O'Farrell, L., et al. (2020). The novel dual glucose-dependent insulinotropic polypeptide and glucagon-like peptide-1 (GLP-1) receptor agonist tirzepatide transiently delays gastric emptying similarly to selective long-acting GLP-1 receptor agonists. *Diabetes Obes. Metab.* 22, 1886–1891. doi:10.1111/dom.14110

Willard, F. S., Douros, J. D., Gabe, M. B., Showalter, A. D., Wainscott, D. B., Suter, T. M., et al. (2020). Tirzepatide is an imbalanced and biased dual GIP and GLP-1 receptor agonist. *CI Insight* 5 (17), e140532. doi:10.1172/jci.insight.140532

Williams, M. E., and Garg, R. (2014). Glycemic management in ESRD and earlier stages of CKD. *Am. J. Kidney Dis.* 63 (2), S22–S38. doi:10.1053/j.ajkd.2013.10.049

Yajima, T., Yajima, K., Hayashi, M., Takahashi, H., and Yasuda, K. (2018). Improved glycemic control with once-weekly dulaglutide in addition to insulin therapy in type 2 diabetes mellitus patients on hemodialysis evaluated by continuous glucose monitoring. *J. Diabetes Complicat.* 32 (3), 310–315. doi:10.1016/J.JDIACOMP.2017.12.005

Zhou, Q., Lei, X., Fu, S., Liu, P., Long, C., Wang, Y., et al. (2023). Efficacy and safety of tirzepatide, dual GLP-1/GIP receptor agonists, in the management of type 2 diabetes: a systematic review and meta-analysis of randomized controlled trials. *Diabetol. Metab. Syndr.* 15, 222. doi:10.1186/s13098-023-01198-4

frontiers | Frontiers in Endocrinology

TYPE Systematic Review
PUBLISHED 04 July 2024
DOI 10.3389/fendo.2024.1429261

Check for updates

OPEN ACCESS

EDITED BY
Divya Bhatia,
NewYork-Presbyterian, United States

REVIEWED BY
Aditya Yashwant Sarode,
Columbia University, United States
Mineaki Kitamura,
Nagasaki University, Japan

*CORRESPONDENCE
Yao-Guang Wang
Yaoguangwa@163.com
Xi Zhao
zhaoxi614@163.com

RECEIVED 07 May 2024
ACCEPTED 17 June 2024
PUBLISHED 04 July 2024

CITATION
Yang S-Q, Zhao X, Zhang J, Liu H, Wang Y-H and Wang Y-G (2024) Comparative efficacy and safety of SGLT2is and ns-MRAs in patients with diabetic kidney disease: a systematic review and network meta-analysis. *Front. Endocrinol.* 15:1429261. doi: 10.3389/fendo.2024.1429261

COPYRIGHT
© 2024 Yang, Zhao, Zhang, Liu, Wang 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.

Comparative efficacy and safety of SGLT2is and ns-MRAs in patients with diabetic kidney disease: a systematic review and network meta-analysis

Si-Qi Yang[1,2,3], Xi Zhao[1,2]*, Jing Zhang[1,2], Huan Liu[1,2,3], Yu-Han Wang[1,2,3] and Yao-Guang Wang[1,2]*

[1]Department of Nephrology, First Teaching Hospital of Tianjin University of Traditional Chinese Medicine, Tianjin, China, [2]Department of Nephrology, National Clinical Research Center for Chinese Medicine Acupuncture and Moxibustion, Tianjin, China, [3]Graduate School, Tianjin University of Traditional Chinese Medicine, Tianjin, China

Objectives: To evaluate the efficacy and safety of non-steroid mineralocorticoid receptor antagonists (ns-MRAs) and sodium-glucose cotransporter 2 inhibitors (SGLT2is) in patients with diabetic kidney disease (DKD).

Methods: Systematic literature searches were performed using PubMed, Embase and Web of Science encompassing inception until January 20, 2024. Randomized control trials (RCTs) comparing ns-MRAs and SGLT2is in DKD were selected. The efficacy outcomes of interest included kidney-specific composite outcome, cardiovascular (CV)-specific composite outcome, end-stage kidney disease (ESKD), and overall mortality. We also investigated safety outcomes, including acute kidney injury (AKI) and hyperkalemia.

Results: A total of 10 randomized clinical trials with 35,786 patients applying various treatments were included. SGLT2is (SUCRA 99.84%) have potential superiority in kidney protection. SGLT2is (RR 1.41, 95%CI 1.26 to 1.57) and ns-MRAs (RR 1.17, 95% CI 1.08 to 1.27) were associated with significantly lower kidney-specific composite outcome than the placebo. Regarding the reduction in CV-specific composite outcome and ESKD, SGLT2is (SUCRA 91.61%; 91.38%) have potential superiority in playing cardiorenal protection. Concerning the CV-specific composite outcome (RR 1.27, 95%CI 1.09 to 1.43) and ESKD (RR 1.43, 95% CI 1.20 to 1.72), SGLT2is significantly reduced the risks compared to placebo. Regarding the reduction in overall mortality, SGLT2is (SUCRA 83.03%) have potential superiority in postponing mortality. Concerning the overall mortality, SGLT2is have comparable effects (RR 1.27, 95%CI 1.09 to 1.43) with placebo to reduce the risk of overall mortality compared to placebo. For AKI reduction, ns-MRAs (SUCRA 63.58%) have potential superiority. SGLT2is have comparable effects (RR 1.24, 95%CI 1.05 to 1.46) with placebo to reduce the risk of AKI. For hyperkalemia reduction, SGLT2is (SUCRA 93.12%) have potential superiority. SGLT2is have comparable effects (RR 1.24, 95%CI 1.05 to 1.46) with placebo to reduce the risk of AKI. Concerning hyperkalemia reduction, nsMRAs (RR 1.24 95% CI 0.39 to 3.72) and SGLT2is (RR 1.01 95%CI 0.40 to 3.02) did not show significant benefit compared to placebo.

Conclusion: Concerning the efficacy and safety outcomes, SGLT2is may be recommended as a treatment regimen for maximizing kidney and cardiovascular protection, with a minimal risk of hyperkalemia in DKD.

Systematic review registration: https://www.crd.york.ac.uk/prospero/, identifier CRD42023458613.

KEYWORDS

diabetic kidney disease, sodium-glucose cotransporter-2 inhibitor, non-steroid mineralocorticoid receptor antagonists, network meta-analysis, cardiorenal

1 Introduction

Diabetes mellitus (DM) constitutes a significant threat to public health and presents a major healthcare burden in most countries. Preventive measures and appropriate management are indispensable to preventing long-term complications (1). As reported in the 10th Edition of the Diabetes Atlas by the International Diabetes Federation (IDF), the total number of DM patients (aged 20 to 79) worldwide has reached 537 million as of 2021, with an anticipated increase to 783 million by 2045 (2).

Sustained chronic hyperglycemia leads to vital damage both to macrovascular and microvascular systems, which are the main causes of cardiovascular and cerebrovascular diseases, peripheral artery disease, retinopathy, and nephropathy (3). Approximately 35% of patients with type 2 diabetes (T2D) are projected to develop chronic kidney disease (CKD). Diabetic kidney disease (DKD) is a critical complication of DM, and it is identified as a major cause of end-stage kidney disease (ESKD) (4). The characteristic of DKD is progressive proteinuria. Initially, the glomerular filtration rate (GFR) will gradually decline, resulting in failure eventually. The process is accompanied by capillary injury, mesangial cell proliferation, accumulation of extracellular matrix, thickening of the glomerular basement membrane, loss of podocytes, glomerulosclerosis, and ultimately renal tubulointerstitial fibrosis (5). DKD is a significant cause of ESKD and also serves as a critical risk factor for cardiovascular events and mortality (6). With the rapid rise in the prevalence of T2D worldwide and the significant number of patients developing DKD, there is a considerable economic burden (7). Therefore, it is crucial to slow down the progression through effective medical interventions, considering both a healthcare system and societal perspectives.

At present, the main therapies aimed at slowing down the progression of DKD involve blood pressure lowering, intensive glucose control, and blood lipid regulation, with angiotensin-converting enzyme inhibitors (ACEI) or angiotensin II receptor blockers (ARB) serving as the cornerstone of DKD management (8). The medications assist in mitigating the effects of the renin-angiotensin system, a key role in the onset and progression of DKD. The renoprotective effect of RAS blockade with ACEIs or ARBs is well established (9, 10). DKD patients have predominantly received treatment with RAS inhibitors, despite their limited efficacy, possibly as a result of angiotensin escape and/or aldosterone escape, which leads to an escalation in renin activity subsequent to the prolonged inhibition of renin-angiotensin-aldosterone system (11).

With the emergence of the new class of anti-hyperglycemia medications, ground breaking impulses were given to the treatment of DKD. Increasing evidence supports that employing new classes of medication, such as sodium-glucose cotransporter 2 (SGLT2is) and non-steroid mineralocorticoid receptor antagonist (ns-MRA) could potentially reduce the predefined composite renal outcomes and cardiovascular (CV) outcomes in patients with DKD. SGLT2is act by blocking the carrier protein SGLT2, which leads to glycosuria and subsequently initiates a cascade of positive effects on both the kidneys and cardiovascular system (12). ns-MRAs exhibit a high degree of specificity for mineralocorticoid receptor (MR). Consequently, this selective binding enables the nsMRAs to effectively counteract the effects of aldosterone, a steroid hormone that is believed to play a pivotal role in the progression of DKD (13).

Since there have been few trials directly comparing the efficacy and safety of SGLT2is and ns-MRAs, the debate continues regarding which interventions demonstrate the most significant renal and cardiovascular protective benefits. Network meta-analysis (NMA) serves as an extension of conventional meta-analysis. NMA enables the simultaneous comparison of multiple drug treatments for a disease, allowing for the decision of the most effective

Abbreviations: ns-MRAs, Non-steroid mineralocorticoid receptor antagonists; SGLT2is, Sodium-glucose cotransporter 2 inhibitors; DKD, Diabetic kidney disease; RCTs, Randomized control trials; CV, Cardiovascular; ESKD, End-stage kidney disease; AKI, Acute kidney injury; DM, Diabetes mellitus; IDF, International Diabetes Federation; T2D, Type 2 diabetes; CKD, Chronic kidney disease; GFR, Glomerular filtration rate; ACEI, Angiotensin-converting enzyme inhibitors; ARB, Angiotensin receptor blockers; MR, Mineralocorticoid receptor; NMA, Network meta-analysis; MI, Myocardial infarction; HHF, Hospitalization for heart failure; DIC, Deviance information criterion; SUCRA, Surface under the cumulative ranking curve; RR, Risk ratio; CI, Confidence interval; KDIGO, Kidney Disease: Improving Global Outcomes; GLP-1 RA, Glucagonlike peptide-1 receptor agonists; ADA, American Diabetes Association.

treatment option, in the absence of direct head-to-head evidence. Our study aims to identify the optimal treatment regimen, in conjunction with ACEI or ARB for patients with DKD. The study mainly centers around the following questions:

(1) Which medication should be administered to a patient with DKD to effectively prevent the progression of renal and cardiovascular composite outcomes?

(2) Which medication effectively decreases the risk of overall mortality, the development of end-stage kidney disease, acute kidney injury, and hyperkalemia?

To answer the questions, we performed a systemic review and NMA, to evaluate the efficacy and safety of SGLT2is and ns-MRAs for the treatment of DKD, and to provide an evidence-based basis for future clinical practice, medical decision-making, and research direction. Thus, the study provides an overview of the current state of DKD treatment and offers evidence-based guidance.

2 Methods

The study was carried out in line with the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) 2020 statement (14) and was registered with PROSPERO (CRD 42023458613) https://www.crd.york.ac.uk/prospero/.

2.1 Data sources and search strategies

Database search was conducted using PubMed, Embase and Web of Science without any limitations on language from inception until January 20, 2024. The major search headings included "diabetic kidney disease", "sodium glucose cotransporter 2 inhibitors" and "nonsteroidal mineralocorticoid receptor antagonists". Mesh terms and free terms were searched (Supplementary Table S1).

2.2 Study selection and data extraction

Two reviewers (YSQ and WYH) independently screened the article titles, abstracts, and full texts to identify potential trials according to the following inclusion criteria:

(1) randomized controlled trials (RCTs) that compared SGLT2is or ns-MRAs with placebo or conventional treatment.

(2) DKD was defined as the presence of CKD in individuals with T2D. Adults (aged>18 years) with T2D and CKD. The clinical diagnosis of CKD is based on either of the following criteria:

- -Persistent high albuminuria defined as UACR≥30 mg/g but <300 mg/g and eGFR≥25 but <60 mL/min/1.73 m2 (Chronic Kidney Disease Epidemiology Collaboration);
- -Persistent very high albuminuria defined as UACR≥300 mg/g and eGFR≥25 but <75 mL/min/1.73 m2 (ChronicA Kidney Disease Epidemiology Collaboration).

(3) at least one of these outcomes must be included in the RCT: kidney-specific composite outcome; cardiovascular (CV)-specific composite outcome; ESKD; overall mortality.

Data extraction was conducted by two reviewers (YSQ and WYH), subsequently the members of the study (ZX, ZJ, LH) checked and eventually discussed the extracted data. The extracted information included trial name, registration number, study period, type of study, study drug, mean age, No. of male patients, sample size, outcome data.

2.3 Risk of bias and quality assessment

The Risk-of-Bias 2 tool (The Cochrane Collaboration's tool for assessing risk of bias) will be used to assess and report the bias associated with the individual studies (15).

2.4 Outcomes

The efficacy outcomes assessed in the NMA include:

(1) kidney-specific composite outcome;

(2) CV-specific composite outcome;

(3) ESKD;

(4) overall mortality.

The kidney-specific composite outcome definitions differed among trials (Supplementary Table S2) but generally involved a combination of worsening eGFR or serum creatinine, ESKD with or without renal replacement therapy or transplant, or death from renal causes. The CV-specific composite outcome definitions varied across trials (Supplementary Table S3) but generally included a combination of CV death, nonfatal myocardial infarction (MI), nonfatal stroke or hospitalization for heart failure (HHF).

The safety outcomes assessed in the NMA include: hyperkalemia and acute kidney injury (AKI).

2.5 Statistical analysis calculated for each study to assess the effect sizes

The BUGSnet package in R (version 4.3.3) was utilized for statistical analysis of Bayesian network meta-analysis. Fixed- or random-effects models were selected for each outcome based on the deviance information criterion (DIC), using the model with the smallest value. Notably, a lower DIC value denotes a superior model fit in relation to the sample size. Risk ratios (RRs) was calculated to assess the effect sizes for dichotomous variables. All results are reported with 95% confidence intervals (CIs). The surface under the cumulative ranking curve (SUCRA) and mean ranks were used to rank the treatments for each outcome. A higher SUCRA value signifies that the treatment is more effective with respect to that outcome. As there were no closed loops, direct estimates could not be obtained in this review. Additionally, a P-value of less than 0.05 is indicative of statistical significance.

3 Results

3.1 Characteristics of included studies

The selection process was illustrated in a flowchart based on the PRISMA guidelines. Figure 1 depicts the selection process. Our literature research initially yielded 1526 publications, out of which 10 RCTs (16–32) were found fully eligible, in total including 35,786 patients. The detailed characteristics of included studies are presented in Table 1. The main baseline information of included studies is summarized in Table 2. All RCTs had placebo as the comparator. The leverage plot was drawn for the option of fixed- or random-effects models (Supplementary Figure S1). The forest plot of NMA was drawn for the comparison for the efficacy and safety outcomes (Supplementary Figure S2).

3.2 Risk of bias in included studies

Six RCTs had an overall low risk of bias. Four RCTs had some concerns. Two RCTs had some concerns for deviations from intended interventions (17, 21), two for missing outcome data (17, 26), one for randomization process (26), one for measurement of the outcome (21), one for selection of the reported result (24). The risk of bias assessments of included studies are shown in Figure 2.

3.3 Efficacy outcomes

Figure 3 shows the network of treatment comparisons in available trials. In this network meta-analysis, none of the six

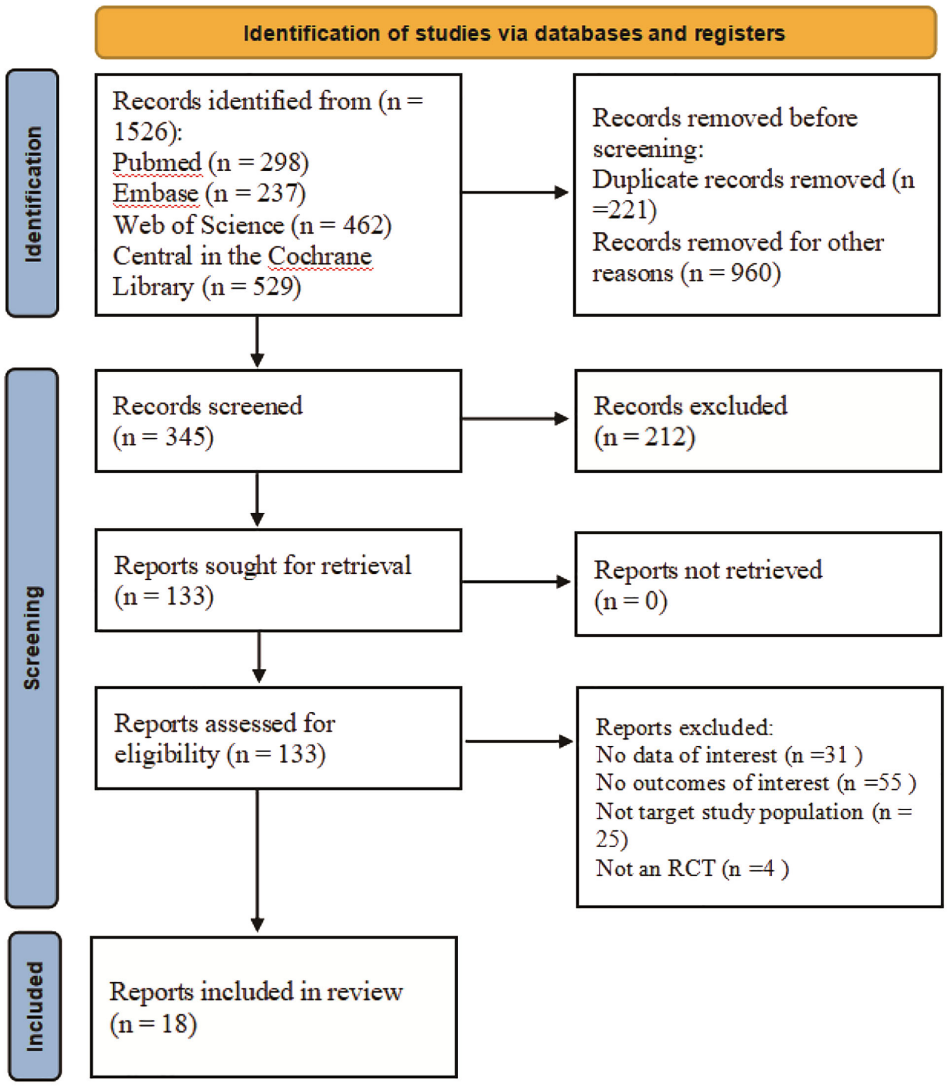


FIGURE 1
Flow diagram of the screening process.

TABLE 1 Characteristics of clinical trials included in the network meta-analysis.

Trial	Sponsor	Country	Follow-up, y	Study phase	Study drug	No. of patients (study group)	Age, y	Sex	Control drug	No. of patients (control group)	Age	Sex
DAPA-CKD	AstraZeneca	21	2.4	phase 3	Dapagliflozin	1455	64.1±9.8	961	placebo	1451	64.7±9.5	980
CREDENCE	Janssen	34	2.62(0.02, 4.53)	phase 3	Canagliflozin	2202	62.9±9.2	1440	placebo	2199	63.2±9.2	1467
CANVAS Program	Janssen	30	3.9	phase 3	Canagliflozin	1322	63.8±8.3	936	placebo	944	64.2±8.3	638
EMPA-REG OUTCOME	Boehringer Ingelheim	42	3.1	phase 3	Empagliflozin	1212	67.1±7.6	816	placebo	607	67.1±8.2	418
SCORED	Sanofi	44	1.18(0.86, 1.58)	phase 3	Sotagliflozin	5292	68.65±8.16	2945	placebo	5292	68.65±8.16	2885
ARTS-DN	Bayer	28	0.25	phase 2B	Finerenone	98	64.94±9.62	77	placebo	94	63.26±8.68	69
FIDELIO-DKD	Bayer	48	2.6	phase 3	Finerenone	2833	65.4±8.9	1953	placebo	2841	65.7±9.2	2030
JapicCTI173695	Daiichi-Sankyo	1	1.1	phase 2B	Esaxerenone	70	64±11	57	placebo	73	66±10	57
ESAX-DN	Daiichi-Sankyo	1	1.1	Phase 3	Esaxerenone	222	66±10	165	placebo	227	66±9	180
FIGARO-DKD	Bayer	48	3.4	phase 3	Finerenone	3686	64.1±9.7	2528	placebo	3666	64.1±10.0	2577

DAPA-CKD, Dapagliflozin and Prevention of Adverse Outcomes in Chronic Kidney Disease; CREDENCE, Canagliflozin and Renal Events in Diabetes with Established Nephropathy Clinical Evaluation; CANVAS, Canagliflozin Cardiovascular Assessment Study; EMPA-REG OUTCOME, Empagliflozin, Cardiovascular Outcomes, and Mortality in Type 2 Diabetes; SCORED, Sotagliflozin Cardiovascular Outcomes Trial in Type 2 Diabetes Mellitus in Patients with Chronic Kidney Disease and Cardiovascular Disease; ARTS-DN, Advanced Renal Therapeutics Study of Diabetic Nephropathy; FIDELIO-DKD, Finerenone in Reducing Kidney Disease and Heart Failure Events in Patients with Type 2 Diabetes Mellitus and Chronic Kidney Disease; JapicCTI173695, Efficacy and Safety of Esaxerenone for the Treatment of Type 2 Diabetes with Microalbuminuria; ESAX-DN, Evaluation of the Effects of Esaxerenone on Renal and Cardiovascular Outcomes in Patients with Type 2 Diabetes Mellitus and Nephropathy; FIGARO-DKD, Finerenone in Reducing Kidney Failure and Disease Progression in Diabetic Kidney Disease; y, years. Sex indicates No. of male patients.
Mean ± SD; Median (interquartile range).

10.3389/fendo.2024.1429261

10.3389/fendo.2024.1429261

TABLE 2 Baseline information of clinical trials included in the network meta-analysis.

Trial	ClinicalTrials.gov number	UACR		eGFR		HbA1c		SBP	
		Study	Control	Study	Control	Study	Control	Study	Control
DAPA-CKD	NCT03036150	#1024.5 (472.5-2111.0)	#1004.5 (493.3-2017.0)	44.0±12.6	43.6±12.6	7.8±1.7	7.8±1.6	138.8±17.6	139.6±17.1
CREDENCE	NCT02065791	#923 (459–1794)	#931 (473–1868)	56.3±18.2	56.0±18.3	8.3±1.3	8.3±1.3	139.8±15.6	140.2±15.6
CANVAS Program	NCT01032639	#67.1 (42.6–127.2)	#69.4 (44.6–120.5)	74.8±20.9	73.9±21.9	8.4±1.0	8.4±0.9	139.4±15.6	139.2±16.2
EMPA-REG OUTCOME	NCT01131676	N	N	48.4±8.2	48.6±7.8	8.07 ±0.86	8.03±0.85	136.1±18.0	136.4±18.7
SCORED	NCT03315143	#74 (18–486)	#75 (17–477)	#44.4 (37.0–51.3)	#44.7 (37.0–51.5)	#8.3 (7.6–9.3)	#8.3 (7.6–9.4)	#138 (127–149)	#139 (127–149)
ARTS-DN	NCT1874431	$249.5 (30.4-3917)	$188.4 (15.0-3056)	67.0±20.9	72.2±20.4	7.7±1.2	7.6±1.3	137.6±14.0	139.9±14.3
FIDELIO-DKD	NCT02540993	N	N	N	N	7.7±1.3	7.7±1.4	138.1±14.3	138.0±14.4
JapicCTI173695	NCT02345057	▲112 (100-126)	▲110 (98-123)	68±19	69±19	7.0±0.6	6.9±0.6	137±13	138±11
ESAX-DN	NCT02345057	$113 (46, 286)	$110 (47, 278)	69±18	69±18	7.0±0.6	7.0±0.6	140±10	140±10
FIGARO-DKD	NCT02545049	#302 (105–749)	#315 (111–731)	67.6±21.7	68.0±21.7	7.7±1.4	7.7±1.4	135.8±14.0	135.7±14.1

DAPA-CKD, Dapagliflozin and Prevention of Adverse Outcomes in Chronic Kidney Disease; CREDENCE, Canagliflozin and Renal Events in Diabetes with Established Nephropathy Clinical Evaluation; CANVAS, Canagliflozin Cardiovascular Assessment Study; EMPA-REG OUTCOME, Empagliflozin, Cardiovascular Outcomes, and Mortality in Type 2 Diabetes; SCORED, Sotagliflozin Cardiovascular Outcomes Trial in Type 2 Diabetes Mellitus in Patients with Chronic Kidney Disease and Cardiovascular Disease; ARTS-DN, Advanced Renal Therapeutics Study of Diabetic Nephropathy; FIDELIO-DKD, Finerenone in Reducing Kidney Disease and Heart Failure Events in Patients with Type 2 Diabetes Mellitus and Chronic Kidney Disease; JapicCTI173695, Efficacy and Safety of Esaxerenone for the Treatment of Type 2 Diabetes with Microalbuminuria; ESAX-DN, Evaluation of the Effects of Esaxerenone on Renal and Cardiovascular Outcomes in Patients with Type 2 Diabetes Mellitus and Nephropathy; FIGARO-DKD, Finerenone in Reducing Kidney Failure and Disease Progression in Diabetic Kidney Disease; UACR, Urine albumin-creatinine ratio; eGFR, Estimated glomerular filtration rate; HbA1c, Hemoglobin glycation; SBP, systolic blood pressure.
Mean ± SD; #Median (interquartile range); $Median (min, max); ▲90%CI.

outcomes had a closed loop. This implies that neither outcomes from direct nor indirect comparisons are considered, which renders the need for assessing coherence redundant.

3.3.1 Kidney-specific composite outcome

The analyses of kidney-specific composite outcome included six studies (four with SGLT2is, two with ns-MRAs), that had enrolled a total of 33,183 participants.

The rankogram (Figure 4A) showed that SGLT2is had the superior probability of reducing the risk of kidney-specific composite outcome (99.84%), followed by ns-MRAs (50.15%). For kidney-specific composite outcome reduction, SGLT2is were most likely to rank best. When compared with placebo, both SGLT2is (RR 1.41, 95% CI 1.26 to 1.57) and ns-MRAs (RR 1.17, 95% CI 1.08 to 1.27) were associated with reductions in kidney-specific composite outcome (Figure 5A). SGLT2is were associated with a lower risk of kidney-specific composite outcome compared with ns-MRAs (RR 1.20, 95% CI 1.05 to 1.38).

3.3.2 CV-specific composite outcome

The analyses of CV-specific composite outcome included eight studies (five with SGLT2is, three with ns-MRAs), that had enrolled a total of 35,194 participants.

The rankogram (Figure 4B) showed that SGLT2is had the superior probability of reducing the risk of CV-specific composite outcome (91.61%), followed by ns-MRAs (55.17%). For CV-specific composite outcome reduction, SGLT2is were most likely to rank best. Compared with the placebo, SGLT2is (RR 1.27, 95% CI 1.09 to 1.43) were associated with reductions in CV-specific composite outcome significantly (Figure 5B). And ns-MRAs (RR 1.14, 95% CI 0.95 to 1.41) were similar in CV-specific composite outcome reduction compared with placebo.

3.3.3 ESKD

The analyses of ESKD included four studies (two with SGLT2is, two with ns-MRAs), that had enrolled a total of 20,333 participants.

The rankogram (Figure 4C) showed that SGLT2is had the superior probability of reducing the risk of ESKD (91.38%), followed by ns-MRAs (57.81%). For ESKD, SGLT2is were most likely to rank best. When compared with placebo, both SGLT2is (RR 1.43, 95% CI 1.20 to 1.72) and ns-MRAs (RR 1.25, 95% CI 1.02 to 1.55) were associated with reductions in ESKD (Figure 5C).

3.3.4 Overall mortality

The analyses of overall mortality included nine studies (five with SGLT2is, four with ns-MRAs), that had enrolled a total of 35,594 participants.

10.3389/fendo.2024.1429261

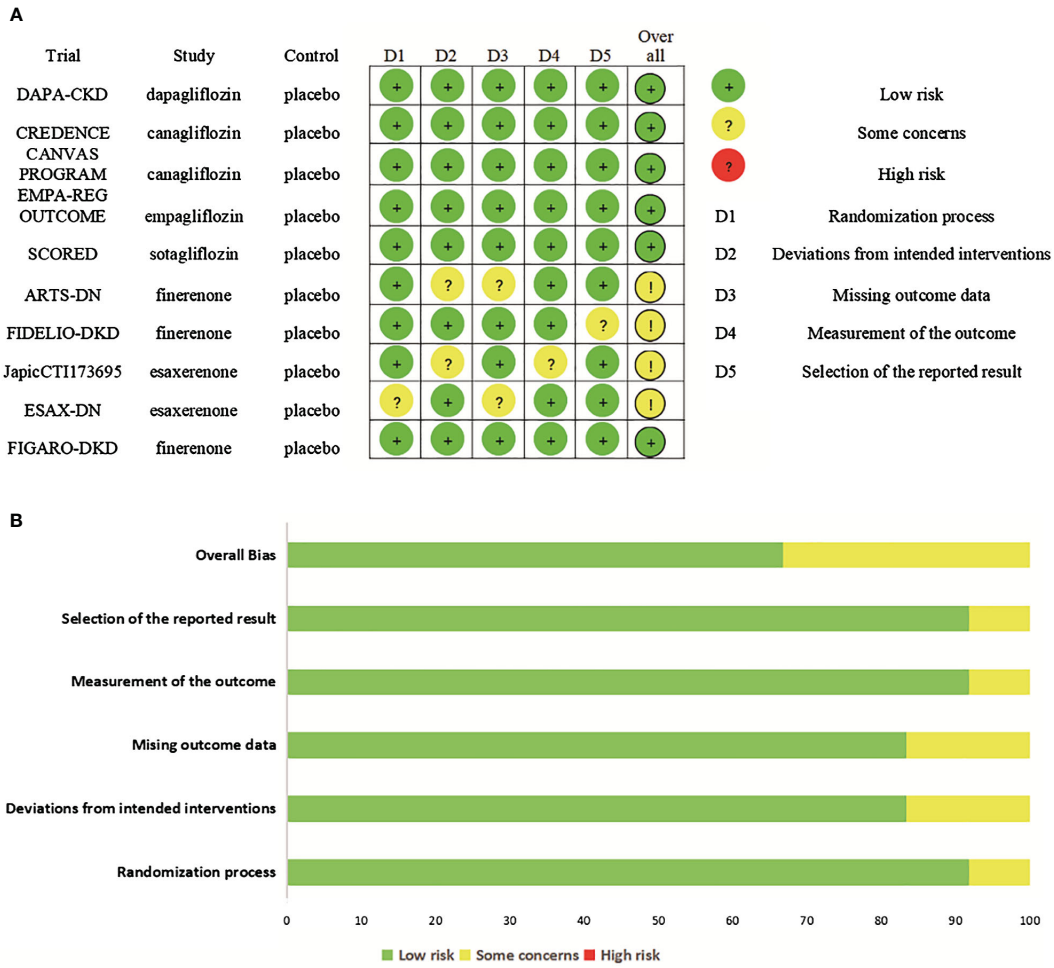


FIGURE 2
Risk of bias. **(A)** Risk of bias summary for included studies, showing each risk of bias item for every included study. **(B)** Risk of bias graph presenting each risk of bias item as percentages across all included studies.

The rankogram (Figure 4D) showed that SGLT2is had the superior probability of reducing the risk of overall mortality (83.03%), followed by ns-MRAs (57.5%). For overall mortality, SGLT2is were most likely to rank best. We found that treatments were similar in overall mortality reduction since compared with placebo, neither of the drugs showed significant changes [SGLT2is (RR 1.19, 95% CI 1.00 to 1.43), ns-MRAs (RR 1.12, 95% CI 0.85 to 1.45)] (Figure 5D).

3.4 Safety outcomes

3.4.1 AKI

The analyses of hyperkalemia included seven studies (four with SGLT2is, three with ns-MRAs), that had enrolled a total of 24,610 participants.

The rankogram (Figure 6A) showed that ns-MRAs had the superior probability of reducing the risk of AKI (63.58%), followed by SGLT2is (44.76%). For AKI reduction, ns-MRAs were most likely to rank best. Compared with the placebo, SGLT2is (RR 1.24, 95% CI 1.05 to 1.46) were associated with reductions in AKI significantly (Figure 7A). And ns-MRAs (RR 1.08, 95% CI 0.90 to 1.28) were similar in AKI reduction compared with placebo.

3.4.2 Hyperkalemia

The analyses of hyperkalemia included nine studies (four with SGLT2is, five with ns-MRAs), that had enrolled a total of 32,880 participants.

The rankogram (Figure 6B) showed that SGLT2is had the superior probability of reducing the risk of hyperkalemia (93.12%), followed by ns-MRAs (46.18%). For hyperkalemia reduction, SGLT2is were most likely to rank best. We found that treatments were similar in hyperkalemia reduction since compared with placebo, neither of the drugs showed significant changes [SGLT2is (RR 1.24, 95% CI 0.39 to 3.72), ns-MRAs (RR 1.01, 95% CI 0.40 to 3.02)] (Figure 7B).

4 Discussion

This Bayesian NMA included 10 RCTs, encompassing 35,786 participants. The efficacy and safety outcomes in the study were

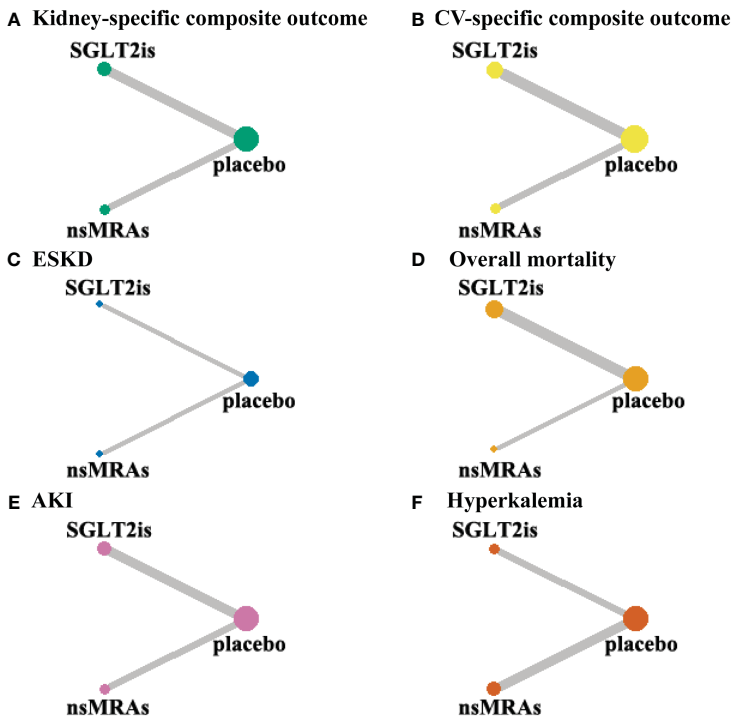


FIGURE 3
Evidence network maps. **(A–F)** were drawn evidence network plots of different treatments for DKD with kidney-specific composite outcome, CV-specific composite outcome, ESKD, overall mortality, AKI, and hyperkalemia as outcomes, respectively. The thickness of line segments in the figure represented the number of studies, that could be directly compared between the two connected interventions, and the size of the points represented the total sample size of the intervention included in the study. SGLT2is, sodium glucose cotransporter 2 inhibitors; nsMRAs, non steroid mineralocorticoid receptor antagonists; CV, cardiovascular; ESKD, end-stage kidney disease; AKI, acute kidney disease.

mainly derived from the DAPA-CKD, CREDENCE, CANVAS Program, EMPA-REG OUTCOME, SCORED, ARTS-DN, FIDELIO-DKD, JapicCTI173695, ESAX-DN, and FIGARO-DKD trials. The main comparison subjects were ns-MRAs (finerenone or esaxerenone) and SGLT2is (canagliflozin, dapagliflozin, empagliflozin or sotagliflozin.

The study compared SGLT2is and ns-MRAs to provide comprehensive evidence of various drug regimens in the DKD population for improving kidney and cardiovascular prognosis. The primary strength of the NMA lies in its targeted focus on DKD patients. This specialized emphasis has facilitated, for the first time, a comparison of the efficacy and safety of innovative antidiabetic drug classes in relation to their impacts on cardiorenal outcomes, specifically within DKD patients.

The findings of the study revealed that, when compared to ns-MRAs, the utilization of SGLT2is resulted in the superior favorable outcomes. SGLT2is have been demonstrated a significant reduction in the risks associated with kidney-specific composite outcome, CV-specific composite outcome, ESKD, overall mortality by 41%, 27%, 43% and 19%, respectively, compared with placebo. While ns-MRAs lowered the risks of kidney-specific composite outcome and ESKD by 17% and 25%, respectively, there was no benefit with regard to the risk of CV-specific composite outcome and overall mortality compared with placebo. As for safety outcomes, the NMA findings suggest that the use of SGLT2is was linked to reduced hyperkalemia; rather, the use of ns-MRAs was associated with reduced AKI. Overall, SGLT2is may be favored over ns-MRAs based on their significant correlation with lower mortality and their additional cardiorenal benefits. SGLT2is ought to be considered an essential addition to the treatment regimen for each patient diagnosed with DKD.

4.1 The potential organ-protective effects of SGLT2is and MRAs

Clinical studies have demonstrated that SGLT2is not only lower blood glucose levels but also have the ability to decrease blood pressure and postpone the onset and/or progression of kidney dysfunction (23, 25, 33). The Kidney Disease: Improving Global Outcomes (KDIGO) diabetes workgroup recommends SGLT2is as a first-line treatment for patients with T2DM who also have CKD (34). The SGLT2is exerts its therapeutic effects by blocking the reabsorption of filtered sodium ions and glucose within the proximal tubule (35). This inhibition increases sodium ion transportation, and the subsequent rise in sodium concentration prompts activation of the tubuloglomerular feedback at the macula densa, which triggers constriction of the afferent arteriole (36). This constriction induces a decline in plasma flow and intra-glomerular capillary pressure, thereby culminating in the reduction of glomerular filtration rate (GFR) observed following the intake of SGLT2is (37).

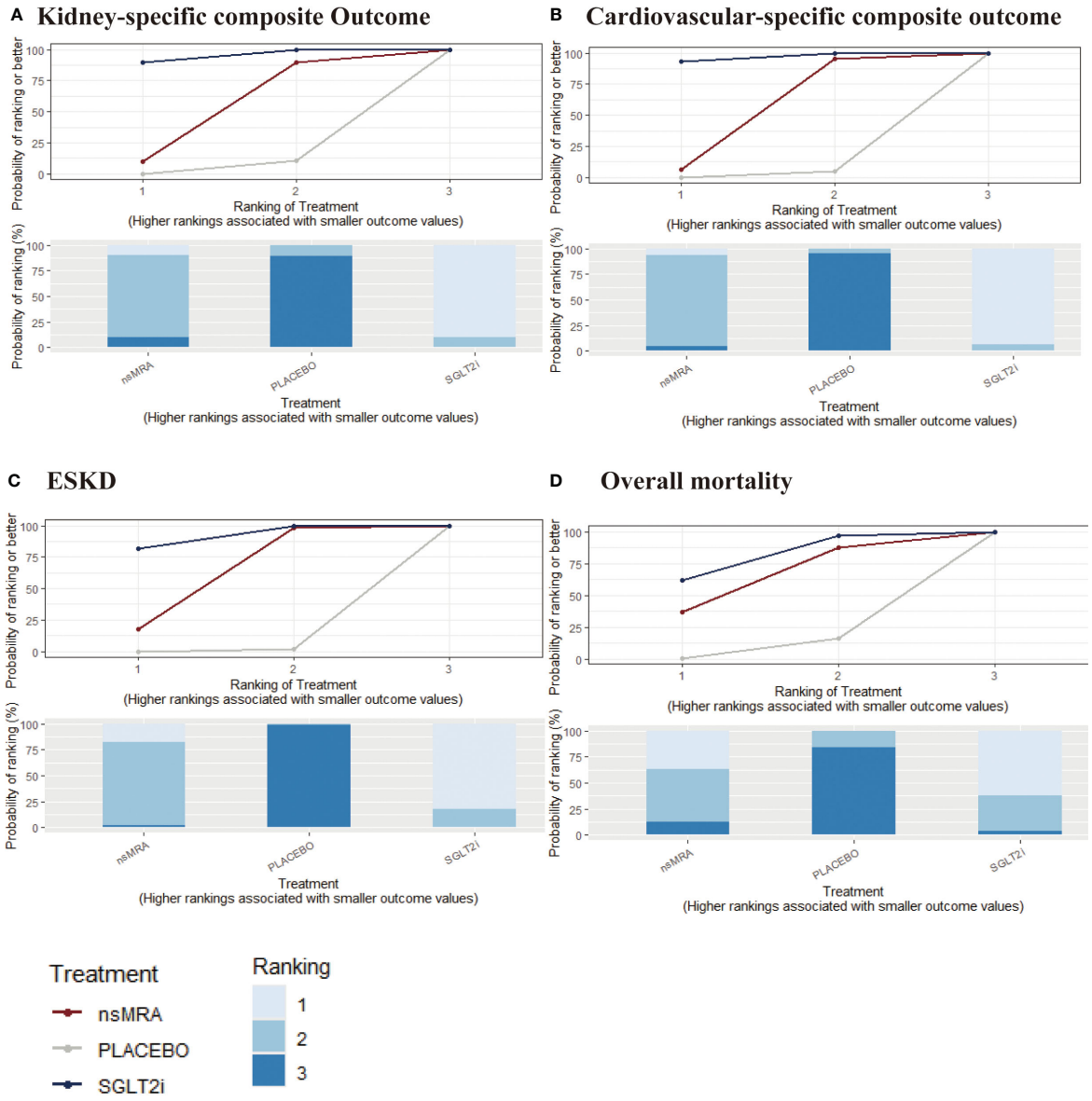


FIGURE 4
SUCRA probabilities for the effectiveness outcomes of interventions. **(A-D)** were drawn SUCRA probabilities plots for the effectiveness outcomes of interventions. **(A)** kidney-specific composite outcome; **(B)** CV-specific composite outcome; **(C)** ESKD; **(D)** overall mortality. A higher SUCRA indicates a lower probability that the drug can reach the endpoint. SGLT2i, sodium glucose cotransporter 2 inhibitor; nsMRA, non steroid mineralocorticoid receptor antagonist; ESKD, end-stage kidney disease.

Patients with T2D are exposed to substantial risks of mortality and the probability of developing complications due to unresolved metabolic, cellular, and inflammatory events (38). Upregulation of the MR has been detected in an assortment of clinical conditions, including hyperglycemia, obesity, insulin resistance, high salt intake, and CKD (39–43). Under pathological conditions, the overactivation of the MR prompts the increase of diverse proinflammatory and profibrotic cytokines (e.g., tumor necrosis factor receptor-α, interleukin-1, fibronectin, and transforming growth factor-α) within the cardiovascular and renal system, ultimately resulting in chronic inflammation and fibrosis (6, 44). Accordingly, MR antagonists have certain benefits in lowering urinary albumin, inhibiting tissue fibrosis, and offering cardiorenal protective effect (44, 45). Traditional MR antagonists, including spironolactone and eplerenone, are not widely used clinically owing to the high incidence of hyperkalemia and gynecomastia (46). Traditional MR antagonists are generally used as diuretics in the management of hypertension, primary aldosteronism, and heart failure (47). Over the past decade, novel and first-in-class ns-MRAs (finerenone, esaxerenone) have been developed and differ significantly with traditional MR antagonists due to their higher selective MRA activity, devoid of androgen antagonism and progesterone agonism (48). The U.S. Food and Drug Administration has granted approval for finerenone to reduce the risks of decline in kidney function, kidney failure,

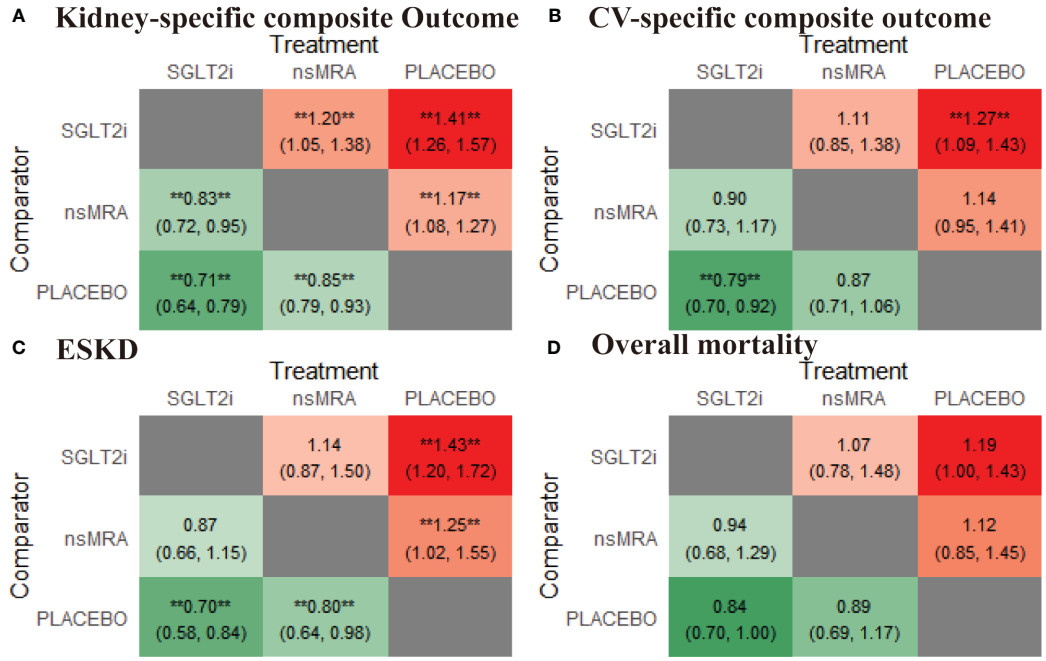


FIGURE 5
Comparative heatmap for effectiveness outcomes of interventions. **(A-D)** drawn comparative heat map of interventions with the kidney-specific composite outcome, CV-specific composite outcome, ESKD, overall mortality. Comparisons between treatments should be read from left to right. The effect size (RR) and 95% confidence interval are in the cell in common between the column-defining treatment and the row-defining treatment. RR>1 favors the row-defining treatment. SGLT2i, sodium glucose cotransporter 2 inhibitor; nsMRA, non steroid mineralocorticoid receptor antagonist; CV, Cardiovascular; ESKD, end-stage kidney disease; RR, Risk ratio. **represents statistical significance.

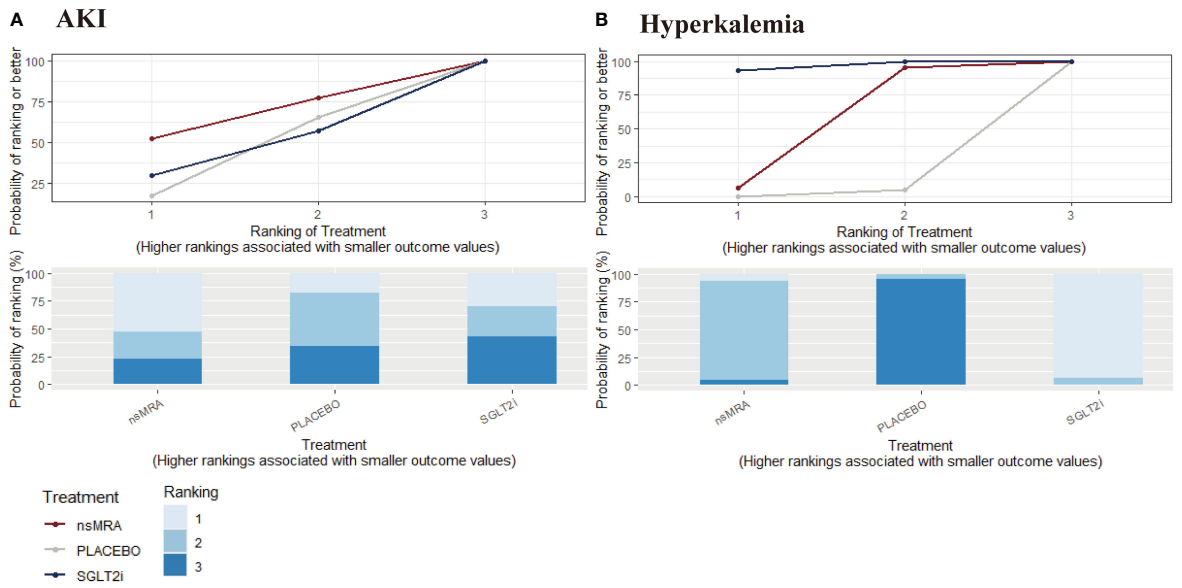


FIGURE 6
SUCRA probabilities for safety outcomes of interventions. **(A, B)** were drawn SUCRA probabilities plots for the safety outcomes of interventions. **(A)** AKI; **(B)** Hyperkalemia. A higher SUCRA indicates a lower probability that the drug can reach the endpoint. SGLT2i, sodium glucose cotransporter 2 inhibitor; nsMRA, non steroid mineralocorticoid receptor antagonist; AKI, acute kidney disease.

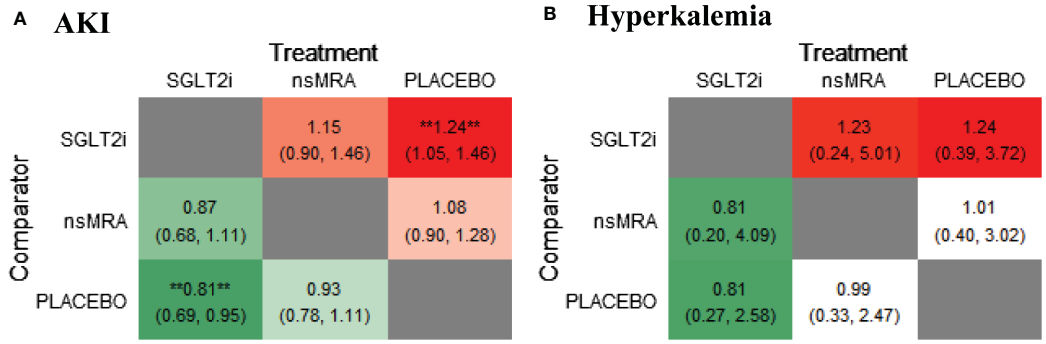


FIGURE 7
Comparative heatmap for safety outcomes of interventions. **(A, B)** drawn comparative heat map of interventions with AKI and hyperkalemia. Comparisons between treatments should be read from left to right. The effect size (RR) and 95% confidence interval are in the cell in common between the column-defining treatment and the row-defining treatment. RR>1 favors the row-defining treatment. SGLT2i, sodium glucose cotransporter 2 inhibitor; nsMRA, non steroid mineralocorticoid receptor antagonist; AKI, Acute kidney injury; RR, Risk ratio. **represents statistical significance.

cardiovascular death, nonfatal myocardial infarctions, and hospitalization for heart failure in adults with CKD associated with T2D (49).

4.2 Advantage of the use of SGLT2is in DKD patients

Based on the available evidence of our study, SGLT2is had the highest SUCRA rank for all efficacy outcomes, indicating that they possess a greater probability than ns-MRAs of reducing risks of both CV and renal outcomes. Obesity is considered to be closely related to insulin resistance. The weight-reducing effect of SGLT2is could improve insulin resistance, regulate energy homeostasis, and thereby exert renal protective effects that go beyond glucose-lowering (50). It has been proposed that SGLT2is maintain the balance of sodium-potassium ion exchange (natriuretic and diuretic effects) and energy metabolism, as well as reduce oxidative stress in cells to provide favorable effects on the risk of renal protection (51, 52). A study by Ravindran et al. (53) suggested that SGLT2is are capable of inhibiting the RAAS activation, playing a role in lowering blood pressure and improving renal hyperfiltration.

4.3 Risks associated with the use of ns-MRAs

Adverse events associated with ns-MRAs are a common concern in clinical practice. For ns-MRAs, some common issues include hyperkalemia, upper respiratory tract infections, pharyngitis, abdominal discomfort, constipation, dizziness, and renal impairment. Among various adverse events, hyperkalemia has always been the major concerning, as the use of ns-MRAs could lead to decreased potassium excretion due to the blockade of aldosterone receptors, resulting in an elevated level of serum potassium (54). Although the incidence of hyperkalemia has increased compared to the placebo, there is study showing that the risk of ns-MRAs is lower than that of traditional steroidal MRA (55). In addition, as some trials included a combination of RAS inhibitors, there is a possibility of increased blood potassium changes. Studies have found that serum potassium elevation most commonly occurs in subsets with low baseline eGFR or high baseline potassium levels. It has been observed that there tends to be a generally stable return to baseline blood potassium levels by the final stages of treatment, or upon treatment completion (56, 57).

4.4 The related meta-analysis

In a comprehensive meta-analysis (58), it was demonstrated that the utilization of SGLT2is can lead to a significant reduction in glycated hemoglobin, blood pressure, body weight, and proteinuria levels. Furthermore, these outcomes have been shown to postpone the decline in the eGFR (with a significant difference when compared to placebo: 1.35 95% CI: 0.78-1.93). Additionally, the study revealed that the risks of renal composite outcome, encompassing the doubling of serum creatinine levels, ESKD, or renal death, were considerably reduced in patients treated with SGLT2is (RR 0.71, 95% CI: 0.53-0.95). Compared to patients who did not use SGLT2is, those who did see a delayed decline in GFR slope. This conclusion also effectively explains the renal protection of SGLT2is.

A NMA (59) based on 18 large trials comparing SGLT2i, finerenone, and glucagonlike peptide-1 receptor agonists (GLP-1 RA) found finerenone can decrease the risk of major adverse cardiovascular events, renal outcome, and HHF, along with the tendency to reduce all-cause death in patients with T2DM and CKD. GLP-1 RA showed a marked impact on reducing cardiovascular events when juxtaposed with exendin-4 analogues. The study further elucidated that finerenone boasts the same potency as SGLT2is in lowering MACE risk. The inconsistency observed may be credited to the different definition of CV- specific composite outcome, specifically the inclusion of unstable angina, or without trials of ESAX-DN.

A comprehensive NMA (60) comprising data from 42346 patients with DKD, had compared all treatments for DKD that

have been used by nephrologists over the last two decades. The study found SGLT2is should be a crucial part of the treatment of DKD patients alongside either ACEI or ARB, concerning mortality and ESKD. The finding aligns with the results observed in our study.

4.5 Limitations

Our study has certain limitations that warrant further discussion. First, our search was confined to placebo-controlled trials only. To date, there have been no RCTs that focus on kidney or cardiovascular outcomes to directly compare the efficacy of SGLT2is and ns-MRAs in patients diagnosed with DKD. Additionally, the absence of closed-loop meta-analyses resulted in findings from our network meta-analysis producing limited insights.

Second, it is of note that FIDELIO-DKD and FIGARO-DKD trials permitted the concurrent administration of finerenone in conjunction with SGLT2is (with 6.4% of patients receiving SGLT2is at baseline), whereas combination therapy with SGLT2is and ns-MRA was limited in CREDENCE and DAPA-CKD trials (as baseline ns-MRA use was prohibited). This could potentially influence the difference in renal and cardiac outcomes between the two agents.

Third, the follow-up period for the trials included varied, ranging from 12 to 104 weeks. Therefore, there may be some differences in kidney-specific composite outcome in patients with DKD in this study.

Finally, there were notable differences in baseline levels of HbA1c and eGFR between patients receiving SGLT2is and ns-MRA treatments. The absence of pooled individual patient data and the small number of eligible trials restricted our capacity to conduct substantial subgroup analyses based on factors like the stage of DKD, the presence of albuminuria. Additionally, since most trials included in this meta-analysis excluded participants with eGFR<25 ml/min/1.73 m^2, our findings may not be applicable to patients with severe renal insufficiency. Future studies are required to clarify the effectiveness of ns-MRAs and SGLT2is in individuals with diverse clinical profiles.

5 Conclusion

The latest Standards of Medical Care in Diabetes guidelines from the American Diabetes Association (ADA) recommend the use of SGLT2is in patients with stage 3 CKD or higher and T2DM regardless of glycemic control, to slow the progression of CKD and reduce CV and renal risks. Our data lend further support to ADA and KDIGO guidelines, which suggest that SGLT2is are needed in DKD.

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.

Author contributions

S-QY: Data curation, Methodology, Visualization, Writing – original draft. XZ: Conceptualization, Funding acquisition, Supervision, Writing – review & editing. JZ: Data curation, Writing – review & editing. HL: Data curation, Writing – review & editing. Y-HW: Data curation, Methodology, Writing – review & editing. Y-GW: Funding acquisition, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This work was supported by National Natural Science Foundation of China (grant numbers 81603544, 81573888), National Famous Traditional Chinese Medicine Expert Inheritance Studio (grant numbers 978022) and Inheritance and Development of Traditional Chinese Medicine: Famous Traditional Chinese Medicine Expert Inheritance Studio of Tianjin (grant numbers 883022).

Acknowledgments

We would like to sincerely express our gratitude for the support of all participants in this study.

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.

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/fendo.2024.1429261/full#supplementary-material

References

1. ElSayed NA, Aleppo G, Aroda VR, Bannuru RR, Brown FM, Bruemmer D, et al. Summary of revisions: standards of care in diabetes—2023. *Diabetes Care*. (2022) 46: S5–9. doi: 10.2337/dc23-Srev

2. Ogurtsova K, Guariguata L, Barengo NC, Ruiz PL-D, Sacre JW, Karuranga S, et al. IDF diabetes Atlas: Global estimates of undiagnosed diabetes in adults for 2021. *Diabetes Res Clin Pract*. (2022) 183:109118. doi: 10.1016/j.diabres.2021.109118

3. Elbatreek MH, PaChado MP, Cuadrado A, Jandeleit-Dahm K, Schmidt HHHW. Reactive oxygen comes of age: mechanism-based therapy of diabetic end-organ damage. *Trends Endocrinol Metab TEM*. (2019) 30:312–27. doi: 10.1016/j.tem.2019.02.006

4. Lim AK. Diabetic nephropathy - complications and treatment. *Int J Nephrol. Renov. Dis*. (2014) 7:361–81. doi: 10.2147/IJNRD.S40172

5. Barrera-Chimal J, Jaisser F. Pathophysiologic mechanisms in diabetic kidney disease: A focus on current and future therapeutic targets. *Diabetes Obes Metab*. (2020) 22 Suppl 1:16–31. doi: 10.1111/dom.13969

6. Alicic RZ, Rooney MT, Tuttle KR. Diabetic kidney disease: challenges, progress, and possibilities. *Clin J Am Soc Nephrol. CJASN*. (2017) 12:2032–45. doi: 10.2215/CJN.11491116

7. Vupputuri S, Kimes TM, Calloway MO, Christian JB, Bruhn D, Martin AA, et al. The economic burden of progressive chronic kidney disease among patients with type 2 diabetes. *J Diabetes Complications*. (2014) 28:10–6. doi: 10.1016/j.jdiacomp.2013.09.014

8. Barrera-Chimal J, Prince S, Fadel F, El Moghrabi S, Warnock DG, Kolkhof P, et al. Sulfenic acid modification of endothelin B receptor is responsible for the benefit of a nonsteroidal mineralocorticoid receptor antagonist in renal ischemia. *J Am Soc Nephrol. JASN*. (2016) 27:398–404. doi: 10.1681/ASN.2014121216

9. Ruggenenti P, Cravedi P, Remuzzi G. The RAAS in the pathogenesis and treatment of diabetic nephropathy. *Nat Rev Nephrol*. (2010) 6:319–30. doi: 10.1038/nrneph.2010.58

10. Warren AM, Knudsen ST, Cooper ME. Diabetic nephropathy: an insight into molecular mechanisms and emerging therapies. *Expert Opin Ther Targets*. (2019) 23:579–91. doi: 10.1080/14728222.2019.1624721

11. Bell DSH, Jerkins T. The potential for improved outcomes in the prevention and therapy of diabetic kidney disease through 'stacking' of drugs from different classes. *Diabetes Obes Metab*. (2024) 26:2046–53. doi: 10.1111/dom.15559

12. Maltese G, Koufakis T, Kotsa K, Karalliedde J. Can sodium-glucose cotransporter 2 inhibitors "spin the thread of life"? *Trends Endocrinol Metab TEM*. (2023) 34:1–4. doi: 10.1016/j.tem.2022.10.002

13. Grune J, Beyhoff N, Smeir E, Chudek R, Blumrich A, Ban Z, et al. Selective mineralocorticoid receptor cofactor modulation as molecular basis for Finerenone's antifibrotic activity. *Hypertens Dallas Tex 1979*. (2018) 71:599–608. doi: 10.1161/HYPERTENSIONAHA.117.10360

14. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ*. (2021) 372:n71. doi: 10.1136/bmj.n71

15. Higgins JPT, TThomas J, Chandler J, Cumpston M, Li T, Page MJ, et al. *Cochrane Handbook for Systematic Reviews of Interventions Version 6.2* (2021). Available online at: www.training.cochrane.org/handbook (Accessed February, 2021).

16. Pitt B, Kober L, Ponikowski P, Gheorghiade M, Filippatos G, Krum H, et al. Safety and tolerability of the novel non-steroidal mineralocorticoid receptor antagonist BAY 94-8862 in patients with chronic heart failure and mild or moderate chronic kidney disease: a randomized, double-blind trial. *Eur Heart J*. (2013) 34:2453–63. doi: 10.1093/eurheartj/eht187

17. Bakris GL, Agarwal R, Chan JC, Cooper ME, Gansevoort RT, Haller H, et al. Effect of finerenone on albuminuria in patients with diabetic nephropathy: A randomized clinical trial. *JAMA*. (2015) 314:884–94. doi: 10.1001/jama.2015.10081

18. Katayama S, Yamada D, Nakayama M, Yamada T, Myoishi M, Kato M, et al. A randomized controlled study of finerenone versus placebo in Japanese patients with type 2 diabetes mellitus and diabetic nephropathy. *J Diabetes Complications*. (2017) 31:758–65. doi: 10.1016/j.jdiacomp.2016.11.021

19. Neuen BL, Ohkuma T, Neal B, Matthews DR, de Zeeuw D, Mahaffey KW, et al. Cardiovascular and renal outcomes with Canagliflozin according to baseline kidney function. *Circulation*. (2018) 138:1537–50. doi: 10.1161/CIRCULATIONAHA.118.035901

20. Bakris GL, Agarwal R, Anker SD, Pitt B, Ruilope LM, Nowack C, et al. Design and baseline characteristics of the finerenone in reducing kidney failure and disease progression in diabetic kidney disease trial. *Am J Nephrol*. (2019) 50:333–44. doi: 10.1159/000503713

21. Ito S, Shikata K, Nangaku M, Okuda Y, Sawanobori T. Efficacy and safety of esaxerenone (CS-3150) for the treatment of type 2 diabetes with microalbuminuria: A randomized, double-blind, placebo-controlled, phase II trial. *Clin J Am Soc Nephrol. CJASN*. (2019) 14:1161–72. doi: 10.2215/CJN.14751218

22. Neuen BL, Ohkuma T, Neal B, Matthews DR, de Zeeuw D, Mahaffey KW, et al. Effect of canagliflozin on renal and cardiovascular outcomes across different levels of albuminuria: data from the CANVAS program. *J Am Soc Nephrol. JASN*. (2019) 30:2229–42. doi: 10.1681/ASN.2019010064

23. Perkovic V, Jardine MJ, Neal B, Bompoint S, Heerspink HJL, Charytan DM, et al. Canagliflozin and renal outcomes in type 2 diabetes and nephropathy. *N Engl J Med*. (2019) 380:2295–306. doi: 10.1056/NEJMoa1811744

24. Bakris GL, Agarwal R, Anker SD, Pitt B, Ruilope LM, Rossing P, et al. Effect of finerenone on chronic kidney disease outcomes in type 2 diabetes. *N Engl J Med*. (2020) 383:2219–29. doi: 10.1056/NEJMoa2025845

25. Heerspink HJL, Stefánsson BV, Correa-Rotter R, Chertow GM, Greene T, Hou F-F, et al. Dapagliflozin in patients with chronic kidney disease. *N Engl J Med*. (2020) 383:1436–46. doi: 10.1056/NEJMoa2024816

26. Ito S, Kashihara N, Shikata K, Nangaku M, Wada T, Okuda Y, et al. Esaxerenone (CS-3150) in patients with type 2 diabetes and microalbuminuria (ESAX-DN). *Clin J Am Soc Nephrol. CJASN*. (2020) 15:1715–27. doi: 10.2215/CJN.06870520

27. Jardine MJ, Zhou Z, Mahaffey KW, Oshima M, Agarwal R, Bakris G, et al. Renal, cardiovascular, and safety outcomes of canagliflozin by baseline kidney function: A secondary analysis of the CREDENCE randomized trial. *J Am Soc Nephrol. JASN*. (2020) 31:1128–39. doi: 10.1681/ASN.2019111168

28. Bhatt DL, Szarek M, Pitt B, Cannon CP, Leiter LA, McGuire DK, et al. Sotagliflozin in patients with diabetes and chronic kidney disease. *N Engl J Med*. (2021) 384:129–39. doi: 10.1056/NEJMoa2030186

29. Pitt B, Filippatos G, Agarwal R, Anker SD, Bakris GL, Rossing P, et al. Cardiovascular events with finerenone in kidney disease and type 2 diabetes. *N Engl J Med*. (2021) 385:2252–63. doi: 10.1056/NEJMoa2110956

30. Wanner C, Inzucchi SE, Lachin JM, Fitchett D, von Eynatten M, Mattheus M, et al. Empagliflozin and progression of kidney disease in type 2 diabetes. *N Engl J Med*. (2016) 375:323–34. doi: 10.1056/NEJMoa1515920

31. Wanner C, Inzucchi SE, Zinman B, Koitka-Weber A, Mattheus M, George JT, et al. Consistent effects of empagliflozin on cardiovascular and kidney outcomes irrespective of diabetic kidney disease categories: Insights from the EMPA-REG OUTCOME trial. *Diabetes Obes Metab*. (2020) 22:2335–47. doi: 10.1111/dom.14158

32. Wheeler DC, Stefánsson BV, Jongs N, Chertow GM, Greene T, Hou FF, et al. Effects of dapagliflozin on major adverse kidney and cardiovascular events in patients with diabetic and non-diabetic chronic kidney disease: a prespecified analysis from the DAPA-CKD trial. *Lancet Diabetes Endocrinol*. (2021) 9:22–31. doi: 10.1016/S2213-8587(20)30369-7

33. Zinman B, Lachin JM, Inzucchi SE. Empagliflozin, cardiovascular outcomes, and mortality in type 2 diabetes. *N Engl J Med*. (2016) 374:1094. doi: 10.1056/NEJMc1600827

34. Kidney Disease: Improving Global Outcomes (KDIGO) Diabetes Work Group. KDIGO 2020 clinical practice guideline for diabetes management in chronic kidney disease. *Kidney Int*. (2020) 98:S1–S115. doi: 10.1016/j.kint.2020.06.019

35. DeFronzo RA, Reeves WB, Awad AS. Pathophysiology of diabetic kidney disease: impact of SGLT2 inhibitors. *Nat Rev Nephrol*. (2021) 17:319–34. doi: 10.1038/s41581-021-00393-8

36. Vallon V, Thomson SC. Renal function in diabetic disease models: the tubular system in the pathophysiology of the diabetic kidney. *Annu Rev Physiol*. (2012) 74:351–75. doi: 10.1146/annurev-physiol-020911-153333

37. Ruggenenti P, Porrini EL, Gaspari F, Motterlini N, Cannata A, Carrara F, et al. Glomerular hyperfiltration and renal disease progression in type 2 diabetes. *Diabetes Care*. (2012) 35:2061–8. doi: 10.2337/dc11-2189

38. Babel RA, Dandekar MP. A review on cellular and molecular mechanisms linked to the development of diabetes complications. *Curr Diabetes Rev*. (2021) 17:457–73. doi: 10.2174/1573399816666201103143818

39. Shibata S, Nagase M, Yoshida S, Kawarazaki W, Kurihara H, Tanaka H, et al. Modification of mineralocorticoid receptor function by Rac1 GTPase: implication in proteinuric kidney disease. *Nat Med*. (2008) 14:1370–6. doi: 10.1038/nm.1879

40. Garg R, Hurwitz S, Williams GH, Hopkins PN, Adler GK. Aldosterone production and insulin resistance in healthy adults. *J Clin Endocrinol Metab*. (2010) 95:1986–90. doi: 10.1210/jc.2009-2521

41. Bădilă E. The expanding class of mineralocorticoid receptor modulators: New ligands for kidney, cardiac, vascular, systemic and behavioral selective actions. *Acta Endocrinol Buchar. Rom*. (2020) 16:487–96. doi: 10.4183/aeb.2020.487

42. Griesler B, Schuelke C, Uhlig C, Gadasheva Y, Grossmann C. Importance of micromilieu for pathophysiologic mineralocorticoid receptor activity-when the mineralocorticoid receptor resides in the wrong neighborhood. *Int J Mol Sci*. (2022) 23:12592. doi: 10.3390/ijms232012592

43. Patera F, Gatticchi L, Cellini B, Chiasserini D, Reboldi G. Kidney fibrosis and oxidative stress: from molecular pathways to new pharmacological opportunities. *Biomolecules*. (2024) 14:137. doi: 10.3390/biom14010137

44. Mende CW, Samarakoon R, Higgins PJ. Mineralocorticoid receptor-associated mechanisms in diabetic kidney disease and clinical significance of mineralocorticoid receptor antagonists. *Am J Nephrol*. (2023) 54:50–61. doi: 10.1159/000528783

45. Lo KB, Rangaswami J, Vaduganathan M. Non-steroidal mineralocorticoid receptor antagonists and cardiorenal outcomes in chronic kidney disease. *Nephrol.*

Dial. Transplant. Off Publ. Eur Dial. Transpl. Assoc - Eur Ren. Assoc. (2023) 38:845–54. doi: 10.1093/ndt/gfac322

46. Chung EY, Ruospo M, Natale P, Bolignano D, Navaneethan SD, Palmer SC, et al. Aldosterone antagonists in addition to renin angiotensin system antagonists for preventing the progression of chronic kidney disease. *Cochrane Database Syst Rev*. (2020) 10:CD007004. doi: 10.1002/14651858.CD007004.pub4

47. Agarwal R, Kolkhof P, Bakris G, Bauersachs J, Haller H, Wada T, et al. Steroidal and non-steroidal mineralocorticoid receptor antagonists in cardiorenal medicine. *Eur Heart J*. (2021) 42:152–61. doi: 10.1093/eurheartj/ehaa736

48. Verma A, Patel AB. Finerenone: A non-steroidal mineralocorticoid receptor blocker for diabetic kidney disease. *Trends Endocrinol Metab TEM*. (2021) 32:261–3. doi: 10.1016/j.tem.2021.02.002

49. Research, C. for D.E. *FDA approves drug to reduce risk of serious kidney and heart complications in adults with chronic kidney disease associated with type 2 diabetes*. FDA (2021). Available online at: https://www.fda.gov/drugs/news-events-human-drugs/fda-approves-drug-reduce-risk-serious-kidney-and-heart-complications-adults-chronic-kidney-disease.

50. Kabir M, Bergman RN, Porter J, Stefanovski D, Paszkiewicz RL, Piccinini F, et al. Dapagliflozin prevents abdominal visceral and subcutaneous adipose tissue dysfunction in the insulin-resistant canine model. *Obes Silver Spring Md*. (2023) 31:1798–811. doi: 10.1002/oby.23771

51. Griffin M, Rao VS, Ivey-Miranda J, Fleming J, Mahoney D, Maulion C, et al. Empagliflozin in heart failure: diuretic and cardiorenal effects. *Circulation*. (2020) 142:1028–39. doi: 10.1161/CIRCULATIONAHA.120.045691

52. Petrie MC, Verma S, Docherty KF, Inzucchi SE, Anand I, Belohlávek J, et al. Effect of dapagliflozin on worsening heart failure and cardiovascular death in patients with heart failure with and without diabetes. *JAMA*. (2020) 323:1353–68. doi: 10.1001/jama.2020.1906

53. Ravindran S, Munusamy S. Renoprotective mechanisms of sodium-glucose co-transporter 2 (SGLT2) inhibitors against the progression of diabetic kidney disease. *J Cell Physiol*. (2022) 237:1182–205. doi: 10.1002/jcp.30621

54. Cohen S, Sternlicht H, Bakris GL. Mineralocorticoid receptor antagonists in the treatment of diabetic kidney disease: their application in the era of SGLT2 inhibitors and GLP-1 receptor agonists. *Curr Diab Rep*. (2022) 22:213-8. doi: 10.1007/s11892-022-01461-4

55. Patel V, Joharapurkar A, Jain M. Role of mineralocorticoid receptor antagonists in kidney diseases. *Drug Dev Res*. (2021) 82:341–63. doi: 10.1002/ddr.21760

56. Barrera-Chimal J, Kolkhof P, Lima-Posada I, Joachim A, Rossignol P, Jaisser F. Differentiation between emerging non-steroidal and established steroidal mineralocorticoid receptor antagonists: head-to-head comparisons of pharmacological and clinical characteristics. *Expert Opin Investig Drugs*. (2021) 30:1141–57. doi: 10.1080/13543784.2021.2002844

57. Grossmann C, Almeida-Prieto B, Nolze A, Alvarez de la Rosa D. Structural and molecular determinants of mineralocorticoid receptor signalling. *Br J Pharmacol*. (2022) 179:3103–18. doi: 10.1111/bph.15746

58. Toyama T, Neuen BL, Jun M, Ohkuma T, Neal B, Jardine MJ, et al. Effect of SGLT2 inhibitors on cardiovascular, renal and safety outcomes in patients with type 2 diabetes mellitus and chronic kidney disease: A systematic review and meta-analysis. *Diabetes Obes Metab*. (2019) 21:1237–50. doi: 10.1111/dom.13648

59. Zhang Y, Jiang L, Wang J, Wang T, Chien C, Huang W, et al. Network meta-analysis on the effects of finerenone versus SGLT2 inhibitors and GLP-1 receptor agonists on cardiovascular and renal outcomes in patients with type 2 diabetes mellitus and chronic kidney disease. *Cardiovasc Diabetol*. (2022) 21:232. doi: 10.1186/s12933-022-01676-5

60. Büttner F, Barbosa CV, Lang H, Tian Z, Melk A, Schmidt BMW. Treatment of diabetic kidney disease. A network meta-analysis. *PloS One*. (2023) 18:e0293183. doi: 10.1371/journal.pone.0293183

frontiers | Frontiers in Pharmacology

TYPE Original Research
PUBLISHED 17 July 2024
DOI 10.3389/fphar.2024.1379821

Check for updates

OPEN ACCESS

EDITED BY
Giuseppe Remuzzi,
Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Italy

REVIEWED BY
Syed Anees Ahmed,
East Carolina University, United States
Quan Hong,
Chinese PLA General Hospital, China

*CORRESPONDENCE
Yanyan Zhao,
fcczhaoyy1@zzu.edu.cn

†These authors have contributed equally to this work

RECEIVED 31 January 2024
ACCEPTED 24 June 2024
PUBLISHED 17 July 2024

CITATION
Yang T, Feng Q, Shao M, Pan M, Guo F, Song Y, Huang F, Linlin Z, Wang J, Wu L, Qin G and Zhao Y (2024), The role of metabolic memory in diabetic kidney disease: identification of key genes and therapeutic targets.
Front. Pharmacol. 15:1379821.
doi: 10.3389/fphar.2024.1379821

COPYRIGHT
© 2024 Yang, Feng, Shao, Pan, Guo, Song, Huang, Linlin, Wang, Wu, Qin and Zhao. 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 role of metabolic memory in diabetic kidney disease: identification of key genes and therapeutic targets

Tongyue Yang[1†], Qi Feng[2,3†], Mingwei Shao[1], Mengxing Pan[1], Feng Guo[1], Yi Song[1], Fengjuan Huang[1], Zhao Linlin[1], Jiao Wang[1], Lina Wu[1], Guijun Qin[1] and Yanyan Zhao[1*]

[1]Division of Endocrinology, Department of Internal Medicine, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, China, [2]Traditional Chinese Medicine Integrated Department of Nephrology, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, China, [3]Research Institute of Nephrology, The First Affiliated Hospital of Zhengzhou University, Zhengzhou University, Zhengzhou, China

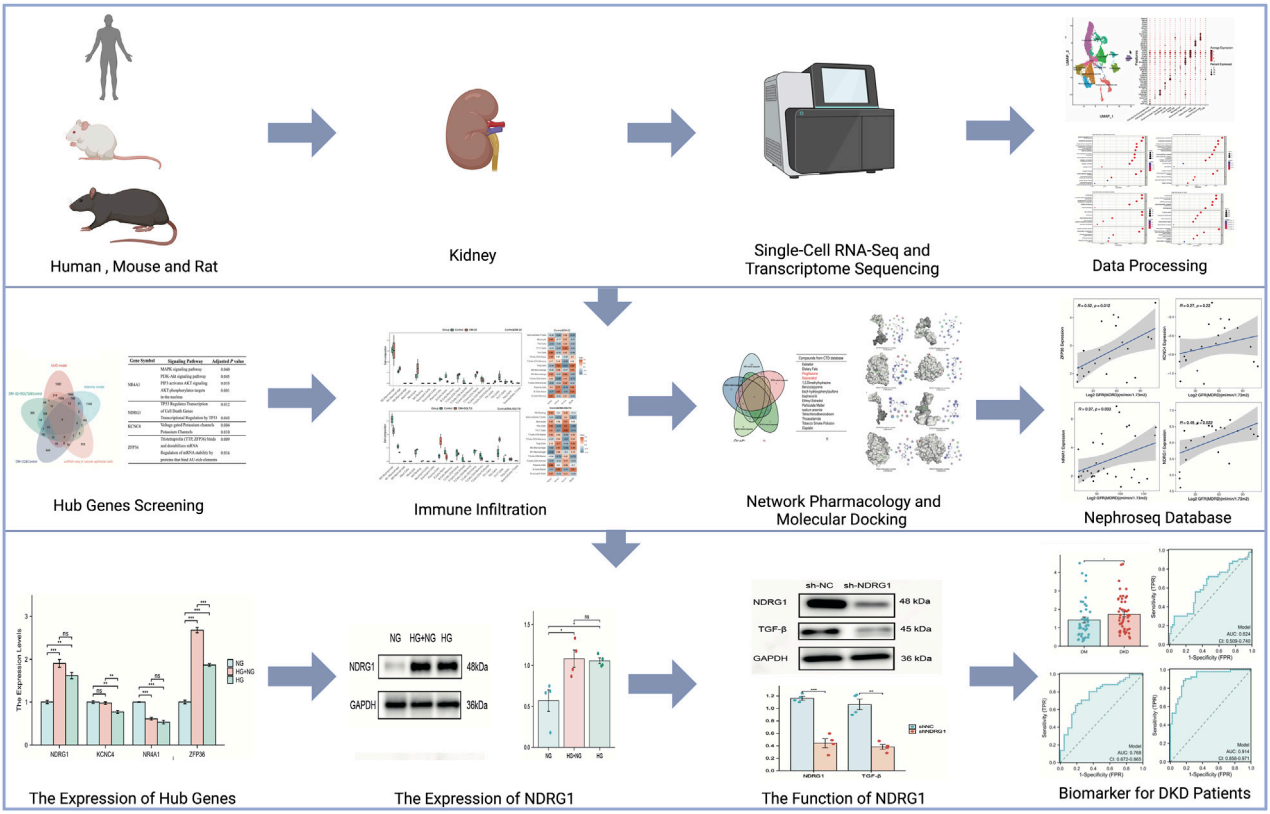
Diabetic kidney disease (DKD) is characterized by complex pathogenesis and poor prognosis; therefore, an exploration of novel etiological factors may be beneficial. Despite glycemic control, the persistence of transient hyperglycemia still induces vascular complications due to metabolic memory. However, its contribution to DKD remains unclear. Using single-cell RNA sequencing data from the Gene Expression Omnibus (GEO) database, we clustered 12 cell types and employed enrichment analysis and a cell–cell communication network. Fibrosis, a characteristic of DKD, was found to be associated with metabolic memory. To further identify genes related to metabolic memory and fibrosis in DKD, we combined the above datasets from humans with a rat renal fibrosis model and mouse models of metabolic memory. After overlapping, NDRG1, NR4A1, KCNC4 and ZFP36 were selected. Pharmacology analysis and molecular docking revealed that pioglitazone and resveratrol were possible agents affecting these hub genes. Based on the *ex vivo* results, NDRG1 was selected for further study. Knockdown of NDRG1 reduced TGF-β expression in human kidney-2 cells (HK-2 cells). Compared to that in patients who had diabetes for more than 10 years but not DKD, NDRG1 expression in blood samples was upregulated in DKD patients. In summary, NDRG1 is a key gene involved in regulating fibrosis in DKD from a metabolic memory perspective. Bioinformatics analysis combined with experimental validation provided reliable evidence for identifying metabolic memory in DKD patients.

KEYWORDS

diabetic kidney disease, metabolic memory, fibrosis, single-cell RNA sequencing, NDRG1

1 Introduction

Diabetic kidney disease (DKD) is the leading cause of end-stage kidney disease (ESKD) in the general population and accounts for approximately 50% of ESKD cases in the industrialized world (Collins et al., 2012; Novak et al., 2016). Although the sodium glucose co-transporter 2 inhibitor (SGLT2i) class has been shown to reduce the risk of kidney events in at-risk patients with type 2 diabetes mellitus (T2DM), efficient therapies arresting or even reversing DKD progression are lacking (Reidy et al., 2014; Wanner et al., 2016; Perkovic et al., 2019; Heerspink et al., 2020). Coincidentally, in 2003, the Diabetes Control and Complications Trial (DCCT)



GRAPHICAL ABSTRACT

with further follow-up in the Epidemiology of Diabetes Interventions and Complications (EDIC) study (DCCT/EDIC) first proposed the concept of "metabolic memory." These findings demonstrated that despite comparable HbA1c levels in both the intensive treatment group and the conventional treatment group, initial hyperglycemia remained a significant factor in elevating the risk of developing long-term diabetic complications, including DKD (Nathan et al., 2013). More recently, Al-Dabet et al. (2022) identified *p21* as the hub gene in hyperglycemic memory and demonstrated that the expression of *p21* was sustained at high levels regardless of glucose levels *in vitro* and *in vivo*, while inhibited *p21* expression could ameliorate high glucose-induced tubulointerstitial fibrosis in DKD.

Single-cell RNA sequencing (scRNA-seq) technology can amplify and sequence the transcriptome or genome at the single-cell level to detect the biological information of single cells in the fields of genomics, transcriptomics and proteomics, thus avoiding many limitations of traditional transcriptome sequencing (Jovic et al., 2022). Considering the diversity of cell types in DKD, an increasing number of studies have applied scRNA-seq to uncover the key genes involved in the onset and progression of DKD. Hirohama et al. (2023) recognized MMP7 as a diagnostic marker of kidney fibrosis through proteomics and scRNA-seq. Song et al. (2023) used scRNA-seq to screen RAC1 and demonstrated that RAC1 was involved in macrophage efferocytosis in DKD. However, at present, no study has screened key genes related to metabolic memory through scRNA-seq or explored the underlying mechanisms involved.

In this study, we used single-cell RNA sequencing (scRNA-seq) data derived from kidney samples of DKD patients, normal tissues adjacent to tumors, rat renal fibrosis models, and hyperglycemic memory mouse models to screen for crucial genes. Subsequently, we conducted cell cluster identification, enrichment analysis, and cell–cell communication analyses on these selected datasets. By intersecting the datasets, we utilized immune infiltrate analysis, network pharmacology, molecular docking, and the Nephroseq database to elucidate the functional roles and clinical implications of the hub genes. Additionally, we employed quantitative real-time PCR (qRT–PCR) and Western blotting to validate the expression levels of these genes in human kidney 2 (HK-2) cells. Furthermore, we assessed their levels in the blood of patients with diabetes for over 10 years but without DKD, as well as in DKD patients, using enzyme-linked immunosorbent assay (ELISA). Drawing from these findings, our objective was to scrutinize genes associated with metabolic memory and fibrosis in DKD, aiming to uncover potential molecular candidates that could illuminate novel avenues for the prevention of DKD.

2 Materials and methods

2.1 Data acquisition

The flowchart of this study is shown in Figure 1. The GEO database (https://www.ncbi.nlm.nih.gov/geo/) was used to obtain three gene expression datasets. The GSE131882 dataset comprises three samples of kidney tissue from DKD patients and three samples from normal tissue adjacent to tumors for scRNA-seq using the GPL24676 platform. The GSE216376 dataset is based on the GPL25947 platform and

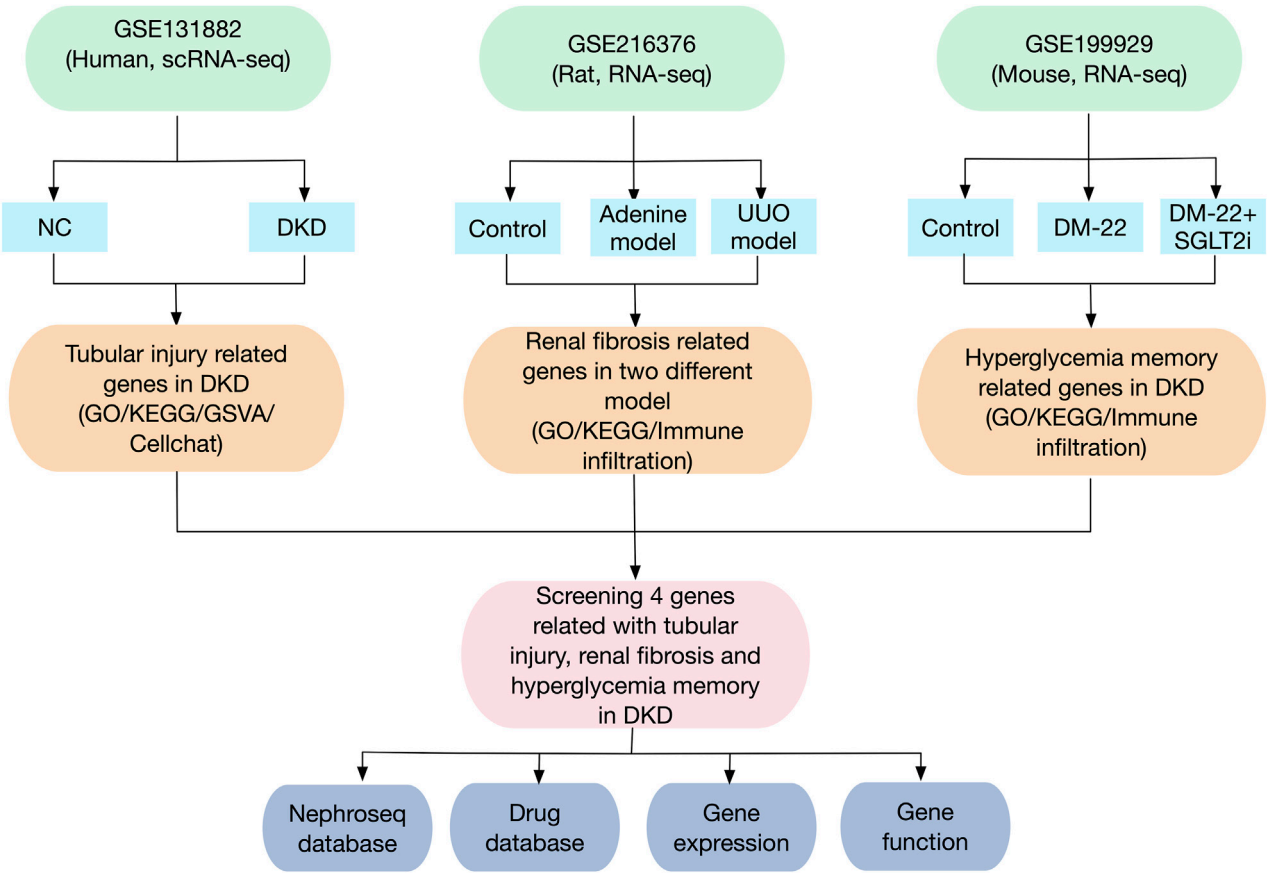


FIGURE 1
Flowchart of the research design and analysis process of the study.

consists of three control models, three sham surgery models, three adenine-induced rat models and three UUO-induced rat models. The GSE199929 dataset (based on the GPL24247 platform) included data from three control models, two diabetic models and two diabetic mice treated with SGLT2i to reduce glucose levels.

2.2 scRNA-seq analysis

In this analysis, the exon, inex and intron sequences of each sample were taken as a single sample to obtain an expression matrix of 373,942 cells * 15,398 features. All cells that displayed nFeatures greater than 200 but less than 4,000 and a percentage of mitochondrial RNA less than 5% were included in the analysis, and a single-cell expression matrix with 2073 features was obtained for the 13,617 cells. The data were standardized by the NormalizeData function. After scale normalization, 2,000 highly variable genes (HVGs) were identified with the FindVariableFeatures function. ScaleData and RunPCA were used in turn to standardize the data, and PCA was performed (npcs = 50). Subsequently, the best PC value of this analysis was selected according to the elbow plot results. The best PC value was 15, and the cluster and tSNE values were obtained through FindNeighbors, FindClusters and RunTSNE. Clusters were then identified using FindClusters (resolution = 0.35). Cell type annotation was performed based on the top 10 HVGs in each cluster via manual checking and the CellMarkers dataset (http://xteam.xbio.top/CellMarker/).

2.3 Enrichment analysis

We used the R package "clusterProfiler" to perform Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses. The packages "enrichGO" and "enrichKEGG" were also used. The "GSVA" Bioconductor package was subsequently used to implement gene set variation analysis (GSVA). An enrichment score was generated for each sample and pathway because of the use of a nonparametric unsupervised method that converted a traditional gene matrix (gene-by-sample) into a gene set by the sample matrix. The "heatmap" package was subsequently used to cluster the GSVA matrix, which was displayed as a heatmap. A p-value of < 0.05 was considered to indicate significant enrichment. In addition, we used the KOBAS 3.0 database (http://kobas.cbi.pku.edu.cn/) to determine the enrichment of KEGG pathways associated with NDRG1, NR4A1, KCNC4 and ZFP36.

2.4 Cell–cell communication analysis

CellChat is a tool that uses network analysis, pattern recognition, and various learning techniques to quantitatively infer and analyze

intercellular communication networks. The primary signaling inputs and outputs of cells, as well as how these cells and signals coordinate for functions, were predicted using a typical workflow. We then calculated the average ligand and receptor expression in a particular cell type by the mean value, which was determined by the proportion of cells between the DKD patients and the control group.

2.5 Immune cell infiltration analysis

RNA sequencing data from GSE199929 were analyzed using CIBERSORT, which was used to estimate the total immune infiltration in each sample and immune cell subset. The boxplots were visualized by the ggplot2 package, and the ggcorplot package was used for heatmap visualization.

2.6 Drug prediction and molecular docking of key target genes

The chemical interactions of the screened genes and disease information were acquired from the Comparative Toxicogenomics Database (CTD, http://ctdbase.org/). First, we created a Venn diagram using the R program VennDiagram to depict the intersection of the obtained drug targets of the 4 target genes and DKD. To investigate the interaction and binding activities of the compounds with the selected genes, molecular docking analysis was subsequently carried out. Protein 3D structures (https://www.rcsb.org/), including ZFP36 (PDB ID: 6NZL), KCNC4 (PDB ID: 1B4G), NR4A1 (PDB ID: 2QW4), and NDRG1 (PDB ID: 6ZMM), were collected from the Protein Data Bank database (PDB database). The molecular structures of pioglitazone and resveratrol were retrieved from the PubChem Compound Database (https://pubchem.ncbi.nlm.nih.gov/). After the legends were energy minimized and the hydrogen atoms of the receptors were removed, the molecular operating environment (MOE) was used for molecular modeling and ligand binding interactions.

2.7 Clinical correlation validation

Spearman rank correlation coefficient analysis was used to validate the association between the hub genes and clinical manifestations of DKD using the Nephroseq v5 online database (http://v5.nephroseq.org/), an integrated data-mining platform for gene expression datasets of kidney illnesses. A p-value of 0.05 indicated statistical significance.

2.8 Cell lines and cell culture

HK-2 cells were obtained from the Chinese Academy of Sciences (Shanghai, China) and cultured at 37°C in Dulbecco's modified Eagle's medium (HyClone, UT) supplemented with 10% fetal bovine serum (FBS; Gibco, CA), 2 mM glutamine, 100 U/mL penicillin, and 100 g/mL streptomycin. The cells were cultured in continuous normal (NG, 5.6 mM) glucose for 48 h, continuous high (HG, 30 mM) glucose for 48 h, or high glucose for 24 h followed by normal glucose for 24 h. The experimental scheme of the *in vitro* experiments is shown in Supplementary Figure S1. The *in vitro* experiments were repeated at least three independent times.

2.9 Knockdown of NDRG1

HK-2 cells were transfected with short hairpin RNA (shRNA) specific for NDRG1 (HANBIO, Shanghai, China) or with nontargeting shRNA (HANBIO, Shanghai, China) as a negative control. The transfection time was 24 h, and the multiplicity of infection (MOI) was 70. The sequence of the shRNA used was CCT GGAGTCCTTCAACAGTTT. The above experiments were carried out following the instructions supplied by the manufacturer.

2.10 qRT–PCR

Total RNA was extracted from HK-2 cells using TRIzol reagent (Invitrogen, United States) according to the manufacturer's instructions. A Transcriptor First Strand cDNA Synthesis Kit (HiScript III All-in-one RT SuperMix Perfect for qRT–PCR, Vazyme) was used to synthesize cDNA from mRNA using OligodT primers. To avoid deterioration, an RNase inhibitor was utilized. Taq Pro Universal SYBR qRT–PCR Master Mix (Vazyme) and specific primer sets (Supplementary Table S1) were used for amplification. The expression of β-actin was used to normalize mRNA expression levels.

2.11 Western blot

HK-2 cells were lysed in RIPA buffer (Cat# P0013B; Beyotime Biotechnology, China) with a protein inhibitor cocktail using the following primary antibodies: anti-NDRG1 (Cell Signaling Technology–#9485), anti-TGF-β (Abcam, ab215715) and anti-GAPDH (Proteintech 10494-1-AP). Western blotting was performed after the sections were incubated with a horseradish peroxidase-conjugated anti-rabbit secondary antibody (D110011; Sangon Biotech, Shanghai, China).

2.12 Mouse model

C57BL/6J mice were obtained from Beijing Charles River (Beijing, China). Six-week-old male C57BL/6J mice were kept in a room at a temperature of 22°C–25°C with a 12-h light/dark cycle. After 2 weeks, streptozotocin (STZ; 50 mg/kg in 0.05 M citrate buffer, pH 4.5; Sigma–Aldrich) was intraperitoneally injected into fasted mice for 12 h to establish a diabetic mouse model. The control mice were injected with citrate buffer. Diabetes was successfully induced by blood glucose levels greater than 16.7 mmol/L according to three consecutive tests when the fasting blood glucose level was tested 72 h after STZ injection. For a total of 16 weeks, body weight and blood glucose levels were monitored every 4 weeks.

2.13 Patients and controls

The participants included 43 patients with T2DM for more than 10 years but without DKD and 51 patients with DKD. Patients were

recruited from the hospitalized patients of the First Affiliated Hospital of Zhengzhou University. The study was not a sex-specific human study. As a result, the sex distribution of the participants was not given special consideration. The sex of the participants was self-reported. The inclusion criteria were shown as follows: 1) Patients aged 20–80 years who have been diagnosed definitively diagnosed with T2DM. 2) DKD group: Patients who received a discharge diagnosis of DKD from the First Affiliated Hospital of Zhengzhou University, fulfilling the criteria of urinary albumin/creatinine ratio (UACR) > 30 mg/g and are accompanied by diabetic retinopathy (DR). 3) In the T2DM group: Patients with a history of diabetes for more than 10 years and a UACR < 30 mg/g were included. The exclusion criteria were shown as follows: 1) Patients who were diagnosed with chronic kidney disease caused by other diseases or triggers. 2) Patients who have been diagnosed with other diseases that lead to increased urinary protein. 3) Patients who were diagnosed with severe liver injury or malignancy were excluded. 4) Patients who have been diagnosed with other diseases that lead to increased urinary protein. All DKD patients were also diagnosed with diabetic retinopathy. The variables included age, sex, body mass index (BMI), systolic blood pressure (SBP), diastolic blood pressure (DBP), HbA1c (Hemoglobin A1c), fasting plasma glucose (FPG), serum creatinine (SCr), blood urea nitrogen (BUN), estimated glomerular filtration rate (eGFR), UACR, 24-h urinary protein quantity (24hUTP), duration of diabetes, and diagnosis of DR and hypertension. eGFR was calculated by the Modification of Diet in Renal Disease (MDRD) formula. The study was approved by the Ethics Committee of the First Affiliated Hospital of Zhengzhou University (Approval number: 2019-KY-228). All participants provided written informed consent in accordance with the Declaration of Helsinki.

2.14 ELISA

NDRG1 ELISA kits (ABclonal, Boston, United States) were used to analyze NDRG1 in the supernatant, and the procedure was performed exactly as directed by the manufacturer.

2.15 Statistical analysis

Comparisons between two and multiple groups were performed using independent t tests and one-way analysis of variance (ANOVA), respectively. R software (version 4.2.1) was used for statistical analysis. A *p*-value less than 0.05 was considered to indicate statistical significance.

3 Results

3.1 Preprocessing and cell cluster identification of scRNA-seq data

As shown in Figure 2A, quality control of the aligned and counted reads was performed according to the *Methods* section. The data quality met the expectations. Next, we used t-distributed stochastic neighbor embedding (tSNE), principal component analysis (PCA), and uniform manifold approximation and projection (UMAP) for dimension reduction (Supplementary Figure S2; Figure 2B). The results suggested that UMAP may be a better choice for dimension reduction, and 12 major cell types were annotated by UMAP (Figure 2C). Different clusters were distinguished by cell markers; for example, proximal tubular cells were identified by CUBN, LRP2, SLC5A12 and TGM4; the loop of Henle was annotated by COL1A2, SLC12A1 and CLDN16; and distal convoluted tubule cells were identified by TMEM52B, TRPM6, and KLHL3 (Figure 2C). Among these cell types, tubular epithelial cells were obviously differentiated. To further verify the identified cell clusters, GO analysis was performed for each cluster. The representative genes and GO terms were shown in Figure 2D. For example, there were a large number of genes with high expression in collecting duct principal cells. The AQP2, SCNN1G, SLC8A1, FXYD4 and PWRN1 were selected as the representative genes, and the functions of these genes included sodium ion transport, sodium ion transmembrane transport and regulation of sodium ion transport, which were all classic functions of collecting duct principal cells.

3.2 Distribution and enrichment of scRNA-seq data

As shown in Figure 3A, the percentage of tubular epithelial cells, including distal convoluted tubule cells, loop of Henle cells and proximal tubular cells, accounted for the largest proportion of cells, which was consistent with the findings of prior studies. In addition, as CREDENCE (Perkovic et al., 2019), EMPA-REG (Wanner et al., 2016) and DAPA-CKD (Heerspink et al., 2020) were carried out, and tubular epithelial cells had gained increased amounts of attention. Thus, we selected tubular epithelial cells as the research objects. After differential gene expression analysis, 3,011 DEGs were found to be specifically expressed in tubular epithelial cells, and 3,656 DEGs were found to be specifically expressed in DKD samples. Subsequently, 2,278 DEGs were identified (Figure 3B). To determine the possible biological functions of the overlapping DEGs, GO analysis, KEGG pathway analysis and GSEA analysis were carried out. The small molecule catabolic process, nuclear speck, actin binding and burn wound healing terms were enriched (Figures 3C, D). In addition, GSVA enrichment was performed to explore the differences between the DKD cluster and the control cluster in tubular epithelial cells, where the most common pathways, such as reactive oxygen species, IL-2, STAT5 and the UV response, were related to oxidative stress, inflammation, and DNA damage repair (Figure 3E).

3.3 Cell–cell crosstalk network of the scRNA-seq data

To clarify the underlying intercellular communications and cell state transitions in DKD, we analyzed the intercellular communication networks from the scRNA-seq data using the

10.3389/fphar.2024.1379821

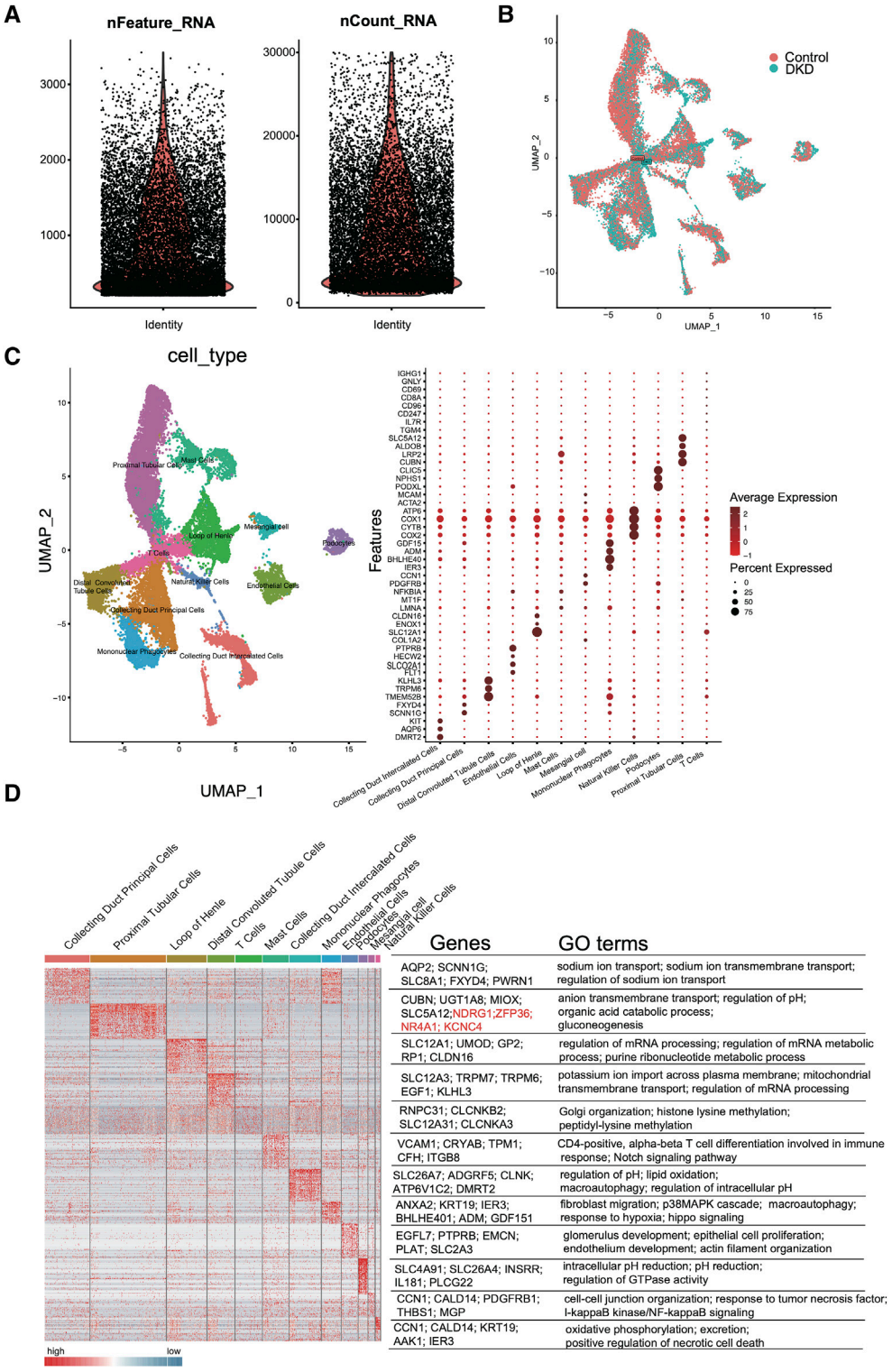


FIGURE 2
scRNA-seq quality control and analysis. **(A)** The number of unique RNA features (nFeatures) detected in each cell (left panel) and the total number of UMIs within a cell across all cells (nCounts) (right panel). **(B)** Uniform manifold approximation and projection (UMAP) for DKD and control samples. **(C)** Heatmap of DEGs in each cluster (left panel). The representative marker genes (middle panel) and the top GO terms (right panel) are shown. **(D)** Cell type UMAP representation (left panel) and dotplot of a selected set of cluster-specific genes (right panel).

CellChat package. In the DKD sample, the number of interactions and interaction weights/strengths of all kinds of cells were greater than those in the control sample, especially for endothelial cells, mast cells and collecting duct principal cells (Figure 3F). In DKD, tubular epithelial cells may be stimulated by various factors, such as hyperglycemia, proteinuria, and oxidative stress; undergo

10.3389/fphar.2024.1379821

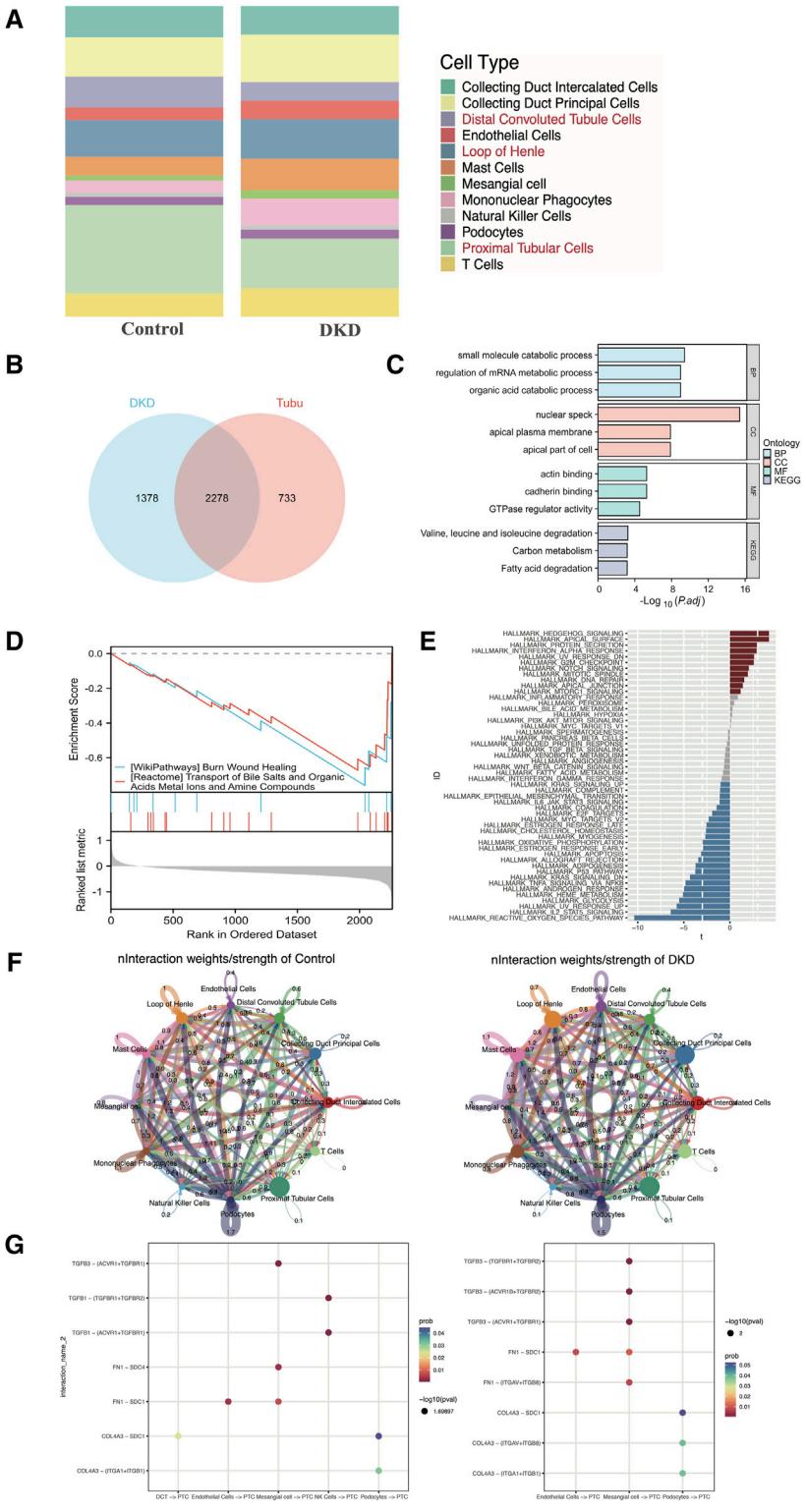


FIGURE 3
Distribution, selection, enrichment, and cell–cell communication in GSE131882. **(A)** Stacked bar chart of the percentage of the cell types in DKD patients and control subjects. **(B)** Venn diagram showing the genes specifically expressed in tubular epithelial cells in DKD patients. **(C)** GO term and KEGG pathway enrichment analysis of the overlapping hub genes. **(D)** GSEA of the overlapping hub genes. **(E)** Differential pathway enrichment between the tubular epithelial cells of DKD patients and controls. **(F)** Interaction weights of tubular epithelial cells from DKD and control samples. **(G)** The intracellular ligand–receptor signaling network in kidney cells from the DKD and control groups. The communication probability indicates the strength of the ligand–receptor connection, and the P-value indicates the number of enriched genes. PCT, proximal tubular cell; DCT, distal convoluted tubulin; NK cells, natural killer cells.

phenotypic changes; and transform into fibroblasts that over synthesize the extracellular matrix, resulting in tubular interstitial fibrosis, which is one of the principal reasons leading to kidney function decline (Zhang et al., 2021; Zheng et al., 2021). To explore intercellular communication between tubular epithelial cells and other cells, we selected TGF-β, FN 1 and collagen as representative signaling pathways to identify receptor–ligand pairs (Figure 3G). In both the DKD and control groups, the expression of TGF-β signaling factors, such as TGFB3-TGFF3R1, increased from mesangial cells to proximal tubular epithelial cells. Collagen signaling molecules, such as COL4A3 − (ITGAV + ITGB8), were increased in DKD samples from podocytes to proximal tubular epithelial cells. However, these findings still needed to be experimentally confirmed.

3.4 Functional analysis of DEGs in mouse models of metabolic memory and rat models of renal fibrosis

Al-Dabet et al. (2022) explored metabolic memory in tubular epithelial cells in DKD, but existing studies were still lacking. Thus, we used the GSE199929 dataset (Al-Dabet et al., 2022). They designed a nondiabetic mouse model (control), a diabetic mouse model without intervention to reduce blood glucose levels (DM-22) and a mouse model of hyperglycemia reversal by sodium-glucose cotransporter 2 inhibitors (SGLT2i, dapagliflozin) (DM-22+SGLT2i) to identify hub genes related to metabolic memory. As shown in Figures 4A, B, even though hyperglycemia was reversed, "phagocytosis," "complement activation" and terms related to the immune response were still enriched, indicating that metabolic memory was present and might be associated with these phenotypes. Their research also indicated that metabolic memory was related to fibrosis, which is characteristic of progressive chronic kidney diseases of any etiology, including DKD, and eventually led to kidney failure (Hung et al., 2021). Therefore, we selected GSE216376, which consisted of two classic renal fibrosis rat models (adenine and UUO), to identify fibrosis-related DEGs. As shown in Figures 4C, D, the GO terms "autophagy," "mRNA processing" and "ubiquitin-like protein ligase binding" were quite similar, suggesting that these two fibrosis models were in good agreement.

3.5 Identification and verification of hub genes related to fibrosis and metabolic memory in DKD

After overlapping the different species datasets from multiple angles, NR4A1, NDRG1, KCNC4 and ZFP36 were screened (Figure 5A). To identify the functions of these key genes, we enriched four genes using KOBAS software (Figure 5B). Among these genes, NR4A1 was related to the MAPK and AKT signaling pathways, NDRG1 was associated with TP53-mediated transcription regulation, KCNC4 was annotated with potassium channels, and ZFP36 may regulate mRNA stability. The expression levels of NR4A1, NDRG1, KCNC4 and ZFP36 were again verified in tubular epithelial cells via the use of scRNA-seq data (Figure 5C). These findings provided clues for further study.

3.6 Analysis of the correlation between the hub genes and immune cell infiltration

As indicated by the results of the functional analysis of the metabolic memory models (Figures 4C, D), hyperglycemic memory might be associated with the immune response. Hence, we further explored the interactions between hub genes (NDRG1, NR4A1, KCNC4, and ZFP36) and immune cells (Figure 5D). M1 macrophages and monocytes were much more abundant in diabetic mice than in control mice, which indicated more pronounced inflammatory injury. After SGLT2i-mediated effects on glucose levels, the percentages of macrophages, monocytes, Th2 cells and Tregs changed, indicating immunosuppression, while the proportions of naive lymphocytes, including naive B cells and T cells, did not improve significantly. According to the heatmap, the expression of these hub genes could not be completely reversed after SGLT2i therapy; for example, the correlation between ZFP36 and naive B cells in the diabetic and control groups was 0.83, while that between the SGLT2i treatment group and the control group was 0.8. Thus, hyperglycemic memory might be correlated with the immune microenvironment in DKD.

3.7 Potential drug prediction and molecular docking of the hub genes

To identify potential drugs against these hub genes, we screened components from the CTD platform (Figures 6A, B). Among the 15 identified compounds, pioglitazone and resveratrol had well-defined kidney protection effects and were selected for molecular docking analysis (Ho et al., 2022; Gu et al., 2022). The binding free energy represented the intermolecular binding ability. The 2D molecular model included detailed information about the ligand–receptor interactions, such as hydrogen bonding, hydrophobic bonding and carbon–hydrogen bonding. A binding free energy <0 kcal/mol indicated that the protein–ligand complex can dock in a natural state, an affinity energy < −1.2 kcal/mol indicated good docking, and a binding energy ≤ −5 kcal/mol indicated strong docking. The affinities of pioglitazone for all four proteins were less than −5 kcal/mol (Figure 6C). Although the binding force of resveratrol was much greater than that of pioglitazone, the affinity of resveratrol was quite close to −5 kcal/mol (Figure 6D). Therefore, pioglitazone and resveratrol might be effective against these key genes and metabolic memory.

3.8 Validation of the clinical significance of the hub genes

To explore the clinical significance of the identified hub genes, correlation analysis between the expression of these genes and the eGFR was conducted with the Nephroseq v5 online tool (Figure 7). All these genes were positively correlated with the GFR, while the *P* values of NDRG1, NR4A1 and ZFP36 were less than 0.05, and the associations were 0.49, 0.37 and 0.52, respectively.

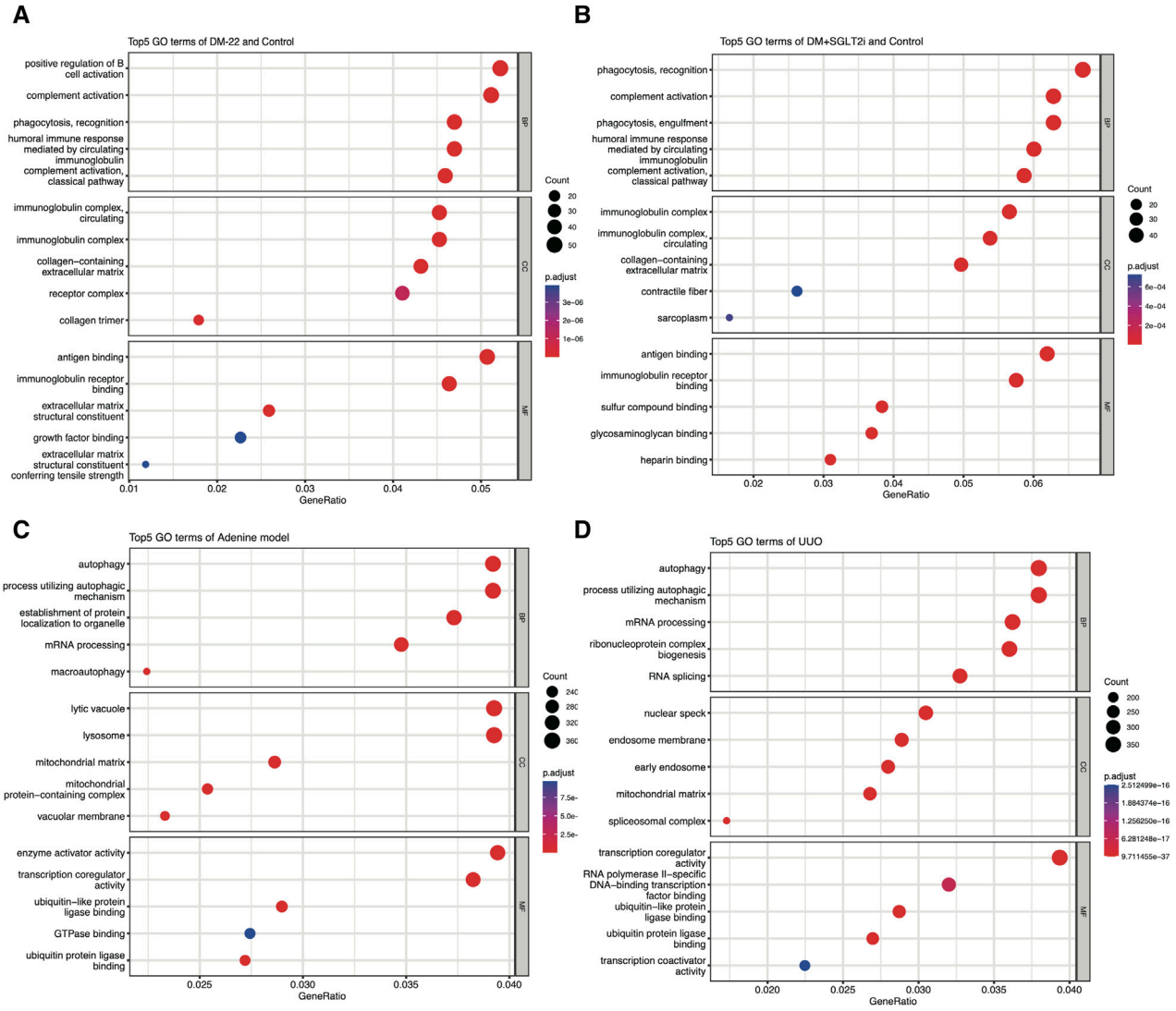


FIGURE 4
Enrichment analysis of GSE199929 and GSE216376. **(A)** Dotplot of the GO analysis of DEGs in nondiabetic mice (control) and diabetic mice (DM-22 model). **(B)** Dotplot of the GO analysis of DEGs in nondiabetic mice (control) and diabetic mice treated with SGLT2i to reduce glucose levels (DM + SGLT2i). **(C)** Dotplot of the GO analysis results for the adenine model. **(D)** Dotplot of the GO analysis results for the UUO model.

3.9 NDRG1 is a metabolic memory- and fibrosis-related hub gene

To examine the expression of these hub genes, we cultured HK-2 cells *in vitro* in the presence of continuous normal glucose (NG) or high glucose (HG) for 24 h followed by HG (HG + NG) or continuous HG. The results indicated that the mRNA and protein levels of NDRG in HK-2 cells remained elevated, even after a further 24-h reduction in glucose concentration (Figures 8A, C). While ZFP36 expression increased under HG conditions, it sharply declined upon transitioning to NG conditions, failing to adhere to metabolic memory criteria. Conversely, NR4A1 expression, though in line with metabolic memory standards, was comparatively low. We also validated NDRG1 expression in diabetic mice with albuminuria, which indicated that NDRG1 was a causative gene in DKD (Figure 8B). Thus, we selected NDRG1 for further validation. Among the fibrosis-related pathways, the TGF-β signaling pathway is one of the most common pathways. In addition, Menezes et al. (2019) illustrated that NDRG1 could inhibit TGF-β signaling to enhance membrane E-cadherin expression in pancreatic cancer. Thus, we knocked down NDRG1 in HK-2 cells, and the results showed that NDRG1 reduced TGF-β expression in HK-2 cells cultured with HG (Figure 8D).

3.10 Diagnostic performance of NDRG1

To further clarify the potential diagnostic value of NDRG1, we collected and conducted a univariate analysis on the general information, main laboratory test results, comorbidities, and NDRG1 expression levels in blood samples from patients with T2DM for more than 10 years but without DKD (T2DM Group) and patients with DKD and DR (DKD Group) (Table 1). The results indicated that the NDRG1 expression level, diabetes duration, age,

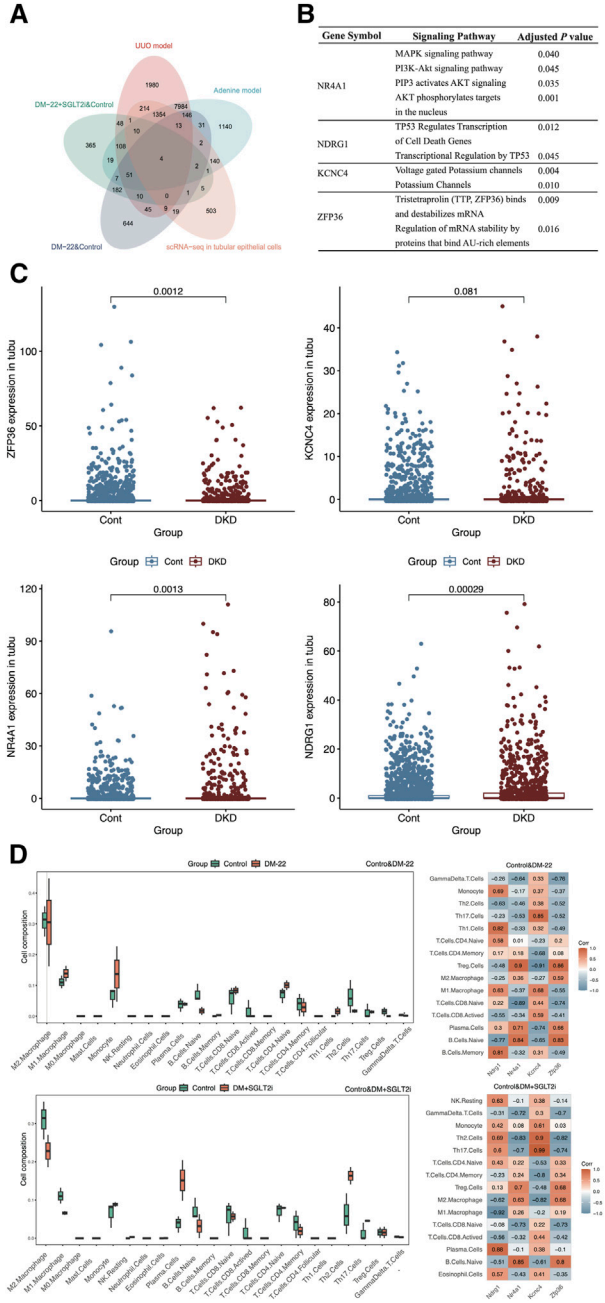


FIGURE 5
Screening hub genes. **(A)** The Venn diagram shows the overlapping genes obtained from five databases. **(B)** Enrichment analysis of ZFP36, KCNC4, NR4A1 and NDRG1. **(C)** Box plots of ZFP36, KCNC4, NR4A1 and NDRG1 expression levels in tubular epithelial cells from DKD and control samples. **(D)** Immune cell proportions and correlations between hub genes and infiltrating immune cells in control mice, diabetic mice (DM-22) and diabetic mice treated with SGLT2i to reduce glucose levels (DM + SGLT2i). *$p < 0.05$ and **$p < 0.01$ vs. the control group. ns, no significance.

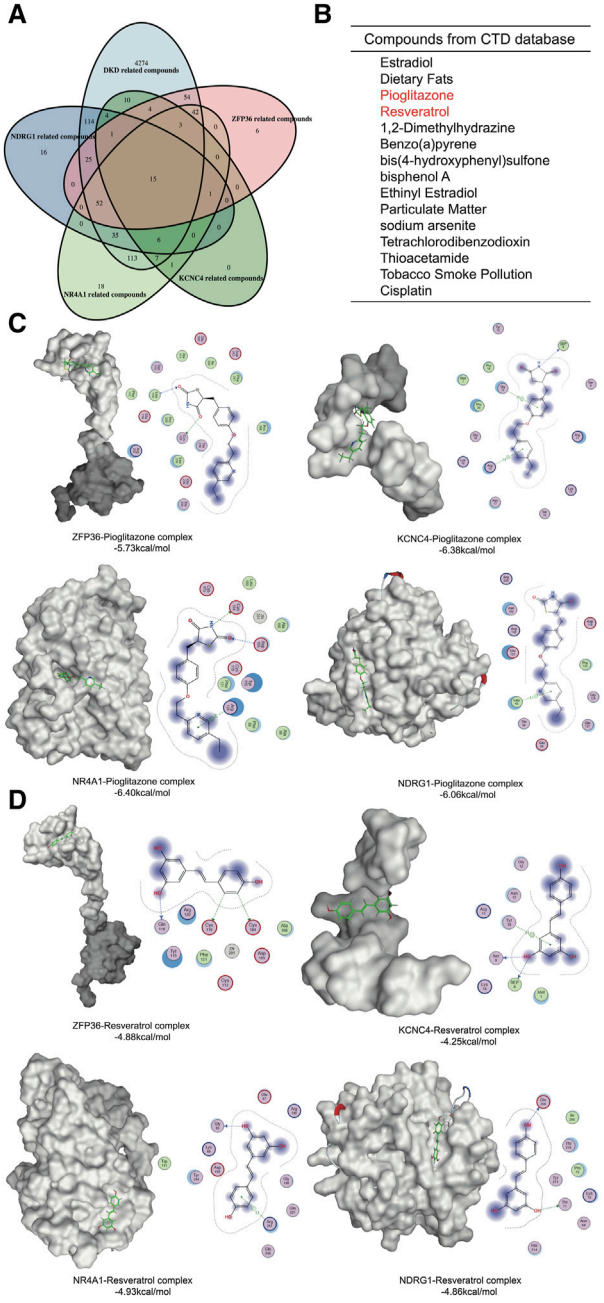


FIGURE 6
Network pharmacology and molecular docking. **(A)** Venn diagram of the gene-related compounds and DKD-related compounds. **(B)** The results of the Venn diagram. **(C)** Molecular docking of pioglitazone and the hub proteins. **(D)** Molecular docking of Resveratrol and the hub proteins.

BMI, SBP, HbA1c, SCr, BUN, UACR, total 24-h urine protein, and the presence of DR in the DKD Group were significantly different from those in the T2DM Group ($P < 0.05$). Since diabetes duration, DR, UACR, and total 24-h urine protein are inclusion criteria for the T2DM Group and DKD Group, these four indicators were not included in subsequent statistical analyses. Among the DKD patients, there were 24 individuals whose eGFR exceeded 100, yet all of them fulfilled the diagnostic criteria for DKD. DKD patients (n = 51) exhibited significantly greater NDRG1 expression than DM patients (Figure 8E). Although the area under the receiver operating characteristic (ROC) curve (AUC) for NDRG1 expression did not reach 0.7, NDRG1 expression combined with demographic and anthropometric indicators (age, BMI and SBP) was 0.768 (95% CI 0.672–0.865) (Figures 8F, G).

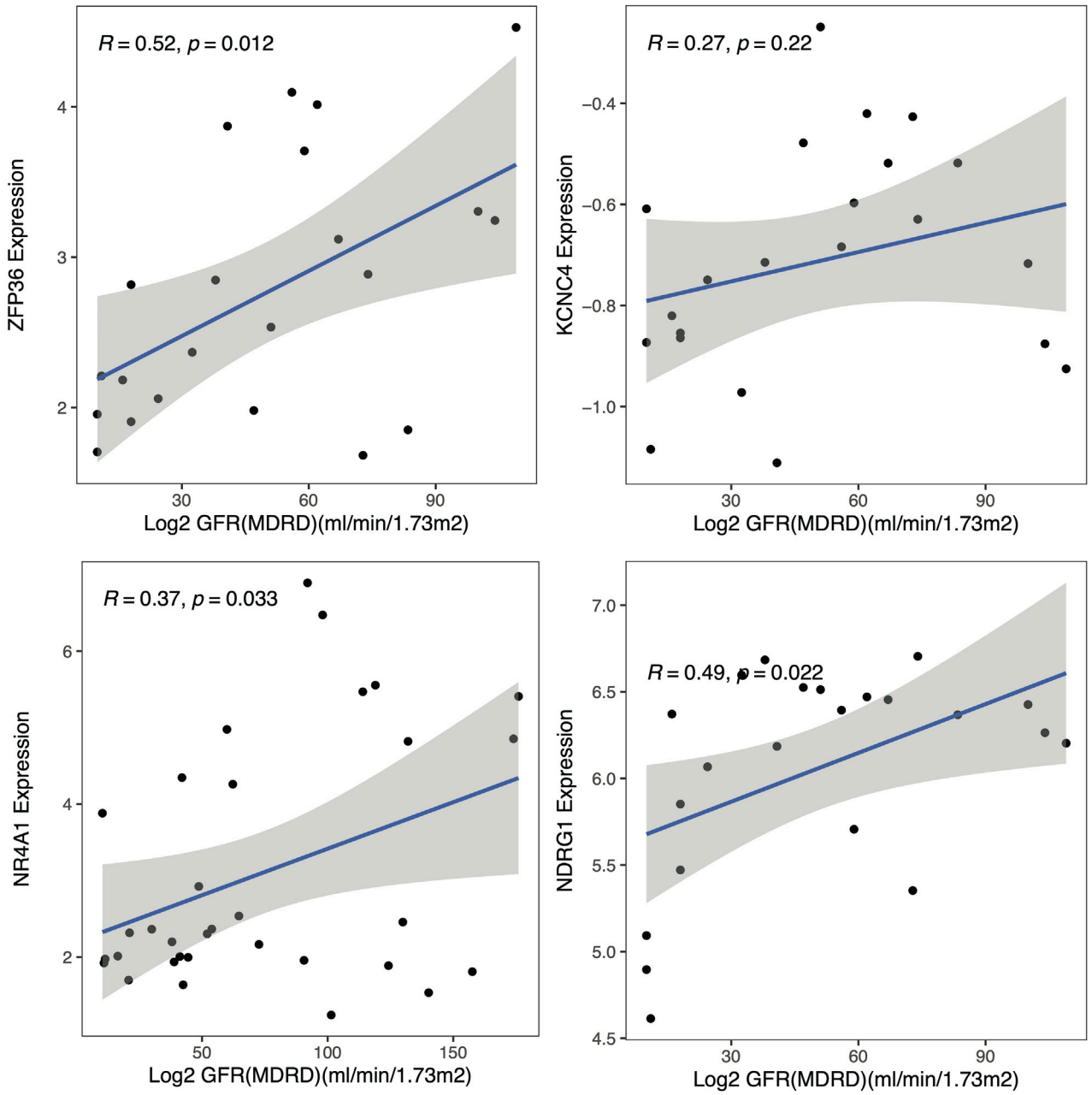


FIGURE 7
Clinical analysis. Correlation analysis of the hub genes with the glomerular filtration rate (GFR).

When further combined with biochemical indicators (SCr, BUN and eGFR), the area under the ROC curve reached 0.914 (95% CI 0.858–0.971) (Figure 8H). The accuracy, sensitivity and specificity were 0.862, 0.882 and 0.837, respectively. Thus, an increase in the blood NDRG1 concentration might be a potential biomarker for DKD diagnosis.

4 Discussion

The pathogenesis of DKD is complex and involves classic mechanisms, such as inflammation (Rayego-Mateos et al., 2023) and oxidative stress (Park et al., 2023), as well as several emerging mechanisms, such as pyroptosis (Qu et al., 2022) and ferroptosis (Li et al., 2021). However, even though multiple hypoglycemic agents are currently available for improving the above mechanisms and reducing blood glucose levels, the initiation of high blood glucose-induced damage in the kidney is still ongoing (Zheng et al., 2021). This latent effect across conditions and time is associated with the occurrence and development of chronic diabetic complications termed “metabolic memory.” Recent studies have shown that metabolic memory plays an important role in the pathogenesis of DKD (Li et al., 2022; Al-Dabet et al., 2022). However, the specific mechanism of metabolic memory in DKD remains unclear. Hence,

10.3389/fphar.2024.1379821

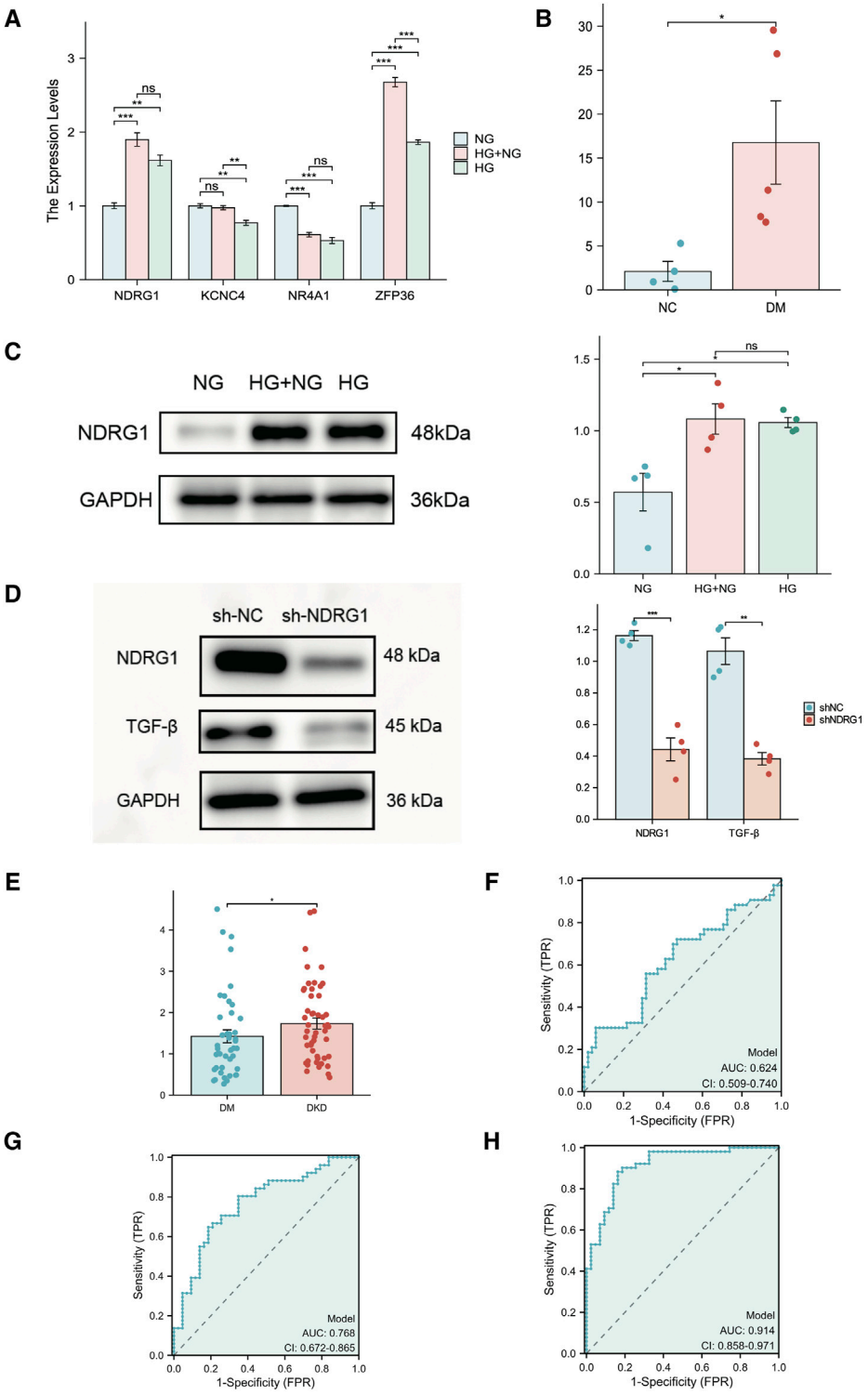


FIGURE 8
Experimental verification of hub genes related to fibrosis and metabolic memory in DKD patients. **(A)** The expression of NR4A1, NDRG1, KCNC4 and ZFP36 was analyzed via RT–PCR in HK-2 cells (n = 3 per sample). **(B)** The expression of NDRG1 in kidney tissue from diabetic mice (DM) and nondiabetic mice (NC) was analyzed via RT–PCR (n = 4 per group). **(C)** Western blotting was used to verify the expression of NDRG1 in HK-2 cells (n = 4 for each). **(D)** Western blotting was used to verify the knockdown efficiency of NDRG1 and to detect the protein expression level after TGF-β was knocked down by NDRG1 (n = 4 per group). **(E)** The expression of NDRG1 in the blood of DKD patients (n = 51) compared with that in the blood of DM patients without DKD for more than 10 years (n = 43). **(F)** ROC curve analysis of NDRG1 expression in the blood of DKD patients and DM patients without DKD for more than 10 years. **(G)** ROC curve analysis of NDRG1 expression combined with age, BMI and SBP. **(H)** ROC curve analysis of NDRG1 expression combined with age, BMI and SBP, Scr, BUN and eGFR. HK-2 cells: human kidney-2 cells; NG: control with continuous normal glucose (5.6 mM glucose) for 48 h; HG: control with continuous high glucose (30 mM glucose) for 48 h; HG + NG: high glucose (30 mM glucose) for 24 h followed by normal glucose (5.6 mM glucose) for 24 h *P < 0.05 or **P < 0.01 or ***P < 0.001.

TABLE 1 Characteristics of participants with T2DM and DKD.

Variables	Total (n = 94)	T2DM group (n = 43)	DKD group (n = 51)	*P-value*
NDRG1 (ng/mL)	1.37 (0.84, 2.03)	1.13 (0.65, 1.87)	1.56 (0.92, 2.4)	0.04
Duration of DM, (years)	12 (10, 18)	13 (10, 18)	10 (5, 17.5)	0.01
Gender, n (%)				0.96
Female	38 (40)	18 (42)	20 (39)	
Male	56 (60)	25 (58)	31 (61)	
Age (years)	59 (51.25, 66)	60 (55, 68)	58 (48, 65.5)	0.05
BMI, (kg/m^2)	22.94 (21.93, 25.81)	23.68 (22.62, 26.81)	22.76 (20.5, 24.32)	0.03
SBP (mmHg)	130.98 ± 19.73	125.56 ± 18.06	135.55 ± 20.08	0.01
DBP (mmHg)	77.65 ± 14.2	74.74 ± 11.89	80.1 ± 15.59	0.06
Haemoglobin (g/L)	127.63 ± 21.96	131.58 ± 16.69	124.29 ± 25.27	0.10
HbA1c (%)	8.6 (7.3, 10.1)	7.7 (7.15, 9.4)	9.2 (7.95, 11.55)	0.01
FPG (mmol/L)	7.98 (5.54, 10.52)	7.54 (5.72, 9.05)	8.59 (5.28, 12.3)	0.27
SCr (umol/L)	70.5 (57.25, 84.75)	64 (55.5, 74.5)	75 (61.5, 103.5)	< 0.01
BUN (mmol/L)	6.94 (5.55, 8.28)	5.62 (4.95, 7.5)	7.05 (6.39, 9.51)	< 0.01
eGFR ($ml/min/1.73m^2$)	108.75 (86.93, 133.4)	120.17 (99.72, 139.08)	97.32 (67.8, 126.5)	< 0.01
UACR (mg/g)	61.38 (5.23, 466.09)	4.9 (2.4, 7.25)	428.06 (134.93, 1,154.05)	< 0.01
24hUTP (g)	0.31 (0.21, 0.64)	0.21 (0.17, 0.26)	0.61 (0.4, 1.31)	< 0.01
DR, n (%)				< 0.01
No	24 (26)	24 (56)	0 (0)	
Yes	70 (74)	19 (44)	51 (100)	
Hypertension, n (%)				1
No	51 (54)	23 (53)	28 (55)	
Yes	43 (46)	20 (47)	23 (45)	

Continuous variables with a normal distribution are expressed as the mean ± SD, and nonnormal data are expressed as the median (interquartile range, IQR).
Abbreviations: DM, diabetes mellitus; DKD, diabetic kidney disease; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HbA1c, hemoglobin A1c; FPG, fasting plasma glucose; SCr, serum creatinine; BUN, blood urea nitrogen; eGFR, estimated glomerular filtration rate; UACR, urinary albumin/creatinine ratio; 24hUTP, 24-h urinary protein quantity; DR, diabetic retinopathy.

we combined scRNA-seq data from humans and high-throughput sequencing data from mice and rats to investigate the possible mechanisms of metabolic memory in tubular epithelial cell fibrosis in DKD. We also used network pharmacology and molecular docking to identify the potential regulatory effects of these metabolic memory-related genes. Our experimental validation revealed that NDRG1 was a metabolic memory-related gene that regulated TGF-β expression and was a potential biomarker for DKD diagnosis.

The concept of metabolic memory was first proposed in 2003; in recent years, numerous studies have started to explore the mechanisms behind this phenomenon. Li et al. (2022) reported that *Sirt7* cooperated with ELK1 to induce inflammation in endothelial cells despite the restoration of normoglycemia. Lizotte et al. (2016) also demonstrated that despite reduced blood glucose levels resulting from insulin treatment for the last 2 months, the expression of SHP-1 remained elevated in the podocytes of diabetic mice. The authors focused on the relationship between metabolic memory and glomerular injury in DKD patients. Although glomerular injury is essential for the progression of DKD, tubular epithelial cells have gained increasing attention in the clinical application of SGLT2is. Several studies have confirmed that tubular epithelial cell injury is the prime and important factor that impacts the progression of DKD (Wang et al., 2023; Shen et al., 2022). Growing evidence has demonstrated that tubular epithelial cells change and become fibrogenic in response to hyperglycemia-induced injury. This results in tubulointerstitial fibrosis, which is one of the most prevalent pathological features of DKD (Cui et al., 2022; Ji et al., 2023). Recent research has shown that methylation of the senescence-related gene *p21* regulates metabolic memory and fibrosis in tubular epithelial cells in DKD (Al-Dabet et al., 2022). Regrettably, studies on the role of metabolic memory in DKD, especially those focused on tubular epithelial cells, are relatively rare.

In our research, we evaluated the potential of pioglitazone and resveratrol as therapeutic agents against metabolic memory in

patients with DKD. While pioglitazone is not conventionally prescribed for DKD treatment, it has demonstrated the ability to hinder renal fibrosis in diabetic mice (Gan et al., 2023). However, the precise therapeutic function of these compounds in addressing metabolic memory remains elusive and demands further exploration. Resveratrol, an antioxidant and kidney-beneficial natural nonflavonoid polyphenolic compound (Blahova et al., 2021; Sattarinezhad et al., 2019), is particularly noteworthy as oxidative stress is a pivotal mechanism underlying metabolic memory, particularly in DR (Lee et al., 2022; Wang et al., 2018; Voronova et al., 2017). Chen et al. (2023) utilized network pharmacology and molecular docking to identify potential target genes of resveratrol in DKD, yet our key genes were not encompassed in their study. Additionally, Xian et al. (2020) hypothesized that resveratrol mitigates oxidative stress induced by metabolic memory in mice, aligning with our findings. Regrettably, cytological experiments were not performed to determine the underlying molecular mechanisms involved.

N-myc downstream-regulated gene 1 (NDRG1) is a member of the NDRG family and is a highly conserved and widely expressed gene located on chromosome 8 at the 8q24.2 locus (Fang et al., 2014). The most studied functions of NDRG1 include tumor metastasis and hypoxia (Park et al., 2020; Joshi et al., 2022). Although NDRG1 has been identified in the mitochondrial inner membrane of proximal tubule cells in the kidney, where it is regulated by HIF, the current literature lacks studies exploring its expression and function in DKD (Lachat et al., 2002; Zhang et al., 2020). Previous studies have confirmed that mitochondrial dysfunction was closely related to fibrosis in DKD and metabolic memory in DR (Forbes and Thorburn, 2018; Wang et al., 2018). For instance, Keshava et al. (2023) demonstrated that reduced NDRG1 expression attenuated pleural fibrosis. In pancreatic cancer, NDRG1 suppressed TGF-β and NF-κB signaling, thereby enhancing membrane E-cadherin expression. Our research revealed that the mRNA level of NDRG1 in kidney tissue was positively related to the eGFR. To validate NDRG1 expression, we employed RT–qPCR and Western blotting in HK-2 cells and diabetic mouse models. Our findings indicate that NDRG1 expression is upregulated by high glucose both *in vitro* and *in vivo*, with a particularly robust increase observed in HK-2 cells after 24 h of exposure to a high glucose culture solution. Moreover, knocking down NDRG1 in HK-2 cells suppressed TGF-β expression, consistent with previous studies. Furthermore, blood samples from DKD patients, but not DKD patients, displayed higher NDRG1 expression than did those from patients with T2DM for over 10 years. Considering demographic, anthropometric, and biochemical parameters, NDRG1 has emerged as a potential biomarker that could aid in distinguishing DKD patients, potentially without interfering with urine test results.

Our study has several strengths. First, we combined scRNA-seq data from humans with high-throughput sequencing data from a mouse model of hyperglycemia and two renal fibrosis models from rats to ensure the stability of NDRG1 in different species. Second, we focused on tubular epithelial cells and performed experimental validation. Third, we selected patients who had diabetes for more than 10 years but without DKD as the control group to ensure the accuracy of the test. Given that our research still needs mechanistic exploration, several potential limitations should be considered. First, although the expression level of NDRG1 was validated at the cellular level in this study, the expression level and underlying mechanisms of NDRG1 in DKD patients and animal models of metabolic memory remain to be further elucidated. Second, despite previous research indicating that resveratrol and pioglitazone can alleviate renal tubular epithelial fibrosis, the specific mechanisms by which they improve metabolic memory in DKD and renal tubular epithelial fibrosis still await experimental verification.

5 Conclusion

In summary, this study analyzed scRNA-seq and high-throughput sequencing data from multiple species and performed potential drug prediction and molecular docking analyses. After experimental validation, we identified NDRG1 as a potential hub gene of metabolic memory in DKD patients. Additionally, NDRG1 may reduce fibrosis in tubular epithelial cells through the TGF-β pathway and may also be a potential biomarker for DKD patients, shedding light on basic and drug research on DKD.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

Ethics statement

The studies involving humans were approved by the Ethics Committee of the First Affiliated Hospital of Zhengzhou University (Approval number: 2019-KY-228). 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. Ethical approval was not required for the studies on animals in accordance with the local legislation and institutional requirements because only commercially available established cell lines were used.

Author contributions

TY: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Resources, Validation, Visualization, Writing–original draft, Writing–review and editing. QF: Conceptualization, Formal Analysis, Writing–review and editing. MS: Methodology, Writing–review and editing. MP: Investigation, Writing–review and editing. FG: Formal Analysis, Writing–original draft. YS: Resources, Validation, Writing–review and editing. FH: Investigation, Writing–original draft. ZL: Formal Analysis, Writing–review and editing. JW: Formal Analysis, Writing–original draft. LW: Data curation, Writing–review and editing. GQ: Project administration, Supervision, Writing–review and editing. YZ: Funding acquisition, Project administration, 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. The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This study was supported by the National Natural Science Foundation of China (U2004116), the Research and Innovation Team Project of the First Affiliated Hospital of Zhengzhou University (QNCXTD2023015) 2021 Henan Province Health Youth and Middle-aged Discipline Leader Training Project, the Key R&D and Promotion Special Projects of Henan Province (No. 242102310009) and the Higher Education Key Research Project of Henan Province (No. 24A320021).

Acknowledgments

The authors would like to thank Dr. Baiquan Ci (State Key Laboratory of Primate Biomedical Research, Institute of Primate Translational Medicine, Kunming University of Science and Technology, Kunming, Yunnan) for helpful assistance in bioinformatics analyses. We also thank all patients who participated in this study and provided us with blood samples.

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.

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.2024.1379821/full#supplementary-material

References

Al-Dabet, M. M., Shahzad, K., Elwakiel, A., Sulaj, A., Kopf, S., Bock, F., et al. (2022). Reversal of the renal hyperglycemic memory in diabetic kidney disease by targeting sustained tubular p21 expression. *Nat. Commun.* 13 (1), 5062. doi:10.1038/s41467-022-32477-9

Blahova, J., Martiniakova, M., Babikova, M., Kovacova, V., Mondockova, V., and Omelka, R. (2021). Pharmaceutical drugs and natural therapeutic products for the treatment of type 2 diabetes mellitus. *Pharm. (Basel)* 14 (8), 806. doi:10.3390/ph14080806

Chen, S., Li, B., Chen, L., and Jiang, H. (2023). Uncovering the mechanism of resveratrol in the treatment of diabetic kidney disease based on network pharmacology, molecular docking, and experimental validation. *J. Transl. Med.* 21 (1), 380. doi:10.1186/s12967-023-04233-0

Collins, A. J., Foley, R. N., Chavers, B., Gilbertson, D., Herzog, C., Johansen, K., et al. United States renal data system 2011 annual data report: atlas of chronic kidney disease and end-stage renal disease in the United States. *Am. J. Kidney Dis.*, 2012. 59, e1-e420. doi:10.1053/j.ajkd.2011.11.015

Cui, X., Shi, E., Li, J., Li, Y., Qiao, Z., Wang, Z., et al. (2022). GPR87 promotes renal tubulointerstitial fibrosis by accelerating glycolysis and mitochondrial injury. *Free Radic. Biol. Med.* 189, 58–70. doi:10.1016/j.freeradbiomed.2022.07.004

Fang, B. A., Kovačević, Ž., Park, K. C., Kalinowski, D. S., Jansson, P. J., Lane, D. J. R., et al. (2014). Molecular functions of the iron-regulated metastasis suppressor, NDRG1, and its potential as a molecular target for cancer therapy. *Biochim. Biophys. Acta* 1845 (1), 1–19. doi:10.1016/j.bbcan.2013.11.002

Forbes, J. M., and Thorburn, D. R. (2018). Mitochondrial dysfunction in diabetic kidney disease. *Nat. Rev. Nephrol.* 14 (5), 291–312. doi:10.1038/nrneph.2018.9

Gan, T., Wang, Q., Song, Y., Shao, M., Zhao, Y., Guo, F., et al. (2023). Canagliflozin improves fatty acid oxidation and ferroptosis of renal tubular epithelial cells via FOXA1-CPT1A axis in diabetic kidney disease. *Mol. Cell. Endocrinol.* 582, 112139. doi:10.1016/j.mce.2023.112139

Gu, W., Wang, X., Zhao, H., Geng, J., Li, X., Zheng, K., et al. (2022). Resveratrol ameliorates diabetic kidney injury by reducing lipotoxicity and modulates expression of components of the junctional adhesion molecule-like/sirtuin 1 lipid metabolism pathway. *Eur. J. Pharmacol.* 918, 174776. doi:10.1016/j.ejphar.2022.174776

Heerspink, H. J. L., Stefánsson, B. V., Correa-Rotter, R., Chertow, G. M., Greene, T., Hou, F. F., et al. (2020). Dapagliflozin in patients with chronic kidney disease. *N. Engl. J. Med.* 383 (15), 1436–1446. doi:10.1056/NEJMoa2024816

Hirohama, D., Abedini, A., Moon, S., Surapaneni, A., Dillon, S. T., Vassalotti, A., et al. (2023). Unbiased human kidney tissue proteomics identifies matrix metalloproteinase 7 as a kidney disease biomarker. *J. Am. Soc. Nephrol.* 34 (7), 1279–1291. doi:10.1681/ASN.0000000000000141

Ho, C. C., Yang, Y. S., Huang, C. N., Wang, Y. H., and Kornelius, E. (2022). The efficacy of pioglitazone for renal protection in diabetic kidney disease. *PLoS One* 17 (2), e0264129. doi:10.1371/journal.pone.0264129

Hung, P. H., Hsu, Y. C., Chen, T. H., and Lin, C. L. (2021). Recent advances in diabetic kidney diseases: from kidney injury to kidney fibrosis. *Int. J. Mol. Sci.* 22 (21), 11857. doi:10.3390/ijms222111857

Ji, J. L., Li, J. Y., Liang, J. X., Zhou, Y., Liu, C. C., Zhang, Y., et al. (2023). Tubular TMEM16A promotes tubulointerstitial fibrosis by suppressing PGC-1α-mediated mitochondrial homeostasis in diabetic kidney disease. *Cell. Mol. Life Sci.* 80 (12), 347. doi:10.1007/s00018-023-05000-6

Joshi, V., Lakhani, S. R., and McCart Reed, A. E. (2022). NDRG1 in cancer: a suppressor, promoter, or both? *Cancers (Basel)* 14 (23), 5739. doi:10.3390/cancers14235739

Jovic, D., Liang, X., Zeng, H., Lin, L., Xu, F., and Luo, Y. (2022). Single-cell RNA sequencing technologies and applications: a brief overview. *Clin. Transl. Med.* 12 (3), e694. doi:10.1002/ctm2.694

Keshava, S., Owens, S., Qin, W., Jeffers, A., Kyei, P., Komatsu, S., et al. (2023). The mTORC2/SGK1/NDRG1 signaling Axis is critical for the mesomesenchymal transition of pleural mesothelial cells and the progression of pleural fibrosis. *Am. J. Respir. Cell. Mol. Biol.* 70, 50–62. doi:10.1165/rcmb.2023-0131OC

Lachat, P., Shaw, P., Gebhard, S., van Belzen, N., Chaubert, P., and Bosman, F. T. (2002). Expression of NDRG1, a differentiation-related gene, in human tissues. *Histochem Cell. Biol.* 118 (5), 399–408. doi:10.1007/s00418-002-0460-9

Lee, Y. J., Jeon, H. Y., Lee, A. J., Kim, M., and Ha, K. S. (2022). Dopamine ameliorates hyperglycemic memory-induced microvascular dysfunction in diabetic retinopathy. *FASEB J.* 36 (12), e22643. doi:10.1096/fj.202200865R

Li, S., Zheng, L., Zhang, J., Liu, X., and Wu, Z. (2021). Inhibition of ferroptosis by up-regulating Nrf2 delayed the progression of diabetic nephropathy. *Free Radic. Biol. Med.* 162, 435–449. doi:10.1016/j.freeradbiomed.2020.10.323

Li, X., Liu, J., Lu, L., Huang, T., Hou, W., Wang, F., et al. (2022). Sirt7 associates with ELK1 to participate in hyperglycemia memory and diabetic nephropathy via modulation of DAPK3 expression and endothelial inflammation. *Transl. Res.* 247, 99–116. doi:10.1016/j.trsl.2022.04.005

Lizotte, F., Denhez, B., Guay, A., Gévry, N., Côté, A. M., and Geraldes, P. (2016). Persistent insulin resistance in podocytes caused by epigenetic changes of SHP-1 in diabetes. *Diabetes* 65 (12), 3705–3717. doi:10.2337/db16-0254

Menezes, S. V., Fouani, L., Huang, M. L. H., Geleta, B., Maleki, S., Richardson, A., et al. (2019). The metastasis suppressor, NDRG1, attenuates oncogenic TGF-β and NF-κB signaling to enhance membrane E-cadherin expression in pancreatic cancer cells. *Carcinogenesis* 40 (6), 805–818. doi:10.1093/carcin/bgy178

Nathan, D. M., Bayless, M., Cleary, P., Genuth, S., Gubitosi-Klug, R., Lachin, J. M., et al. (2013). Diabetes control and complications trial/epidemiology of diabetes interventions and complications study at 30 years: advances and contributions. *Diabetes* 62 (12), 3976–3986. doi:10.2337/db13-1093

Novak, M., Mucsi, I., Rhee, C. M., Streja, E., Lu, J. L., Kalantar-Zadeh, K., et al. (2016). Increased risk of incident chronic kidney disease, cardiovascular disease, and mortality in patients with diabetes with comorbid depression. *Diabetes Care* 39 (11), 1940–1947. doi:10.2337/dc16-0048

Park, J., Seo, E., and Jun, H. S. (2023). Bavachin alleviates diabetic nephropathy in db/db mice by inhibition of oxidative stress and improvement of mitochondria function. *Biomed. Pharmacother.* 161, 114479. doi:10.1016/j.biopha.2023.114479

Park, K. C., Paluncic, J., Kovacevic, Z., and Richardson, D. R. (2020). Pharmacological targeting and the diverse functions of the metastasis suppressor, NDRG1, in cancer. *Free Radic. Biol. Med.* 157, 154–175. doi:10.1016/j.freeradbiomed.2019.05.020

Perkovic, V., Jardine, M. J., Neal, B., Bompoint, S., Heerspink, H. J. L., Charytan, D. M., et al. (2019). Canagliflozin and renal outcomes in type 2 diabetes and nephropathy. *N. Engl. J. Med.* 380 (24), 2295–2306. doi:10.1056/NEJMoa1811744

Qu, X., Zhai, B., Liu, Y., Chen, Y., Xie, Z., Wang, Q., et al. (2022). Pyrroloquinoline quinone ameliorates renal fibrosis in diabetic nephropathy by inhibiting the pyroptosis pathway in C57BL/6 mice and human kidney 2 cells. *Biomed. Pharmacother.* 150, 112998. doi:10.1016/j.biopha.2022.112998

Rayego-Mateos, S., Rodrigues-Diez, R. R., Fernandez-Fernandez, B., Mora-Fernández, C., Marchant, V., Donate-Correa, J., et al. (2023). Targeting inflammation to treat diabetic kidney disease: the road to 2030. *Kidney Int.* 103 (2), 282–296. doi:10.1016/j.kint.2022.10.030

Reidy, K., Kang, H. M., Hostetter, T., and Susztak, K. (2014). Molecular mechanisms of diabetic kidney disease. *J. Clin. Investig.* 124 (6), 2333–2340. doi:10.1172/JCI72271

Sattarinezhad, A., Roozbeh, J., Shirazi Yeganeh, B., Omrani, G. R., and Shams, M. (2019). Resveratrol reduces albuminuria in diabetic nephropathy: a randomized double-blind placebo-controlled clinical trial. *Diabetes Metab.* 45 (1), 53–59. doi:10.1016/j.diabet.2018.05.010

Shen, S., Ji, C., and Wei, K. (2022). Cellular senescence and regulated cell death of tubular epithelial cells in diabetic kidney disease. *Front. Endocrinol. (Lausanne)* 13, 924299. doi:10.3389/fendo.2022.924299

Song, Y., Liu, Y., Guo, F., Zhao, L., and Qin, G. (2023). Single-cell RNA sequencing reveals RAC1 involvement in macrophages efferocytosis in diabetic kidney disease. *Inflammation* 47, 753–770. doi:10.1007/s10753-023-01942-y

Voronova, V., Zhudenkov, K., Helmlinger, G., and Peskov, K. (2017). Interpretation of metabolic memory phenomenon using a physiological systems model: what drives oxidative stress following glucose normalization? *PLoS One* 12 (2), e0171781. doi:10.1371/journal.pone.0171781

Wang, Y., Jin, M., Cheng, C. K., and Li, Q. (2023). Tubular injury in diabetic kidney disease: molecular mechanisms and potential therapeutic perspectives. *Front. Endocrinol. (Lausanne)* 14, 1238927. doi:10.3389/fendo.2023.1238927

Wang, Z., Zhao, H., Guan, W., Kang, X., Tai, X., and Shen, Y. (2018). Metabolic memory in mitochondrial oxidative damage triggers diabetic retinopathy. *BMC Ophthalmol.* 18 (1), 258. doi:10.1186/s12886-018-0921-0

Wanner, C., Inzucchi, S. E., and Zinman, B. (2016). Empagliflozin and progression of kidney disease in type 2 diabetes. *N. Engl. J. Med.* 375 (4), 1801–1802. doi:10.1056/NEJMc1611290

Xian, Y., Gao, Y., Lv, W., Ma, X., Hu, J., Chi, J., et al. (2020). Resveratrol prevents diabetic nephropathy by reducing chronic inflammation and improving the blood glucose memory effect in non-obese diabetic mice. *Naunyn Schmiedeb. Arch. Pharmacol.* 393 (10), 2009–2017. doi:10.1007/s00210-019-01777-1

Zhang, Y., Jin, D., Kang, X., Zhou, R., Sun, Y., Lian, F., et al. (2021). Signaling pathways involved in diabetic renal fibrosis. *Front. Cell. Dev. Biol.* 9, 696542. doi:10.3389/fcell.2021.696542

Zhang, Z. Y., Zhang, S. L., Chen, H. L., Mao, Y. Q., Li, Z. M., Kong, C. Y., et al. (2020). The up-regulation of NDRG1 by HIF counteracts the cancer-promoting effect of HIF in VHL-deficient clear cell renal cell carcinoma. *Cell. Prolif.* 53 (7), e12853. doi:10.1111/cpr.12853

Zheng, W., Guo, J., and Liu, Z. S. (2021). Effects of metabolic memory on inflammation and fibrosis associated with diabetic kidney disease: an epigenetic perspective. *Clin. Epigenetics* 13 (1), 87. doi:10.1186/s13148-021-01079-5

frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 29 August 2024
DOI 10.3389/fendo.2024.1418575

Check for updates

OPEN ACCESS

EDITED BY
Divya Bhatia,
NewYork-Presbyterian, United States

REVIEWED BY
Jasna Klen,
University Medical Centre Ljubljana, Slovenia
Ning Jie,
Shenzhen Longhua District Central Hospital, China

*CORRESPONDENCE
Gailing Liu
liugailing7@126.com

RECEIVED 16 April 2024
ACCEPTED 14 August 2024
PUBLISHED 29 August 2024

CITATION
Liu G (2024) Assessment of the effect of the SLC5A2 gene on eGFR: a Mendelian randomization study of drug targets for the nephroprotective effect of sodium-glucose cotransporter protein 2 inhibition.
Front. Endocrinol. 15:1418575.
doi: 10.3389/fendo.2024.1418575

COPYRIGHT
© 2024 Liu. 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.

Assessment of the effect of the SLC5A2 gene on eGFR: a Mendelian randomization study of drug targets for the nephroprotective effect of sodium-glucose cotransporter protein 2 inhibition

Gailing Liu*

Department of Nephrology, People's Hospital of Zhengzhou University, He'nan Provincial People's Hospital, He'nan Provincial Key Laboratory of Kidney Disease and Immunology, Zhengzhou, China

Aim: Sodium-glucose cotransporter protein 2 (SGLT2) inhibitors have been shown to have renoprotective effects in clinical studies. For further validation in terms of genetic variation, drug-targeted Mendelian randomization (MR) was used to investigate the causal role of SGLT2 inhibition on eGFR effects.

Methods: Genetic variants representing SGLT2 inhibition were selected as instrumental variables. Drug target Mendelian randomization analysis was used to investigate the relationship between SGLT2 inhibitors and eGFR. The IVW method was used as the primary analysis method. As a sensitivity analysis, GWAS pooled data from another CKDGen consortium was used to validate the findings.

Results: MR results showed that hemoglobin A1c (HbA1c) levels, regulated by the *SLC5A2* gene, were negatively correlated with eGFR (IVW β -0.038, 95% CI -0.061 to -0.015, P = 0.001 for multi-ancestry populations; IVW β -0.053, 95% CI -0.077 to -0.028, P = 2.45E-05 for populations of European ancestry). This suggests that a 1-SD increase in HbA1c levels, regulated by the SLC5A2 gene, is associated with decreased eGFR. Mimicking pharmacological inhibition by lowering HbA1c per 1-SD unit through SGLT2 inhibition reduces the risk of eGFR decline, demonstrating a renoprotective effect of SGLT2 inhibitors. HbA1c, regulated by the *SLC5A2* gene, was negatively correlated with eGFR in both validation datasets (IVW β -0.027, 95% CI -0.046 to -0.007, P=0.007 for multi-ancestry populations, and IVW β -0.031, 95% CI -0.050 to -0.011, P=0.002 for populations of European origin).

Conclusions: The results of this study indicate that the *SLC5A2* gene is causally associated with eGFR. Inhibition of *SLC5A2* gene expression was linked to higher eGFR. The renoprotective mechanism of SGLT2 inhibitors was verified from the perspective of genetic variation.

KEYWORDS

sodium-glucose cotransporter protein 2 (SGLT2) inhibition, *SLC5A2*, eGFR, drug target Mendelian randomization, insulin resistance

1 Introduction

Chronic Kidney Disease (CKD) is a disease that seriously jeopardizes human health. In recent years, cardiorenal protection studies have made significant progress in patients with CKD, and the introduction of sodium-glucose cotransporter protein 2 (SGLT2) inhibitors in particular has provided an important new tool in the treatment of CKD (1, 2). These inhibitors target SGLT2, which is responsible for the reabsorption of glucose and sodium in the proximal tubules of the kidneys. SGLT2 inhibitors lower glucose levels and blood pressure by lowering the renal threshold for glucose excretion and by promoting negative salt and water homeostasis. They also have an effect on glomerular hemodynamics, leading to a decrease in glomerular filtration rate, which is reversible when treatment is stopped. This phenomenon is known as "glomerular feedback". In type 2 diabetes mellitus (T2DM), SGLT2 inhibitors affect glomerular ultrafiltration by decreasing vascular resistance and inducing vasodilation. In addition, these inhibitors reduce fibrosis in the proximal tubule by decreasing energy expenditure and inhibiting the mammalian target of rapamycin (mTOR) pathway (3). Another growing area of research is the ability of SGLT2 inhibitors to induce a fasting state (4). All of these effects reduce cellular stress and inflammation in cardiac and renal tissues, which is thought to be the pathophysiologic reason for the beneficial effects of SGLT2 inhibitors on cardiac and renal function (1).

SGLT2 inhibitors, including canagliflozin, dapagliflozin, and empagliflozin, are widely approved antihyperglycemic agents that improve insulin resistance (IR) (5), and are used to reduce glycemia and cardiovascular risk in T2DM patients (6). Large clinical trials provide compelling evidence to support their beneficial effects on major adverse cardiovascular events, hospitalization for heart failure (7–11) and renal outcomes (12, 13). However, studies on genetic variants related to SGLT2 inhibitors' renoprotective effects are limited. The *SLC5A2* gene encodes SGLT2, crucial for renal glucose reabsorption. Despite promising results in clinical studies, challenges such as variable patient responses and long-term effects remain. Addressing these challenges is essential to optimize clinical application.

In the field of precision medicine, finding effective molecular targets is a key task. Traditional methods to study the mechanism of action of target proteins through pharmacology are inefficient and time-consuming. The drug target Mendelian randomization (MR) has emerged as a novel and powerful research method. Novel methods such as gene co-localization and gene variants associated with drug target mRNA expression (expression quantitative trait loci (*eQTL*)) can be used to create MR tool variables for drug exposure (14). The *eQTL* is a type of expressed quantitative trait locus, which can be classified into *cis-eQTL* and *trans-eQTL* by analyzing the relationship between the amount of gene expression and the locus. The former regulates genes that are closer together, while the latter regulates genes that are farther apart. Drug target MR utilizes genetic instrumental variables to explore the causal relationship between molecules and diseases. This approach is based on the downstream products of target proteins, using Single Nucleotide Polymorphisms (SNPs) near the coding genes of the target proteins that have significant effects on these downstream products (protein Quantitative Trait Loci (*pQTL*) or *eQTL*) as the instrumental variables. The concentration of the downstream products of the target proteins serves as the exposure, while disease serves as the endpoint. MR is then performed to verify the effect of the target proteins on the studied diseases, providing new ideas for drug development and effect prediction. This strategy can be used to investigate the biological mechanism of the effect of SGLT2 inhibition on eGFR.

Given that the genetic mechanisms of SGLT2 inhibition in CKD protection are poorly understood, we hypothesized that SGLT2 inhibitors may be causally related to eGFR. Therefore, our study was designed to assess the genetic causal effect of SGLT2 inhibition on eGFR. We performed a two-sample MR analysis using genetic variants associated with *SLC5A2* gene expression and hemoglobin A1c (HbA1c) levels as instrumental variables. This helps to expand our understanding of the mechanisms of SGLT2 inhibitors in renal protection and provides evidence for this.

2 Methods

2.1 Study design

Figure 1 illustrates a schematic of the study design. The study was conducted in the following five steps: 1) Genetic variants representing the effects of SGLT2 inhibition were selected as instrumental variables. 2) Pooled GWAS data for T2DM was selected as a positive control. 3) The outcome factor, eGFR, was

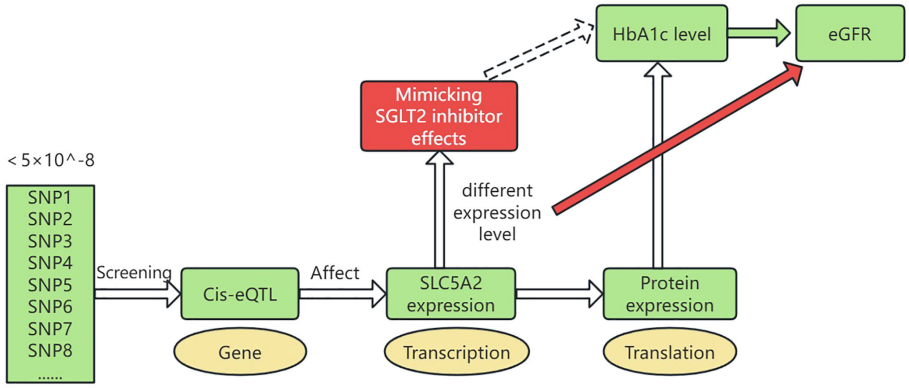


FIGURE 1
Drug-targeted MR study design. SGLT2, Sodium–glucose cotransporter 2; HbA1c, hemoglobin A1c; eGFR, estimated Glomerular Filtration Rate; cis-eQTL, cis-expression Quantitative Trait Locus. Genetic variants representing SLC5A2 gene expression levels were selected by screening cis-eQTLs with a genotype frequency of less than 5×10^-8. These variants influence SLC5A2 gene expression at the RNA transcription level. The expression further affects protein levels, which in turn influence HbA1c levels. Thus, different expression levels of the SLC5A2 gene can mimic the therapeutic effects of SGLT2 inhibitors (glucose-lowering effects, reduced HbA1c). We studied the impact of these varying expression levels on eGFR.

selected. 4) MR analyses were performed to estimate the causal effects of SGLT2 inhibition on eGFR.

2.2 Data sources

2.2.1 Selection of genetic variants of SGLT2 inhibitors

The process of selecting genetic variants for SGLT2 inhibitors is described in detail in the study of Xu M. et al. (15). It involves four steps: First, genetic variants associated with *SLC5A2* gene expression were selected by Genotype-Tissue Expression (GTEx) (16) and *eQTL*Gen Consortium (17) data; second, the correlation of each *SLC5A2* variant with HbA1c levels was assessed using UK Biobank data (18); then, the causal variants of *SLC5A2* with HbA1c were verified by the co-localization method (10); and finally, six genetic variants closely associated with HbA1c and SGLT2 inhibition were screened for MR analysis by the standard clustering process and F-statistic estimation (Supplementary Table 1).

2.2.2 Study outcome

The GWAS data used in this study included the year of publication, sample size, SNP counts, and population information, as detailed in Table 1. The outcome GWAS data in this study were extracted from specialized disease databases. GWAS data for T2DM were obtained from the Diabetes Genetics Replication and Meta-analysis (DIAGRAM) consortium, and GWAS data for eGFR were obtained from the CKDGen consortium.

2.2.2.1 Positive control data

Positive control MR analysis justifies the genetic tool of a drug by demonstrating the expected effect on an outcome that has an established causal relationship with the target drug (19). SGLT2 inhibitors are used as a glucose-lowering drug with the intended indication of T2DM. Therefore, T2DM GWAS pooled data was selected as a positive control. The T2DM data in this study was obtained from one of the largest diabetes case-control studies published in 2022 in the DIAGRAM consortium. This study was

TABLE 1 Description of GWAS data sources.

Variable	Phenotype	Sample size	Ancestry	Cohort/ Consortium	First author	Year	PubMed ID
Exposure	SLC5A2	344182	European	UK Biobank	NA	2018	NA
Positive control	T2DM	673025	Multi-ancestry	DIAGRAM	Mahajan A	2022	35551307
Outcome-ALL	eGFR	1,046,070	Multi-ancestry	CKDGen	Wuttke M	2019	31152163
Outcome-EA	eGFR	1,046,070	European	CKDGen	Wuttke M	2019	31152163
Validation dataset-ALL	eGFR	1201929	Multi-ancestry	CKDGen, UK Biobank	Stanzick KJ	2021	34272381
Validation dataset-EA	eGFR	1004040	European	CKDGen, UK Biobank	Stanzick KJ	2021	34272381

GWAS, Genome-Wide Association Study; T2DM, Diabetes Mellitus Type 2; eGFR, estimated Glomerular Filtration Rate; DIAGRAM, Diabetes Genetics Replication and Meta- analysis; CKDGen, Chronic Kidney Disease Gen; ALL, Multi-ancestry population; EA, European population.
NA, Not Available.

conducted by Mahajan et al. The GWAS meta-analysis included 22 GWAS studies involving 180,834 patients with T2DM and 492,191 controls, as well as five lineage groups (51.1% of European ancestry) (20).

2.2.2.2 Study outcome data

Genetic associations for eGFR were obtained from a meta-analysis of publicly available CKDGen studies (21). All participants were of European ancestry, with a mean age of 54 years. All genetic correlations were adjusted for sex, age, study center, genetic rationale, components, correlations, and other study-specific characteristics (22). To confirm the robustness of the results of this study, we performed MR analysis using eGFR meta-analysis GWAS data from a diverse ancestry cohort (n ≥ 61 studies) and from a European ancestry cohort (n ≥ 42 studies). Detailed information is provided in Table 1.

2.2.2.3 Validation of outcome data

In addition, as a sensitivity analysis, the results of this study were validated using GWAS pooled data from another CKDGen meta-analysis using eGFR GWAS data from Stanzick et al, which is publicly accessible on the official website of CKDGen (23). Also, this data has been integrated into the GWAS Catalog database (GCST90103633, GCST90103634) (Table 1).

The GWAS summary statistics used in this study can be found in the IEU OpenGWAS project (https://gwas.mrcieu.ac.uk/), the GTEx portal (https://www.gtexportal.org/), the *eQTL*Gen consortium (https://eqtlgen.org/), the DIAGRAM consortium (https://diagram-consortium.org/), CKDGen (https://ckdgen.imbi.uni-freiburg.de/), UKB Research (https://www.nealelab.is/uk-biobank, ukb-d-30750_irnt), and the GWAS Catalog (https://www.ebi.ac.uk/gwas/downloads/summary-statistics, GCST008058, GCST008064, GCST90103633, GCST90103634).

2.3 Statistical analysis

In the first place, genetic variant screening for SGLT2 inhibitors applied a generalized inverse variance weighting (IVW) method to consider the correlation of six predictors. MR effects were estimated by the LD matrix and IVW and MR-Egger methods to improve the efficiency and stability of the analysis (24). Subsequently, in MR analysis, the IVW method was used as the primary method to explore the effect of SGLT2 inhibition on the positive control T2DM and the study outcome eGFR (19). Various methods (including MR-Egger, weighted median, simple, and weighted models) were applied to test the reliability and stability of the results. The Strengthening the Reporting of Observational Studies in Epidemiology-Mendelian Randomization (STROBE-MR) guidelines were applied to guide the design of this study and the writing of the results (19). All statistical analyses were performed using the "Two-sample MR" (version 0.5.6) and "MR" (version 0.5.1) packages in the R software (version 4.3.2) environment. To more accurately interpret causality, we set the level of statistical significance to the Bonferroni-corrected level, that is, $P < 0.0083$. All MR analyses were performed using the Mendelian randomization R package and the TwoSampleMR R package (github.com/MRCIEU/TwoSampleMR). We plotted the results as forest plots using code from the ggplot 2 package in R.

As a sensitivity analysis, we applied an independent validation with a separate dataset. The validation data came from GWAS pooled data from another CKDGen meta-analysis, details of which are shown in Table 1.

To control for heterogeneity, we applied the MR multivalent residuals and outliers (MR-PRESSO) method. To test potential MR hypotheses, such as assessing the effect of directional pleiotropy, we applied the generalized MR-Egger regression method. Both tests of pleiotropy using the MR-Egger intercept term and tests of heterogeneity among predictors (Cochran Q statistic for IVW and global test for MR-PRESSO) were used to quantify the level of pleiotropy in the MR analysis. Compared to the MR-Egger, the MR-PRESSO method has higher accuracy for identifying levels of multivalence and outliers (25). The stability of outliers and results was also tested using the leave-one-out sensitivity analysis method, funnel plots, and scatter plots.

For a more rigorous interpretation of causality, we also used the Bonferroni correction based on the number of SNPs analyzed in the final MR. We used the significance level after performing the Bonferroni correction, which was set at 0.0083 (0.05 divided by 6 SNPs), to control for the rate of false discovery of multiple comparisons. In each independent SNP analysis, we compared the P value to 0.0083 to determine whether statistical significance existed.

3 Results

3.1 Effect of positive control-*SLC5A2* on T2DM

To conduct a positive control, T2DM GWAS data were selected as outcome data and analyzed using MR with *SLC5A2*. As shown in Table 2, the MR results indicated that a 1-SD increase in HbA1c regulated by *SLC5A2* was associated with an 83% increase in the risk of T2DM (IVW OR 1.83, 95% CI: 1.180-2.846, p = 0.007). This result effectively mimics the pharmacological inhibition mechanism of SGLT2 inhibitors, demonstrating that SGLT2 inhibition reduces the risk of T2DM. SGLT2 inhibitors, as glucose-lowering drugs, have an established causal relationship with T2DM, and the results of the positive control analysis were consistent with the expected outcomes. This suggests that SGLT2 inhibition decreases the risk of T2DM and demonstrates that the target gene, *SLC5A2*, can serve as a genetic tool for the drug target of SGLT2 inhibitors.

3.2 Effect of *SLC5A2* on eGFR

The IVW method showed evidence to support a causal association between *SLC5A2* and eGFR (Table 2, Figures 2, 3). As shown in Table 2, the MR results indicated that the *SLC5A2* gene, was negatively correlated with eGFR (IVW β -0.038, 95% CI -0.061 to -0.015, P = 0.001 for multi-ancestry populations; IVW β -0.053,

TABLE 2 MR estimation of the effect of SLC5A2 on T2DM and eGFR.

Exposure	Group	Outcome	Method	nSNP	β (95% CI)	SE	OR (95% CI)	P-value	P_h	$P_{intercept}$
SLC5A2	Positive control	T2DM	MR Egger	6	-1.456 (-3.641~0.729)	1.115	0.233 (0.026~2.072)	0.262	0.913	0.132
			Weighted median	6	0.512 (-0.080~1.103)	0.302	1.668 (0.941~2.955)	0.090		
			IVW	6	0.606 (0.165~1.046)	0.225	1.832 (1.180~2.846)	0.007	0.474	
	Outcome-ALL	eGFR	MR Egger	6	0.049 (-0.051~0.149)	0.051		0.389	0.476	0.157
			Weighted median	6	-0.028 (-0.055~-0.002)	0.013		0.035		
			IVW	6	-0.038 (-0.061~-0.015)	0.012		0.001	0.258	
	Outcome-EA	eGFR	MR Egger	6	-0.006 (-0.124~0.113)	0.060		0.932	0.412	0.471
			Weighted median	6	-0.064 (-0.098~-0.030)	0.017		2.507E-04		
			IVW	6	-0.053 (-0.077~-0.028)	0.012		2.451E-05	0.468	
	Validation dataset-ALL	eGFR	MR Egger	6	0.027 (-0.072~0.127)	0.051		0.620	0.969	0.340
			Weighted median	6	-0.025 (-0.049~-0.001)	0.012		0.041		
			IVW	6	-0.027 (-0.046~-0.007)	0.010		0.007	0.887	
	Validation dataset-EA	eGFR	MR Egger	6	-0.009 (-0.109~0.091)	0.051		0.867	0.948	0.688
			Weighted median	6	-0.031 (-0.055~-0.006)	0.012		0.014		
			IVW	6	-0.031 (-0.050~-0.011)	0.010		0.002	0.969	

T2DM, Diabetes Mellitus Type 2; eGFR, estimated Glomerular Filtration Rate; 95% CI, 95% confidence interval; IVW, Inverse Variance Weighted; nSNP, Number of Single Nucleotide Polymorphism; SE, standard deviation; P_h, Heterogeneity P-value; $P_{intercept}$, MR-Egger regression intercept P-value; ALL, Multi-ancestry population; EA, European population.

95% CI -0.077 to -0.028, P = 2.45E-05 for populations of European ancestry). This suggests that each 1-SD increase in HbA1c predicted by the SLC5A2 gene is associated with a decrease in eGFR.

Conversion to mimic pharmacological inhibition, that is, lowering genetically predicted HbA1c per 1-SD unit by SGLT2 inhibition, reduces the risk of eGFR decline, which means that SGLT2 inhibitors are demonstrated to have a nephroprotective effect. All of the above P-values were less than 0.083 (Bonferroni-corrected significance level), suggesting statistical significance and robust results.

3.3 Validation and sensitivity analysis

First, as a sensitivity analysis, this study was validated using GWAS pooled data from another CKDGen meta-analysis, which yielded consistent findings across both multi-ancestry and European populations (Table 2, Supplementary Figures 1, 2). The validation data indicated a negative correlation between HbA1c predicted by the gene SLC5A2 and eGFR. Specifically, for multi-ancestry populations, IVW β was -0.027 (95% CI -0.046 to -0.007, P=0.007), and for populations of European ancestry, IVW β was -0.031 (95% CI -0.050 to -0.011, P=0.002). These results are consistent with the primary study outcomes.

Secondly, as shown in Table 2, MR analyses applying the weighted median method in the positive control, primary outcome, and validation outcome groups yielded consistent findings.

Third, the IVW heterogeneity test using the Cochran Q test in each group separately showed that neither the Q statistic nor the P value was significant (P > 0.05), implying that there was no evidence of heterogeneity in the effect of SGLT2 inhibition on eGFR. The test of pleiotropy using the MR-Egger intercept term showed that the P-value for the intercept was greater than 0.05, which implies that horizontal pleiotropy was not shown (Table 2).

Fourth, as shown in Supplementary Figures 3-6, the leave-one-out analysis in the primary outcome data and validation data for

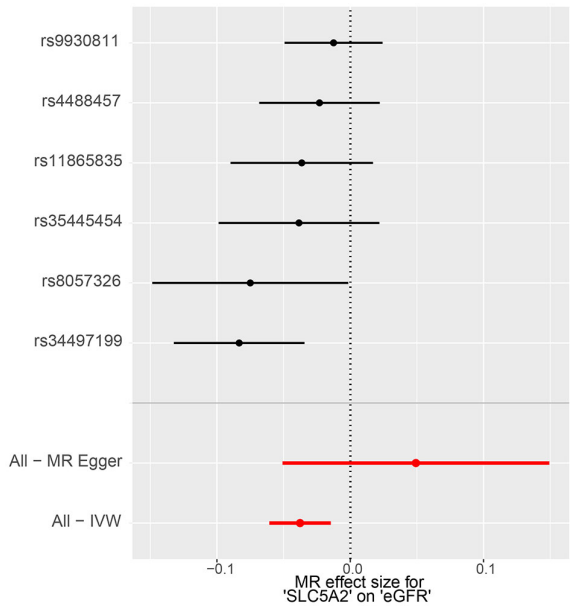


FIGURE 2
MR forest plot of the effect of SLC5A2 on eGFR in multi-ancestry populations. eGFR, estimated Glomerular Filtration Rate; IVW, Inverse Variance Weighted; MR, Mendelian randomization.

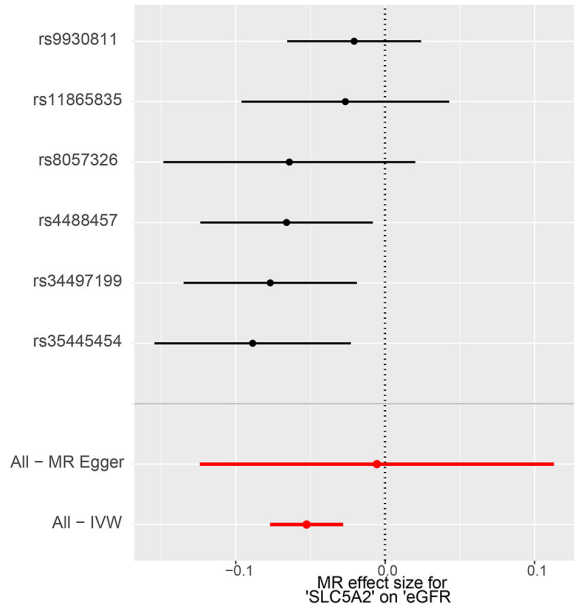


FIGURE 3
MR forest plot of the effect of SLC5A2 on eGFR in European populations. eGFR, estimated Glomerular Filtration Rate; IVW, Inverse Variance Weighted; MR, Mendelian randomization.

both multi-ancestry and European populations indicated that the effect sizes remained on the same side of the reference line 0, demonstrating robust results. This analysis confirms the negative correlation between the SLC5A2 gene and eGFR.

Overall, the results of the MR analysis in this study were robust.

4 Discussion

In this study, we performed MR analysis of drug targets and determined the genetic causal effect of SGLT2 inhibitors on eGFR. This finding provides genetic validation of the mechanism of action of SGLT2 inhibitors in improving renal function and delaying CKD progression.

Several large clinical observational or randomized controlled studies have found that canagliflozin, dapagliflozin, and empagliflozin produce a sustained improvement in kidney disease prognosis, including slowing eGFR decline, reducing albuminuria, and lowering the risk of renal failure (1, 13, 26, 27). This improvement is not only seen in T2DM patients, but patients without diabetes can also benefit. Based on extensive evidence of SGLT2 inhibitors in patients with CKD, KDIGO updated its clinical practice guideline in 2022 to recommend SGLT2 inhibitors as preferred therapeutic agents for managing T2DM combined with CKD (2). SGLT2 inhibitors are crucial for managing T2DM combined with atherosclerotic cardiovascular disease (ASCVD). They are the first-line therapeutic agents for patients with T2DM combined with ASCVD, cardiovascular risk factors, heart failure (HF), and CKD. Additionally, SGLT2 inhibitors are recommended for treating patients with HF or CKD who do not have comorbid diabetes (28).

It is worth noting that the currently completed randomized controlled trials of various SGLT2 inhibitors have different requirements for minimum eGFR levels in the enrollment criteria. Moreover, there are differences in the design of clinical studies for different SGLT2 inhibitors, and the respective protocols may be heterogeneous, so the findings of clinical studies corresponding to one drug may not be directly generalizable to other drugs of the same class. Randomized controlled trials are generally costly and lengthy. In contrast, a new research approach in recent years, drug-targeted MR, has significant advantages. MR is a method that utilizes genetic variants closely associated with exposure as a potentially unconfounded tool to infer a causal relationship between exposure and outcome. New approaches, such as genetic co-localization and genetic variation (*eQTL*) associated with drug target mRNA expression, can be used to create MR instrumental variables for use in drug targets (14), which has become a new research hotspot.

Previous MR studies have further explored the benefits of SGLT2 inhibitors. Xu M et al. (15) investigated the role of SGLT2 inhibition in reducing coronary artery disease (CAD) and T2DM. They found that SGLT2 inhibition lowered the risk of both conditions, with choline metabolites partly mediating these effects. Another study focused on HF and identified that SGLT2 inhibition reduced HF risk through anti-inflammatory mechanisms, specifically via the CXCL10 biomarker (29). These studies support our findings and highlight the broader therapeutic potential of SGLT2 inhibitors in managing complex diseases like CKD, T2DM, CAD, and HF.

To the best of our knowledge, few relevant MR studies have explored the genetic causal effect of SGLT2 inhibition on the effects of eGFR. A recently published study by Wang Z et al. investigated the role of circulating metabolites in the renoprotective effects of

SGLT2 inhibition, specifically identifying small high-density lipoprotein (HDL) particles as mediators in this process (30). However, our study differs in that it focuses on the direct genetic causal effect of SGLT2 inhibition on eGFR using MR analysis without considering metabolic intermediates. Our MR analysis revealed that genetically predicted SGLT2 inhibition resulted in a reduced risk of eGFR decline. This result supports the association between IR and renal function found in previous observational studies, using genetic data to provide further causal evidence. The EMPA-KIDNEY trial (27), the most extensive international multicenter, randomized, double-blind, placebo-controlled clinical trial in CKD to date, confirmed that empagliflozin treatment significantly reduced the risk of the primary endpoint event, which includes kidney disease progression and cardiovascular death, compared to placebo. An updated subgroup analysis of the EMPA-KIDNEY study confirmed that Engeletin significantly slows the progression of chronic kidney function in a broad population of CKD patients regardless of CKD etiology, diabetes status, eGFR and urinary ACR levels (31, 32). Our findings provide timely and robust evidence for the role of SGLT2 inhibition in reducing CKD risk.

The potential mechanisms linking SLC5A2 inhibition and eGFR may involve reduced expression of SLC5A2, inhibition of SGLT2 receptors, leading to decreased reabsorption of glucose and sodium in renal tubules, which results in osmotic diuresis and lower blood pressure (1). Additionally, these inhibitors may exert anti-inflammatory effects, alleviate renal oxidative stress, and modulate metabolic pathways, collectively supporting renal function (3, 4).

However, there are some limitations to this study. First, this study could only predict the effect of drugs on eGFR by interfering with the encoded proteins (targeting effect). We cannot rule out the possibility that drugs alter eGFR risk through other proteins (off-target effects). Second, our MR analyses estimated SGLT2 inhibition based on targeted reductions in genetically predicted HbA1c levels rather than direct effects of SGLT2 inhibitors. This is based on the assumption that the effect of SGLT2 inhibitors on HbA1c levels is proportional to their overall effect, which may be inconsistent with the actual mechanism of SGLT2 inhibitors. In addition, the drug formulation, dosage, and mode of administration of SGLT2 inhibitors can influence their effects on eGFR. These factors should be taken into account when interpreting our findings. Third, genetic variation reflects the effect of lifelong exposure to a biomarker on outcome and therefore cannot be used to directly estimate the expected impact of short-term pharmacologic changes in that biomarker (33). This means that the effect sizes in our study may not be comparable to those reported in trials or observational studies. Fourth, although the MR-Egger method is dependent on the orientations of SNPs and can influence the outcome of the analysis, our study conducted a pleiotropy test using the MR-Egger intercept term, which showed no evidence of horizontal pleiotropy ($P > 0.05$). Finally, our analyses were conducted mainly in European populations. Therefore, this limits the generalizability of our findings to other ethnicities. The potential for confounding factors in observational data must also be considered, as they might affect the accuracy and applicability of the results.

Future studies should explore the genetic causal effect of SGLT2 inhibition on eGFR in diverse populations to enhance the generalizability of the findings. Additionally, future research should aim to integrate genetic evidence with experimental and clinical studies to provide a comprehensive understanding of how SGLT2 inhibitors can be leveraged to protect against CKD progression.

5 Conclusions

Our results show that *SLC5A2* is negatively correlated with eGFR, identifying a causal role of SGLT2 inhibition with eGFR. By modeling the drug-targeted effects of SGLT2 inhibitors, it was thus concluded that inhibition of *SLC5A2* gene expression was causally associated with higher eGFR. The renoprotective effect of SGLT2 inhibitors was validated from the perspective of genetic variation, and also provided MR evidence for SGLT2 inhibitors as first-line therapeutic agents for CKD patients.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary material.

Ethics statement

This research has been conducted using the publicly available data. No original data were collected for the MR study. Ethical approval for each of the studies included in the investigation can be found in the original publications (including informed consent from each participant). 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

GL: Writing – original draft, Writing – review & editing.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Acknowledgments

The author would like to express his gratitude to all colleagues in the department. I sincerely acknowledge Dr. Wentao Ni (People's Hospital of Peking University) for his guidance and assistance with the Mendelian randomization analysis method.

Conflict of interest

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

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/fendo.2024.1418575/full#supplementary-material

References

1. Maxson R, Starr J, Sewell J, Lyas C. SGLT2 inhibitors to slow chronic kidney disease progression: A review. *Clin Ther*. (2024) 46:e23–8. doi: 10.1016/j.clinthera.2023.10.014

2. Kidney Disease: Improving Global Outcomes (KDIGO) Diabetes Work Group. KDIGO 2022 clinical practice guideline for diabetes management in chronic kidney disease. *Kidney Int*. (2022) 102:S1–S127. doi: 10.1016/j.kint.2022.06.008

3. Scurt FG, Ganz MJ, Herzog C, Bose K, Mertens PR, Chatzikyrkou C. Association of metabolic syndrome and chronic kidney disease. *Obes Rev*. (2024) 25:e13649. doi: 10.1111/obr.13649

4. Packer M. Critical reanalysis of the mechanisms underlying the cardiorenal benefits of SGLT2 inhibitors and reaffirmation of the nutrient deprivation signaling/autophagy hypothesis. *Circulation*. (2022) 146:1383–405. doi: 10.1161/CIRCULATIONAHA.122.061732

5. Kullmann S, Hummel J, Wagner R, Dannecker C, Vosseler A, Fritsche L, et al. Empagliflozin improves insulin sensitivity of the hypothalamus in humans with prediabetes: A randomized, double-blind, placebo-controlled, phase 2 trial. *Diabetes Care*. (2022) 45:398–406. doi: 10.2337/dc21-1136

6. Heerspink HJL, Perkins BA, Fitchett DH, Husain M, Cherney DZI. Sodium glucose cotransporter 2 inhibitors in the treatment of diabetes mellitus: cardiovascular and kidney effects, potential mechanisms, and clinical applications. *Circulation*. (2016) 134:752–72. doi: 10.1161/CIRCULATIONAHA.116.021887

7. Zinman B, Wanner C, Lachin JM, Fitchett D, Bluhmki E, Hantel S, et al. Empagliflozin, cardiovascular outcomes, and mortality in type 2 diabetes. *N Engl J Med*. (2015) 373:2117–28. doi: 10.1056/NEJMoa1504720

8. Neal B, Perkovic V, Mahaffey KW, de Zeeuw D, Fulcher G, Erondu N, et al. Canagliflozin and cardiovascular and renal events in type 2 diabetes. *N Engl J Med*. (2017) 377:644–57. doi: 10.1056/NEJMoa1611925

9. Wiviott SD, Raz I, Bonaca MP, Mosenzon O, Kato ET, Cahn A, et al. Dapagliflozin and cardiovascular outcomes in type 2 diabetes. *N Engl J Med*. (2019) 380:347–57. doi: 10.1056/NEJMoa1812389

10. Cannon CP, Pratley R, Dagogo-Jack S, Mancuso J, Huyck S, Masiukiewicz U, et al. Cardiovascular outcomes with ertugliflozin in type 2 diabetes. *N Engl J Med*. (2020) 383:1425–35. doi: 10.1056/NEJMoa2004967

11. McMurray JJV, Solomon SD, Inzucchi SE, Køber L, Kosiborod MN, Martinez FA, et al. Dapagliflozin in patients with heart failure and reduced ejection fraction. *N Engl J Med*. (2019) 381:1995–2008. doi: 10.1056/NEJMoa1911303

12. Wanner C, Inzucchi SE, Lachin JM, Fitchett D, von Eynatten M, Mattheus M, et al. Empagliflozin and progression of kidney disease in type 2 diabetes. *N Engl J Med*. (2016) 375:323–34. doi: 10.1056/NEJMoa1515920

13. Perkovic V, Jardine MJ, Neal B, Bompoint S, Heerspink HJL, Charytan DM, et al. Canagliflozin and renal outcomes in type 2 diabetes and nephropathy. *N Engl J Med*. (2019) 380:2295–306. doi: 10.1056/NEJMoa1811744

14. Walker VM, Kehoe PG, Martin RM, Davies NM. Repurposing antihypertensive drugs for the prevention of Alzheimer's disease: a Mendelian randomization study. *Int J Epidemiol*. (2020) 49:1132–40. doi: 10.1093/ije/dyz155

15. Xu M, Zheng J, Hou T, Lin H, Wang T, Wang S, et al. SGLT2 inhibition, choline metabolites, and cardiometabolic diseases: A mediation mendelian randomization study. *Diabetes Care*. (2022) 45:2718–28. doi: 10.2337/dc22-0323

16. GTEx Consortium. The GTEx Consortium atlas of genetic regulatory effects across human tissues. *Science*. (2020) 369:1318–30. doi: 10.1126/science.aaz1776

17. Võsa U, Claringbould A, Westra H-J, Bonder MJ, Deelen P, Zeng B, et al. Large-scale cis- and trans-eQTL analyses identify thousands of genetic loci and polygenic scores that regulate blood gene expression. *Nat Genet*. (2021) 53:1300–10. doi: 10.1038/s41588-021-00913-z

18. Elsworth B, Lyon M, Alexander T, Liu Y, Matthews P, Hallett J, et al. The MRC IEU OpenGWAS data infrastructure. *Genetics*. (2020). doi: 10.1101/2020.08.10.244293. preprint.

19. Burgess S, Davey Smith G, Davies NM, Dudbridge F, Gill D, Glymour MM, et al. Guidelines for performing Mendelian randomization investigations: update for summer 2023. *Wellcome Open Res*. (2019) 4:186. doi: 10.12688/wellcomeopenres.15555.3

20. Mahajan A, Spracklen CN, Zhang W, Ng MCY, Petty LE, Kitajima H, et al. Multi-ancestry genetic study of type 2 diabetes highlights the power of diverse populations for discovery and translation. *Nat Genet*. (2022) 54:560–72. doi: 10.1038/s41588-022-01058-3

21. Köttgen A, Pattaro C. The CKDGen Consortium: ten years of insights into the genetic basis of kidney function. *Kidney Int*. (2020) 97:236–42. doi: 10.1016/j.kint.2019.10.027

22. Wuttke M, Li Y, Li M, Sieber KB, Feitosa MF, Gorski M, et al. A catalog of genetic loci associated with kidney function from analyses of a million individuals. *Nat Genet*. (2019) 51:957–72. doi: 10.1038/s41588-019-0407-x

23. Stanzick KJ, Li Y, Schlosser P, Gorski M, Wuttke M, Thomas LF, et al. Discovery and prioritization of variants and genes for kidney function in >1. *2 million individuals. Nat Commun*. (2021) 12:4350. doi: 10.1038/s41467-021-24491-0

24. Burgess S, Zuber V, Valdes-Marquez E, Sun BB, Hopewell JC. Mendelian randomization with fine-mapped genetic data: Choosing from large numbers of correlated instrumental variables. *Genet Epidemiol*. (2017) 41:714–25. doi: 10.1002/gepi.22077

25. Verbanck M, Chen C-Y, Neale B, Do R. Detection of widespread horizontal pleiotropy in causal relationships inferred from Mendelian randomization between complex traits and diseases. *Nat Genet*. (2018) 50:693–8. doi: 10.1038/s41588-018-0099-7

26. Heerspink HJL, Stefánsson BV, Correa-Rotter R, Chertow GM, Greene T, Hou F-F, et al. Dapagliflozin in patients with chronic kidney disease. *N Engl J Med*. (2020) 383:1436–46. doi: 10.1056/NEJMoa2024816

27. The EMPA-KIDNEY Collaborative Group, Herrington WG, Staplin N, Wanner C, Green JB, Hauske SJ, et al. Empagliflozin in patients with chronic kidney disease. *N Engl J Med*. (2023) 388:117–27. doi: 10.1056/NEJMoa2204233

28. Chen TK, Hoenig MP, Nitsch D, Grams ME. Advances in the management of chronic kidney disease. *BMJ*. (2023) 383:e074216. doi: 10.1136/bmj-2022-074216

29. Guo W, Zhao L, Huang W, Chen J, Zhong T, Yan S, et al. Sodium-glucose cotransporter 2 inhibitors, inflammation, and heart failure: a two-sample Mendelian randomization study. *Cardiovasc Diabetol*. (2024) 23:118. doi: 10.1186/s12933-024-02210-5

30. Wang Z, Wei J, Zhao W, Shi R, Zhu Y, Li X, et al. SGLT2 inhibition, high-density lipoprotein, and kidney function: a mendelian randomization study. *Lipids Health Dis*. (2024) 23:84. doi: 10.1186/s12944-024-02072-6

31. EMPA-KIDNEY Collaborative Group. Impact of primary kidney disease on the effects of empagliflozin in patients with chronic kidney disease: secondary analyses of the EMPA-KIDNEY trial. *Lancet Diabetes Endocrinol*. (2024) 12:51–60. doi: 10.1016/S2213-8587(23)00322-4

32. EMPA-KIDNEY Collaborative Group. Effects of empagliflozin on progression of chronic kidney disease: a prespecified secondary analysis from the empa-kidney trial. *Lancet Diabetes Endocrinol*. (2024) 12:39–50. doi: 10.1016/S2213-8587(23)00321-2

33. Ference BA. How to use Mendelian randomization to anticipate the results of randomized trials. *Eur Heart J*. (2018) 39:360–2. doi: 10.1093/eurheartj/ehx462

frontiers | Frontiers in Pharmacology

TYPE Review
PUBLISHED 04 October 2024
DOI 10.3389/fphar.2024.1432724

Check for updates

OPEN ACCESS

EDITED BY
Swayam Prakash Srivastava,
Weill Cornell Medical Center, NewYork-Presbyterian, United States

REVIEWED BY
Jiaoyi Chen,
University Health Network (UHN), Canada
David Ribet,
Institut National de la Santé et de la Recherche Médicale (INSERM), France

*CORRESPONDENCE
Lifan Wang,
wlf4648374@163.com
Peng Liu,
drliupeng@sina.cn
Ping Li,
lp8675@163.com

†These authors have contributed equally to this work

RECEIVED 14 May 2024
ACCEPTED 20 September 2024
PUBLISHED 04 October 2024

CITATION
Wang J, Zhang R, Wu C, Wang L, Liu P and Li P (2024) Exploring potential targets for natural product therapy of DN: the role of SUMOylation.
Front. Pharmacol. 15:1432724.
doi: 10.3389/fphar.2024.1432724

COPYRIGHT
© 2024 Wang, Zhang, Wu, Wang, Liu 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.

Exploring potential targets for natural product therapy of DN: the role of SUMOylation

Jingjing Wang[1†], Rui Zhang[1†], Chenguang Wu[1†], Lifan Wang[1*], Peng Liu[2*] and Ping Li[3*]

[1]Renal Division, Heilongjiang Academy of Chinese Medicine Sciences, Harbin, China, [2]Shunyi Hospital, Beijing Hospital of Traditional Chinese Medicine, Beijing, China, [3]China-Japan Friendship Hospital, Beijing, China

Diabetic nephropathy (DN) is a common and serious micro-vascular complication of diabetes and a leading cause of end-stage renal disease globally. This disease primarily affects middle-aged and elderly individuals, especially those with a diabetes history of over 10 years and poor long-term blood glucose control. Small ubiquitin-related modifiers (SUMOs) are a group of reversible post-translational modifications of proteins that are widely expressed in eukaryotes. SUMO proteins intervene in the progression of DN by modulating various signaling cascades, such as Nrf2-mediated oxidative stress, NF-κB, TGF-β, and MAPK pathways. Recent advancements indicate that natural products regulating SUMOylation hold promise as targets for intervening in DN. In a previous article published in 2022, we reviewed the mechanisms by which SUMOylation intervenes in renal fibrosis and presented a summary of some natural products with therapeutic potential. Therefore, this paper will focus on DN. The aim of this review is to elucidate the mechanism of action of SUMOylation in DN and related natural products with therapeutic potential, thereby summarising the targets and candidate natural products for the treatment of DN through the modulation of SUMOylation, such as ginkgolic acid, ginkgolide B, resveratrol, astragaloside IV, etc., and highlighting that natural product-mediated modulation of SUMOylation is a potential therapeutic strategy for the treatment of DN as a potential therapeutic strategy.

KEYWORDS

diabetic nephropathy, sumoylation, natural products, ginkgolic acid, ginkgolide B, resveratrol, astragaloside IV

1 Introduction

Diabetes represents a significant challenge to global health. This issue affects over 500 million individuals worldwide. The global prevalence of this condition is on a startling upward trajectory, with projections indicating a rise to 700 million by the year 2045 (Smith et al., 2022). Particularly, the elderly demographic faces a heightened risk of developing diabetes-related complications, including Diabetic Nephropathy (DN) (Lin et al., 2022). DN is a prevalent micro-vascular complication among individuals with diabetes and serves as a primary mechanism leading to end-stage renal disease (Shoeib et al., 2023). The pathological characteristics of DN encompass thickening of the glomerular basement membrane, mesangial matrix expansion, interstitial fibrosis in the renal tubules, and podocyte loss (Harlan et al., 2018; Wang H. et al., 2021). In individuals with diabetes, the lifetime prevalence of DN exceeds 50% (Pop-Busui et al., 2022). Factors such as hypertension

(Skov et al., 2016), hyperglycemia (Wu et al., 2023), oxidative stress (Ma et al., 2021), advanced glycation end products (AGEs), and angiotensin II can induce the onset of DN by activating pathways like transforming growth factor β (TGF-β), nuclear factor kappa B (NF-κB), Nrf2-mediated oxidative stress, and mitogen-activated protein kinases (MAPK) (Hwang et al., 2019). To date, the treatment for DN has primarily focused on correcting the metabolic dysregulation and hemodynamic abnormalities in patients with diabetes. However, clinical studies have demonstrated that these therapeutic measures only partially alleviate the pathology of DN (Gross et al., 2005). Evidence suggests that standard treatments aimed at strict control of blood sugar and blood pressure are insufficient to halt the progression of DN to end stage renal disease (ESRD) (Arora and Singh, 2013). Consequently, the search for new therapeutic targets to prevent and treat DN has emerged as a critical challenge in this field.

SUMOylation is a reversible post-translational modification (PTM) process (Han et al., 2018), intricately linked with various cellular processes including nucleocytoplasmic transport (Melchior et al., 2003), transcriptional regulation (Muller et al., 2004), apoptosis (Choi et al., 2017), protein stability, stress response, and cell cycle progression (Eifler and Vertegaal, 2015). It has been demonstrated that SUMO can alter and influence the function of specific metabolic enzymes in the pathway, thereby regulating the entire metabolic pathway (Gareau and Lima, 2010). Previous studies have demonstrated that SUMO is associated with a number of diseases related to metabolic disorders, including Type I diabetes (Li et al., 2005; Wang and She, 2008), Type II diabetes (Dou et al., 2022), and diabetes-mediated cardiovascular diseases (Chang and Abe, 2016). The SUMOylation E2 conjugase UBC9 was first reported to be expressed in the kidneys in 2003 (Golebiowski et al., 2003). A growing body of research suggests that SUMOylation is associated with the progression of DN through the modulation of multiple signalling pathways (Gao et al., 2014). It is also linked to the damage process of podocyte (Li et al., 2019). Although some natural products have been proven to inhibit enzymes involved in the SUMOylation process, progress in developing more selective and potent inhibitors of SUMOylation remains limited (Brackett et al., 2020). In previous publications, we have reviewed the mechanisms involved in modulating SUMOylation for the treatment of renal fibrosis, as well as candidate natural products (Liu P. et al., 2022). This article connects the mechanisms of SUMOylation with natural products, exploring the therapeutic prospects of SUMOylation in the progression of DN and the candidate natural products. Figure 1 depicts the chemical structures of the pertinent natural compounds. Table 1 lists the targets associated with SUMOylation for these compounds.

2 SUMOylation

2.1 SUMO proteins

Small ubiquitin-related modifiers (SUMOs) are members of the ubiquitin-like protein family and can conjugate with a wide array of proteins. Predominantly localized in the nucleus, SUMOs are crucial

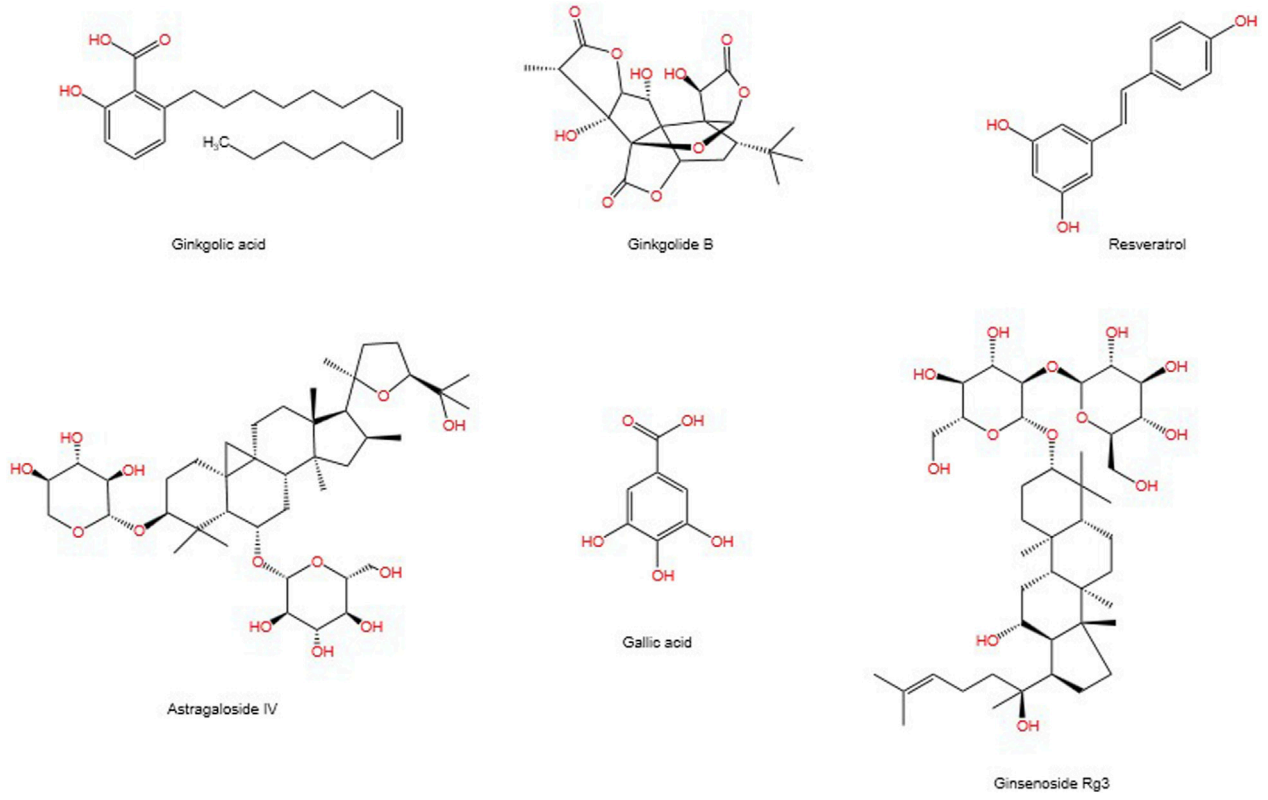


FIGURE 1
Chemical structures of selected natural compounds.

TABLE 1 Natural products and therapeutic targets against DN SUMOylation.

Compounds	Resource	SUMO proteins or enzymes	Therapeutic target	References
Ginkgolic acid	Ginkgo	SUMO1, SUMO2/3	SUMO1-p53	Tan et al. (2022)
Resveratrol	Vitis L., Veratrum L., Arachis, Polygonum	SUMO1 SUMO1	COX-2 β-catenin	Cheng et al. (2018), Wang et al. (2020)
Astragaloside IV	Astragalus membranaceus	SUMO1 --	HIF-1α PPARγ	Wang et al. (2021c), Xing et al. (2021)
Gallic acid	gallnut, sumac, tea leaves, and oak bark	SENP1	--	Taghvaei et al. (2022)
Ginsenoside Rg3	Panax ginseng	E3	NF-κB	Zou et al. (2018)

for various cellular processes, including cell cycle progression, genome stability, and transcription (Vertegaal, 2022). Sumoylation of proteins can influence their stability and enzymatic activity, alter their localization, or mediate novel interactions with other proteins containing SUMO-interacting motifs (SIMs) (Geiss-Friedlander and Melchior, 2007). Research has shown that the PTM of proteins by SUMOs can regulate chromatin structure and function at multiple levels, influencing gene expression and maintaining genomic integrity through various mechanisms. Both SUMO ligases and deconjugating enzymes are key factors in modulating chromatin structure. Sumoylation plays a multifaceted role in regulating chromatin architecture, gene expression, and genomic stability. Part of its function lies in the diverse downstream effects that occur when SUMO is conjugated to different proteins (Cubenas-Potts and Matunis, 2013).

SUMOs, approximately 11 kDa in size and slightly larger than ubiquitin, can bind to target proteins as single or multiple monomers or in various polymeric forms. Like ubiquitin, SUMO can undergo self-modification, such as phosphorylation and/or acetylation. The SUMO conjugation mechanism involves three types of enzymes: the activating E1 enzyme, the conjugating E2 enzyme, and the E3 ligase. In addition to covalent attachment to substrates, SUMO can interact with other proteins through SIMs. Non-covalent protein-protein interactions between sumoylated proteins containing SIMs and SUMO readers can alter the subcellular localization of the sumoylated proteins, including facilitating SUMO-mediated phase separation. SIMs in SUMO-targeted ubiquitin ligases (STUbLs) enable binding to poly-sumoylated proteins, which are subsequently ubiquitinated and degraded, forming a SUMO-mediated protein turnover pathway. Additionally, when SUMO monomers effectively compete with ubiquitin for lysine residues on target proteins, they can prevent the degradation of these proteins (Vertegaal, 2022). The specificity of the SUMO pathway is achieved through redox regulation, acetylation, phosphorylation, or other post-translational modifications of SUMOylation and deSUMOylation enzymes.

SUMO-1 typically modifies substrates as a monomer; however, SUMO-2/3 can form poly-SUMO chains. Monomeric SUMO-1 or poly-SUMO chains can interact with other proteins via SIMs, thus providing a platform to enhance protein interactions. The consequences of SUMOylation include changes in cellular localization, protein activity, or protein stability. Moreover, SUMO can facilitate protein degradation by binding ubiquitin through STUbLs (Chang and Yeh, 2020). To date, five genes encoding SUMO paralogs, known as SUMO1, SUMO2, SUMO3, SUMO4, and SUMO5, have been identified in the human genome (Wu and Huang, 2023). SUMO-1 shares only 56% identity with SUMO-2 and -3. SUMO2 and SUMO3 are 95% homologous, collectively referred to as SUMO2/3 (Kim and Baek, 2009). Mice with a knockout of the SUMO1 gene can survive, thanks to compensatory mechanisms involving SUMO2 or SUMO3 (Wang et al., 2014a; Evdokimov et al., 2008). Under different physiological and pathological conditions, the conjugation of the same substrate protein with different SUMO isoforms can lead to distinct functional outcomes. For instance, the modification of Drp1 by SUMO1 and SUMO2/3 has opposing effects on mitochondrial apoptosis regulation. SUMO1-conjugated Drp1 significantly accelerates mitochondrial fission, ultimately promoting the apoptotic process. In contrast, SUMO2/3-conjugated Drp1 effectively delays mitochondrial fission and inhibits apoptosis (Sheng et al., 2021). Similarly, in the regulation of the stability and function of the circadian rhythm protein Period2 (PER2), SUMO2 modification promotes proteasomal degradation of PER2, whereas SUMO1 conjugation inhibits its degradation and enhances its function as a transcriptional repressor (Chen et al., 2021). Additionally, the poly-SUMO2/3 modification of mutant CFTR protein associated with cystic fibrosis promotes its degradation, while SUMO1 conjugation enhances CFTR stability (Gong et al., 2019). Generally, SUMO1 modification promotes the dissolution of aberrant proteins and inhibits their aggregation, thereby protecting cells, a function particularly significant in preventing protein misfolding-related diseases. On the other hand, SUMO2/3, in coordination with STUBL and the ubiquitin-proteasome system, facilitates substrate degradation, helping cells eliminate damaged or unnecessary proteins. This mechanism is especially important in response to oxidative stress, DNA damage, and other stress conditions (Wang and Matunis, 2023). Although SUMO1, SUMO2, and SUMO3 all play important roles in cellular regulation, current studies suggest that SUMO2/3 may be more critical in the progression of DN. Unlike SUMO1, SUMO2/3 is more readily induced under conditions of cellular stress, particularly in response to oxidative stress and inflammation, which are closely associated with the high-stress environment of DN. SUMO2/3 has the ability to form poly-SUMO chains, thereby influencing a broader network of proteins. This feature gives SUMO2/3 a unique advantage in regulating complex cellular functions such as cell proliferation, differentiation, and apoptosis, making it especially relevant in the context of DN progression. SUMO4 is most similar to

SUMO2/3 but contains a unique proline residue (P90) at its C-terminus, which impedes its effective maturation (Owerbach et al., 2005). Under normal culture conditions, SUMO4 is rapidly degraded; however, during cellular stress, SUMO4 can mature through stress-induced endogenous hydrolases and covalently conjugate to its substrate proteins (Wei et al., 2008). Genetic studies have linked SUMO4 to both type 1 and type 2 diabetes, suggesting that the unbound form of SUMO4 may play a role (Guo et al., 2004; Lin et al., 2007), although this association remains controversial (Podolsky et al., 2009). Nonetheless, recent research by Nisha Sinha and colleagues, through gene polymorphism analysis in diabetic patients without nephropathy (DM) and those with DN, has demonstrated that the SUMO4 c.163 G> A polymorphism is associated with an increased susceptibility to DN in North Indian patients with type 2 diabetes (Sinha et al., 2016). A recent meta-analysis has confirmed that the SUMO4 (M55V) variant is associated with both type 1 and type 2 diabetes in Asian and European populations (Li et al., 2017). The newest member of the SUMO family, SUMO5, highly homologous to SUMO1, has been shown to regulate the dynamics of PML nuclear bodies when exogenously expressed in cells (Liang et al., 2016).

2.2 SUMOylation and deSUMOylation process

SUMOylation is a reversible PTM involving the covalent attachment of small ubiquitin-like modifier proteins to substrate proteins (Tokarz and Wozniak, 2021). The attachment of SUMO1-SUMO3 to proteins, known as SUMOylation, is mediated by a mechanism that involves the dimeric SUMO activating enzyme subunits 1 and 2 (SAE1-SAE2; also known as UBA2-AOS1), the E2 conjugating enzyme UBC9 (also referred to as UBE2I), and E3 SUMO ligases, whereas deSUMOylation is regulated by the sentrin/SUMO-specific protease (SENP) family (Kunz et al., 2018; Yeh, 2009). The first SUMO proteases described were the ubiquitin-like proteases 1 and 2 (Ulp1 and Ulp2) in yeast (Li and Hochstrasser, 1999; Li and Hochstrasser, 2000). The mammalian SENP family consists of six SUMO proteases (SENP1-3 and SENP5-7), which were identified based on their sequence similarity to the Ulp family. These enzymes are all cysteine proteases and contain a catalytic triad (Cys-His-Asp) within a conserved protease domain (Jia et al., 2019). Based on sequence homology, substrate specificity, and subcellular localization, these six SENPs can be categorized into three subfamilies (Kumar and Zhang, 2015). SENP1 and SENP2 form the first subfamily. SENP1 is primarily localized in the nucleus, where it is involved in the maturation of SUMO precursors and the deconjugation of SUMOylated proteins. SENP2 is found in both the nucleus and the nuclear envelope, regulating the SUMOylation of nuclear pore components and other nuclear proteins. Both SENP1 and SENP2 exhibit broad specificity for SUMO-1, SUMO-2, and SUMO-3 and are highly homologous to the yeast Ulp1 protease. By processing the precursors of SUMO-1, SUMO-2, and SUMO-3, SENP1 and SENP2 play a multifunctional role in the SUMOylation process, which is crucial for the regulation of gene expression, DNA repair, and cell cycle progression (Kunz et al., 2018). SENP3 and SENP5 belong to the second subfamily of SUMO proteases and are also located in the nucleus. They exhibit a preference for SUMO-2 and SUMO-3 over SUMO-1, thereby regulating the function and localization of SUMOylated proteins (Yun et al., 2008; Gong and Yeh, 2006; Di Bacco et al., 2006). SENP6 and SENP7 are members of the third subfamily, typically localized in the nucleus. Their catalytic domains contain four conserved loop insertions that are absent in other SENPs, which are believed to confer their SUMO subtype specificity and preference for cleaving SUMO2/3 chains (Claessens and Vertegaal, 2024). Subsequently, the SUMO proteases deSUMOylating isopeptidase 1 (DESI1), DESI2, and USPL1 were identified, which share little sequence homology with the Ulp/SENP family (Schulz et al., 2012; Shin et al., 2012). SUMO is conjugated to lysine residues on target proteins through an isopeptide bond, catalyzed by SUMO-specific activating (E1), conjugating (E2), and ligating (E3) enzymes (Chang and Yeh, 2020). In mammalian cells, SUMOs are translated as inactive precursors and are processed into their mature forms by specific neutral proteases known as SENPs. The maturation of SUMO involves the hydrolysis of a peptide bond at the C-terminus, thereby exposing a diglycine (GG) motif. This motif subsequently forms a thioester bond with the SUMO E1 enzyme (SAE1/UBA2) in an ATP-dependent manner. Subsequently, SUMO is transferred to the catalytic cysteine residue of the SUMO E2 enzyme UBC9 through a transesterification reaction. UBC9 then conjugates SUMO to a lysine residue on the substrate, either with the assistance of a SUMO-E3 ligase or independently (Claessens and Vertegaal, 2024). The complete process of SUMOylation and deSUMOylation is illustrated in Figure 2.

3 Mechanisms of SUMOylation intervention in DN

DN is a principal cause of End-Stage Renal Disease (ESRD) worldwide and is a major contributor to the morbidity and mortality among individuals with diabetes (Reutens and Atkins, 2011). The clinical intervention for DN primarily focuses on controlling risk factors such as hyperglycemia, dyslipidemia, hypertension, and proteinuria, aiming to alleviate symptoms and slow the progression of DN. However, these interventions have limited efficacy (Tang et al., 2021; Xiong and Zhou, 2019; Magee et al., 2017). SUMOylation has been found to be extensively involved in the pathogenesis of DN. Under normal physiological conditions, SUMOylation and deSUMOylation are maintained in dynamic balance, regulating critical cellular functions such as protein stability, transcriptional control, and DNA repair. However, in the context of DN, this balance is disrupted, leading to abnormal increases or decreases in SUMOylation levels. Modification of the NR5A2 protein, a key mediator in the transcriptional regulation of the calreticulin gene, by SUMO exacerbates renal fibrosis (Arvaniti et al., 2016). Furthermore, SUMO may play a protective role against the apoptosis of podocyte (Yang et al., 2019). These findings have been summarized in detail (Figure 3; Table 2).

3.1 SUMOylation interferes with insulin secretion

The SUMO pathway is associated with the production and secretion of insulin (Kamynina and Stover, 2017). In a diabetic

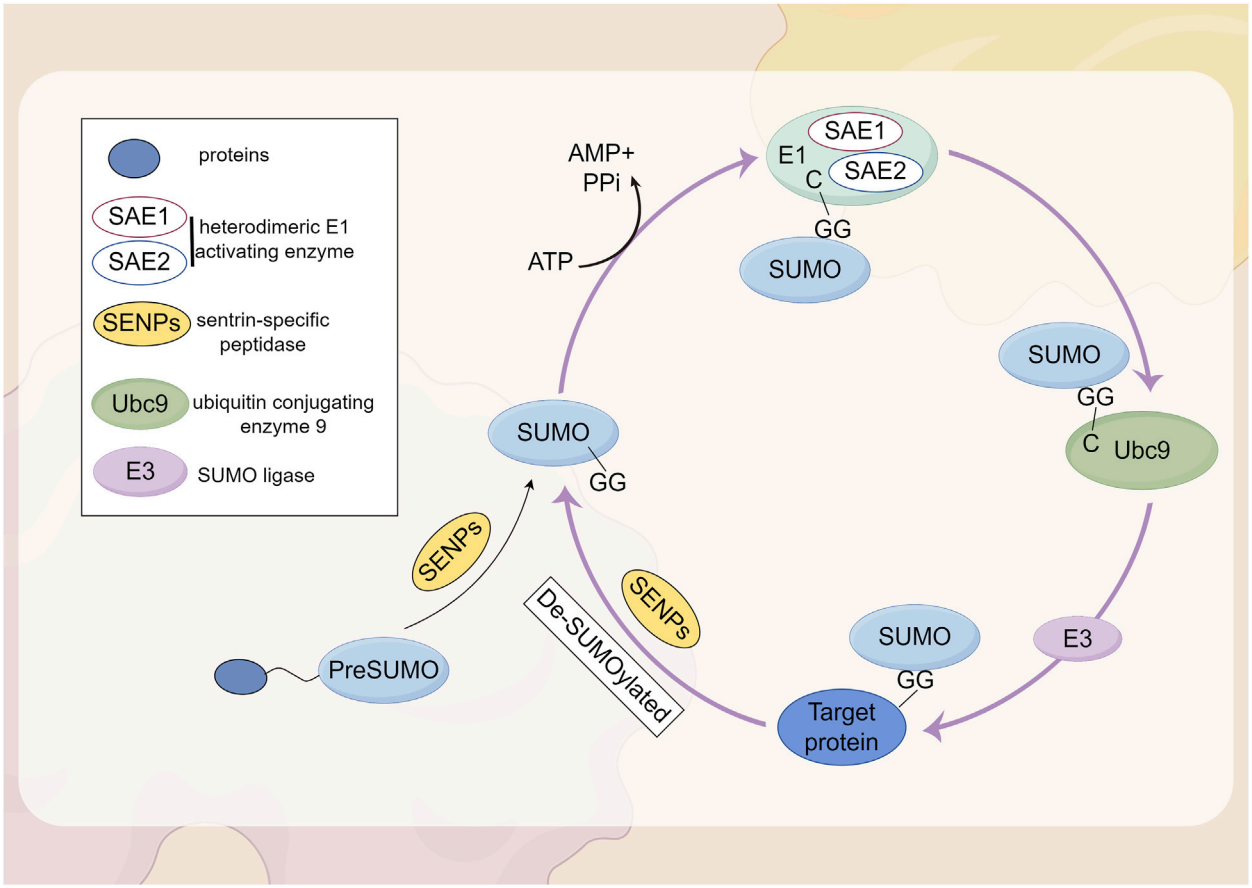


FIGURE 2
Biochemical processes by which proteins undergo SUMOylation.

mouse model established through UBC9 deficiency, β-cells exhibited impaired antioxidant capacity, and Ubc9 deficiency resulted in reduced NRF2 activity and decreased expression of its downstream antioxidant genes, leading to Reactive Oxygen Species (ROS) accumulation and oxidative stress (He et al., 2018). SENP1 plays a crucial role in insulin secretion in type 2 diabetes (Ferdaoussi et al., 2015). SENP1 is essential for glucose-dependent insulin secretion. SUMOylation responds to intracellular Ca^{2+} elevation by directly and reversibly inhibiting β-cell exocytosis, thereby acutely regulating insulin secretion (Dai et al., 2011; Manning et al., 2012). NADPH equivalents and reduced glutathione activate SENP1 function, thereby increasing insulin secretion (Ferdaoussi et al., 2015). Conversely, over-expression of SENP1 reduces insulin secretion and impairs the handling of Ca^{2+}-induced cell death (Hajmrle et al., 2014). Glucagon-like peptide-1 (GLP-1) is an incretin hormone secreted by intestinal L-cells, crucial for postprandial insulin secretion (Drucker et al., 1987). In mouse pancreatic β-cell lines and primary mouse β-cells, cAMP produced by GLP-1R stimulation was shown to be downregulated by SUMO. Therefore, SUMO modification of GLP-1R may be a contributing factor to the diminished incretin response (Rajan et al., 2012). Oxidative stress-mediated inactivation of SENP1 may be a critical step in impaired insulin secretion in type 2 diabetes (Shoeib et al., 2023; Attie, 2015). Studies indicate that SENP2 is involved in adipocyte differentiation (Liang et al., 2019) and adipogenesis (Chung et al., 2010), adipose tissue thermogenesis (Liu et al., 2021), and insulin sensitivity (Zheng et al., 2018). Expression of SENP2 in insulin-secreting cells is triggered by chronic high glucose stimulation. *In vitro*, the increase in SENP2 levels under high glucose conditions was more pronounced in the cytoplasm than in the nucleus (Jung et al., 2016). Experiments have shown that SENP2 regulates mitochondrial function and insulin secretion in pancreatic β-cells, with the regulation of DRP1 sumoylation-mediated phosphorylation being a potential mechanism (Nan et al., 2022). Endoplasmic reticulum protein 44 (ERp44) is closely related to glucose and lipid metabolism (Nyirimigabo et al., 2019). Interfering with ERp44 SUMOylation enhances ERp44 degradation and impairs ER retention by Ero1α, thereby ameliorating ER stress and maintaining adipose tissue homeostasis. Therefore, modulating ERp44 SUMOylation in adipocyte may be a viable strategy to combat obesity and insulin resistance (Xie et al., 2023). A synopsis of the preceding discussion is provided in Figure 4.

3.2 Improvement of the inflammatory response

Inflammation is closely related to the development and progression of DN (Deepa and Venkatraman, 2013). A significant body of research indicates that the activation of

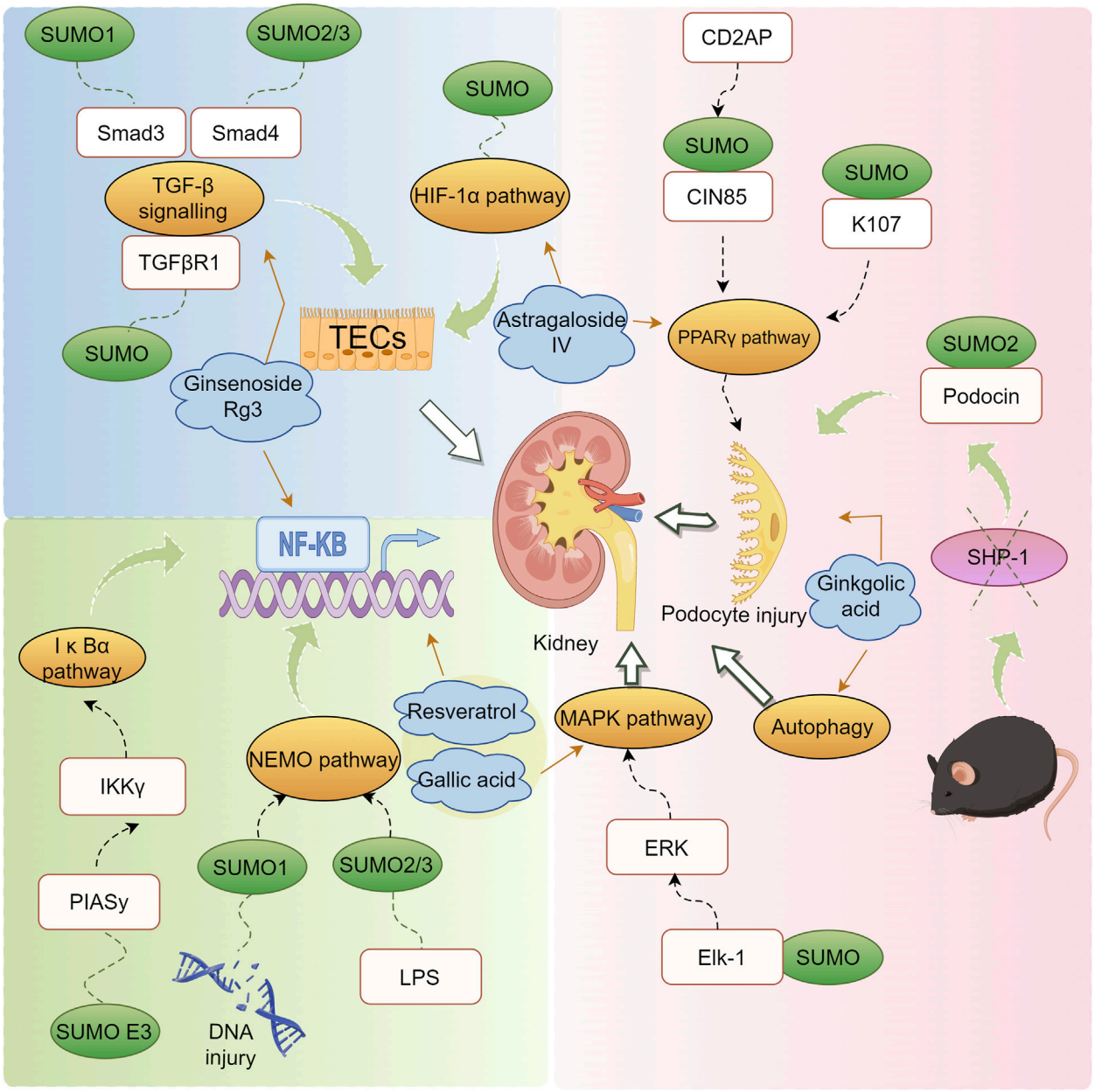


FIGURE 3
Mechanism of action of natural products in the treatment of DN through modulation of the SUMO pathway.

inflammatory signaling and the infiltration of inflammatory cells are crucial for the development of DN (Galkina and Ley, 2006). Increasing evidence suggests that SENPs play roles in modulating macrophage function and inflammation. For instance, Lao et al. (2018) reported that SENP3 enhances lipopolysaccharide (LPS) induced TLR4 signal transduction via the deSUMOylation of MKK7, and myeloid-specific deletion of SENP3 can alleviate LPS-induced endotoxic shock and acute lung injury (Chen et al., 2020). During high-fat diet (HFD)-induced obesity in mice, SENP3 expression in adipose tissue macrophages (ATMs) is significantly increased, while myeloid-specific SENP3 deficiency protects mice from HFD-induced obesity and systemic inflammation. This protective effect is partly mediated by alterations in YAP1 sumoylation. The YAP1 signaling pathway plays a role in regulating macrophage function and inflammation (Zhou et al., 2019; Liu M. et al., 2020). In the context of HFD-induced obesity in mice, SENP3 regulates the deSUMOylation of YAP1, while the absence of SENP3 can abolish the upregulation of YAP1 induced by IL-1β. Myeloid-specific deletion of SENP3 attenuates macrophage infiltration in adipose tissue and reduces serum inflammatory factor levels during diet and age-related obesity (Jiang et al., 2023).

3.2.1 NF-κB signaling

As a pivotal coordinator of inflammatory responses, NF-κB controls the expression of various inflammatory cytokines

TABLE 2 The SUMOylation of transcription factors and key mediators in DN.

In vivo/In vitro	Model	SUMO proteins or enzymes	Target genes	References
In vivo	Inducible beta cell-specific Ubc9- mice	UBC9	NRF2	He et al. (2018)
In vivo/In vitro	Human islets and C57BL/6 mice	SENP1	NADPH and GSH	Ferdaoussi et al. (2015)
In vitro	islets and β-cells, INS-1 832/13 cells	SENP1	synaptotagmin VII	Dai et al. (2011)
In vitro	MIN6 cell	SUMO-1	GLP-1R	Rajan et al. (2012)
In vivo/In vitro	Senp2knockout mice, C3H10T1/2 cell line	SENP2	CREB	Liang et al. (2019)
In vivo/In vitro	C57BL/6 mice, ob/ob mice, db/db mice, INS1 cells and the human islets	SENP2	Cyclin D1 and Mafa	Jung et al. (2016)
In vivo/In vitro	ancreatic β cell-specific Senp2 knockout mice, NIT-1 cells	SENP2	DRP1	Nan et al. (2022)
In vivo	inducible adipocyte-specific Ubc9 knockout mice	Ubc9	ERp44	Xie et al. (2023)
In vivo/In vitro	C57BL/6 Senp3$^{+/-}$ mice and Senp3flox mice, RAW264.7, HEK293T, and TLR4 HEK293 cells	SENP3	MKK7 kinase	Lao et al. (2018)
In vivo/In vitro	C57BL/6 SENP3$^{fl/fl}$ mice and Lyz2-Cre SENP3 cKO mice, RAW264.7 cells	SENP3	JNK	Chen et al. (2020)
In vivo	C57BL/6 mice, Senp3$^{flox/flox}$ control mice, and Senp3$^{flox/flox}$; Lyz2-Cre mice	SENP3	YAP1	Jiang et al. (2023)
In vivo/In vitro	C57BL/6 mice, HEK293T, MEF and RAW264.7 cells	SENP6	NEMO	Liu et al. (2013a)
In vitro	HepG2 cells, SMMC7721 cells	SUMO1	p65	Liu et al. (2016)
In vivo/In vitro	IB3-1 cell lines, A549 and 16HBE cell lines, CF mice	SUMO1	TG2	Luciani et al. (2009)
In vitro	Rat glomerular mesangial cells	E3 Ligase PIASy	NF-κB	Huang et al. (2017)
In vivo	Rat GMCs (HBZY-1)	SUMO1 and SUMO2/3	IκBα	Huang et al. (2013)
In vitro	HEK293, COS7 and 3T3 cells	SUMO4	IκBα	Guo et al. (2004)
In vivo/In vitro	UUO.Smad3-null mice, renal tubular epithelial cells, bone marrow monocytes	Smad3	TGF-β	Sato et al. (2003)
In vivo	Smad3$^{ex8/ex8}$ mice and streptozotocin-inducedCL57/Bl6J mice	Smad3	TGF-β	Fujimoto et al. (2003)
In vivo	Smad3-KO mouse	Smad3	TGF-β	Wang et al. (2007)
In vivo/In vitro	Smad4 KO mice, mouse model of unilateral ureteral obstruction, Smad4 KO macrophages and fibroblasts	Smad4	Smad3 and Smad7	Meng et al. (2012)
In vitro	Rat glomerular mesangial cells (HBZY-1)	SUMO2/3	Smad4	Zhou et al. (2014)
In vitro	Hep3B cell line, HaCaT cell line and 293T cell line	Smad3	Smad3	Imoto et al. (2008)
In vitro	NIH 3T3 cells	Smad4	TGF-β	Long et al. (2004)
In vivo	SENP2 deficiency mice	SENP2	ERK5 and p53	Heo et al. (2013)
In vivo/In vitro	C57/Bl6 mice, mouse podocytes and human embryonic kidney 293 cells	SUMO-1 and SUMO-2/3	nephrin	Tossidou et al. (2014)
In vitro	conditionally immortalized mouse CD2AP$^{+/+}$and CD2AP$^{-/-}$podocytes	SUMO-1 and SUMO-2/3	CIN85	Tossidou et al. (2012)
In vivo/In vitro	C57BL/6 mice and the MPC5 cell line	SENP6	Notch1, EDN1	Guo et al. (2023)
In vivo	SHP-1-deficient mice	SUMO2	SHP-1	Lizotte et al. (2023)
In vitro	Immortalized mouse podocytes	SENP1	p53	Wang et al. (2014b)
In vitro	GEnC cell line	SENP1	HIF-1α	Wang et al. (2015a)
In vitro	SRA01/04 cells	E3	HIF-1α	Han et al. (2015)

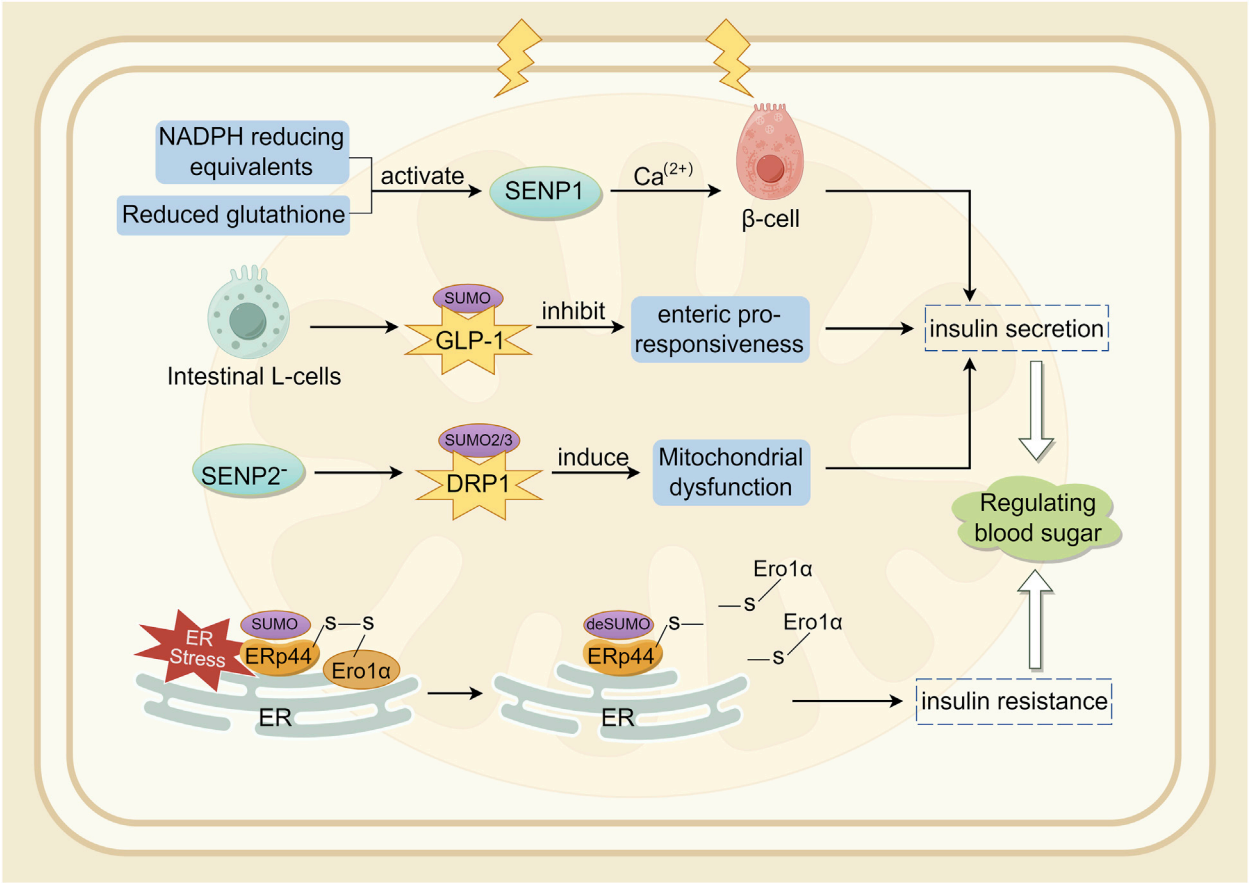


FIGURE 4
Involvement of SUMOylation in insulin secretion.

associated with the pathogenesis of DN (Perez-Morales et al., 2019; Wu et al., 2016). Some components of the NF-κB pathway can be modified by the SUMOylation process (Mabb and Miyamoto, 2007). NEMO can be considered a potential target for downregulating NF-κB activity, as NF-κB activation requires SUMO modification of NEMO before its accumulation in the nucleus (Hamdoun and Efferth, 2017). SUMO1 is conjugated by Ubc9 to K21 and K22 lysines of IκBα, preventing its ubiquitination and degradation, and further inhibiting the activation of NF-κB. Specifically, the NEMO kinase, a part of the IKK complex essential for NF-κB activation, is modified by SUMO1 in response to DNA damage, while exposure to lipopolysaccharide induces the modification of NEMO by SUMO2/3. This modification prevents the deubiquitinase CYLD from binding to NEMO, thereby enhancing the activation of the NF-κB essential modifier (IKK) (Liu X. et al., 2013). P100 can also activate the NF-κB pathway upon SUMOylation, with NEMO SUMOylation involving SUMO-1, mainly regulating the activation of NF-κB in genotoxic responses (Liu et al., 2016). PIAS proteins enhance the SUMOylation of tissue TG2, keeping TG2 stable and avoiding ubiquitin-mediated degradation, thereby sustaining oxidative stress and chronic inflammation in cells (Luciani et al., 2009). It has been elucidated that elevated glucose upregulates PIASy expression and that the SUMO E3 ligase PIASy mediates high-glucose-induced NF-κB inflammatory signalling, suggesting that PIASy is a potential therapeutic target for DN (Huang et al., 2017). Studies have found that high glucose stimulation enhances the expression of SUMO1 and SUMO2/3 in a dose- and time-dependent mannerand significantly reduces SUMOylation of IκBα, thereby activating NF-κB signalling (Huang et al., 2013). SUMO 4 binds to IκBα and negatively regulates NF-κB transcriptional activity (Guo et al., 2004).

3.2.2 TGF-β signaling

TGF-β is a multifunctional cytokine recognized, with Smad4 identified as a central channel in the TGF-β signaling mechanism (Wang L. et al., 2021). In the unilateral ureteral obstruction (UUO) mouse model, the absence of Smad3 significantly reduces renal fibrosis (Sato et al., 2003). Regarding DN, in streptozotocin (STZ)-induced DN models, mice lacking Smad3 were found to avoid renal fibrosis, including glomerular basement membrane (GBM) thickening and excessive extracellular matrix (ECM) production (Fujimoto et al., 2003), although the suppression of albuminuria was consistently observed (Wang et al., 2007). Studies have shown that Smad4 knockdown enhances pro-inflammatory NF-κB signalling while inactivating fibrotic Smad3 signalling (Meng et al., 2012). High glucose significantly increases the expression of SUMO1 and SUMO2/3 and promotes the conjugation of SUMO2/3 to Smad4

(Zhou et al., 2014). In addition to Smad4, Smad3 also plays an important role in coordinating TGF-β-mediated signalling processes. Studies have verified that PIASy, acting as a SUMOylation E3 ligase, intervenes in TGF-β signaling by binding to and SUMOylating Smad3, thus serving as a regulatory checkpoint (Derynck et al., 1998; Imoto et al., 2008). PIASy regulates TGF-β/Smad3-mediated signaling by stimulating the SUMOylation and nuclear export of Smad3. SUMOylation also inhibits the transcriptional activity of Smad4 (Gao et al., 2014; Long et al., 2004).

3.2.3 MAPK signaling

The Mitogen-Activated Protein Kinase (MAPK) pathway is a crucial conduit for transmitting external signals to internal cellular responses and is recognized as a prototypical pro-inflammatory signaling pathway. The over-activation of the MAPK pathway has been closely linked to numerous inflammatory diseases. Indeed, several MAPK inhibitors have undergone preclinical evaluation in a wide range of disease models, showing significant therapeutic potential (Yong et al., 2009). This pathway primarily participates in various cell functions by regulating the phosphorylation of its substrates, a process closely associated with SUMOylation (Guo et al., 2007). In conditions of chronic hyperglycemia, the MAPK pathway is activated, leading to localized inflammatory responses (Sakai et al., 2005). Recent research has revealed that the inhibition of the transcription factor Elk-1 due to SUMOylation can be counteracted by activating the ERK pathway (Besnard et al., 2011). Moreover, the SUMOylation of key proteins such as p53 and ERK5 has profound effects, influencing the apoptosis of endothelial cells under disturbed blood flow and regulating inflammatory processes (Heo et al., 2013).

3.3 The SUMOylation of podocyte apoptosis pathway

Clinical and experimental studies have shown that podocytopenia leads to proteinuria and/or glomerulosclerosis. And apoptosis is the main reason for the decrease in podocyte numbers (Asanuma, 2015; Nagata, 2016). Podocyte injury is an early critical event in the progression of DN, in which autophagy is an important factor (Xu et al., 2020; Deretic, 2021). This degradation process involves pathways such as mTOR, AMPK, and PI3K, playing a crucial role in inhibiting inflammation and kidney damage, thereby slowing the progression of DN (Zhang Z. et al., 2022).

Podocyte injury, including ultra-structural changes and reduced expression of slit diaphragm components like nephrin, podocin, and CD2AP, underlies many glomerular diseases. Nephrin is a SUMO-1 and SUMO-2/3 SUMOylated target protein and the presence of SUMOylation increases nephrin stability (Tossidou et al., 2014). CD2AP affects the post-translational structure of CIN85 in podocyte, enhancing its modification by SUMO-1, -2, and -3. Converting lysine 598 to arginine eliminates this modification and enhances the interaction between CIN85 and nephrin, indicating a newly discovered regulatory function of CD2AP (Tossidou et al., 2012). SENP6 deficiency enhances podocyte loss induced by high glucose (HG) through activation of the Notch1 signaling. Simultaneously, the lack of SENP6 promotes the production of EDN1 by regulating the transcription of EDN1 in podocyte, thereby exacerbating HG-induced glomerular endothelial cell injury and dysfunction. This suggests that the protective role of SENP6 may be related to its deSUMOylation function (Guo et al., 2023).

SHP-1 is a cell membrane protein tyrosine phosphatase expressed primarily in haematopoietic and epithelial cells. In the kidney, increased expression of SHP-1 in mouse and human podocytes exposed to HG leads to decreased insulin and nephrin action and podocyte dysfunction (Drapeau et al., 2013; Lizotte et al., 2016). Research has demonstrated that SHP-1 can decrease the PTM of podocin by SUMO2. In renal tissues of diabetic mice and patients, a reduction in SUMO2 and an increase in SUMO1 are observed. Upon deletion of SHP-1 in podocyte, the SUMOylation of structural proteins is restored, thereby maintaining their integrity and mitigating the progression of DN. Thus, targeting SHP-1 could represent a strategic approach to prevent podocyte injury caused by diabetes (Lizotte et al., 2023).

In kidney disease, p53 has been identified as a key factor in regulating podocyte apoptosis (Wada et al., 2005). Data indicate that SENP1 expression was low in normal podocyte and significantly increases in podocyte undergoing apoptosis and oxidative stress induced by puromycin aminonucleoside (PAN). The absence of SENP1 results in the accumulation of SUMOylated p53, which directly mediates increased apoptosis in podocytes treated with PAN (Wang et al., 2014b).

Renal tubular hypoxia is a major driving factor in DN proximal tubular pathology (Miyata and de Strihou, 2010). Hypoxia-inducible factor (HIF)-1 plays a crucial role under hypoxic conditions. There is increasing evidence that HIF-1α expression is elevated in renal proximal tubule cells under hyperglycaemic conditions (Bessho et al., 2019). Elevated levels of ROS may lead to renal cell damage, resulting in renal dysfunction (Liang et al., 2021). It was shown that HIF-1α attenuates high glucose-mediated associated renal tubular cell injury by promoting Parkin/PINK1-mediated mitosis (Yu et al., 2021). Hypoxia might promote the stability and activation of HIF-1α by increasing the expression of SENP1 in podocyte, thereby inducing survival and angiogenesis in Glomerular Endothelial Cells to combat hypoxia. The deSUMOylation of HIF-1α signaling protects glomerular endothelial cells from hypoxic injury (Wang L. et al., 2015). Over-expression of SUMO or SUMO E3 ligase can enhance the stability and transcriptional activity of HIF-1α in Human Lymphatic Endothelial Cells (Han et al., 2015).

Although the current study reveals beneficial effects of SENP1, it is important to consider the insights from other studies. For instance, Meinecke *et al.* demonstrated that elevated levels of SUMO acetylation in promyelocytic leukaemia proteins result in resistance to Fas-induced apoptosis (Meinecke et al., 2007). In a study conducted by Li et al. (2008) it was demonstrated that the deSUMOylation of HIPK1 by SENP1 in TNF-treated human umbilical vein endothelial cells resulted in the enhancement of ASK1-dependent apoptosis. These contradictory results are intriguing and, aside from the different types of stimuli and signaling pathways studied, might also relate to the different cell types investigated. Therefore, the relationship between SUMOylation and apoptosis warrants further exploration.

4 Natural products that combat DN by regulating SUMOylation

In the clinical treatment of DN, pharmacological interventions are being explored alongside natural products. Traditional drug therapies focus primarily on blood glucose control, blood pressure management, and inhibition of the renin-angiotensin-aldosterone system (RAAS). Antidiabetic medications like metformin and SGLT2 inhibitors have shown some degree of nephroprotective effects. Additionally, ACE inhibitors (ACEIs) and angiotensin II receptor blockers (ARBs) are effective in reducing proteinuria and slowing the progression of renal dysfunction (Hu et al., 2023a). However, these synthetic drugs are often accompanied by side effects, including ketoacidosis, weight gain, polyuria, liver damage, renal impairment, and cardiovascular complications (Roborel et al., 2020; Blahova et al., 2021; Kushwaha et al., 2020a). This has prompted further exploration of phytochemicals as alternative therapeutic approaches (Kushwaha et al., 2020b). Phytochemicals are considered potential natural modulators due to their ability to interact with multiple targets, slow disease progression, and their relatively low toxicity (Khatoon et al., 2022). Among these, natural compounds like polyphenols, flavonoids, and saponins possess antioxidant, anti-inflammatory, and antifibrotic properties, offering protective effects on the kidneys (Hu et al., 2023b). Research suggests that certain natural compounds and herbal extracts may exert therapeutic effects on DN through mechanisms such as SUMOylation, highlighting their potential as a treatment option for DN.

Ginkgo biloba is a traditional herbal medicine with thousands of years of clinical use history and stands as one of the most widely used botanical treatment products globally (Howes and Houghton, 2012). It is primarily utilized for treating conditions such as cough, asthma, and enuresis (Nash and Shah, 2015). Previously, researchers found that ginkgo biloba alleviated the worsening of proteinuria in patients with type 2 diabetes, suggesting that ginkgo biloba is a promising option for early protection of the kidneys in patients with DN (Howes and Houghton, 2012). Modern pharmacological research indicates that Ginkgo biloba possesses anti-oxidative, anti-inflammatory (Mohanta et al., 2014), neuroprotective (Zuo et al., 2017), and anti-platelet aggregation effects (Koch, 2005). Animal studies have confirmed Ginkgo biloba's preventive and therapeutic effects on DN (Avula et al., 2015; Qiu et al., 2015; Tang et al., 2009). Ginkgolic acid (GA), an alkylphenol constituent found in Ginkgo biloba leaves and fruits (Isah, 2015), significantly reduces and reverses the modulation induced by oxidized low-density lipoprotein, indicating its anti-inflammatory action through the NF-κB pathway (Zhang and Yan, 2019). GA is a SUMO chemical inhibitor that binds directly to the E1 enzyme and inhibits the formation of the E1-SUMO intermediate, thereby effectively inhibiting the SUMO chemical pathway (Fukuda et al., 2009). Research has demonstrated that this compound can downregulate the expression of SUMO1 and SUMO2/3 in macrophages, an effect consistently replicated in both experimental and clinical models (Liu X. et al., 2022). The application of GA to inhibit SUMOylation or suppress SUMO1 expression can regulate the levels of SUMO1 and its conjugation with p53, promoting autophagy while inhibiting cell proliferation. These results reveal the ability of GA to control the multifaceted regulation of complex cellular processes (Tan et al., 2022). Beyond its cell regulatory functions, SUMOylation is also essential for the exchange on slit diaphragms, a critical component of the renal unit in kidney architecture. Treatment with the SUMOylation inhibitor GA reduces the expression of nephrin on podocyte membranes, highlighting the importance of SUMOylation in renal homeostasis and its potential as a therapeutic intervention target (Tossidou et al., 2014).

Research indicates that polyphenols hold promise for treating various chronic diseases, including diabetes and its complications, with minimal toxic effects observed *in vitro* and in animal models (Kabir et al., 2021). Resveratrol (RES) is a naturally occurring non-flavonoid polyphenolic compound derived mainly from natural plants such as grapes, cranberries, lingonberries and certain herbs (Nanjan and Betz, 2014). The pharmacological actions of RSV have been studied, including its anti-oxidative, anti-inflammatory, immunomodulatory, hepatoprotective, anticancer, and anti-atherosclerotic effects (Pandey and Rizvi, 2009; Prasad, 2012; Bo et al., 2013; Carrizzo et al., 2013; Carter et al., 2014; Wang Y. et al., 2015; Peiyuan et al., 2017). Studies have shown that RSV treatment has a protective effect on DN, with RSV treatment in animals with DN alleviating hyperglycemia, hyperlipidemia, and improving kidney structure integrity and renal function (Den Hartogh and Tsiani, 2019). Resveratrol effectively inhibits carcinogen-induced expression of cyclooxygenase-2 (COX-2). This process requires the phosphorylation of extracellular signal-regulated kinases 1 and 2 (ERK1/2) and the translocation of the enzyme to the nucleus by SUMO-1. COX-2 then accumulates in the nucleus, forming a complex with SUMO-1, which binds to phosphorylated p53, potentially influencing the carcinogenic process (Cheng et al., 2018). Furthermore, resveratrol can mediate the downregulation of SUMO1, inhibit the expression of Wnt5a, deactivate β-catenin, and prevent their entry into the cell nucleus. This molecular cascade attenuates the Wnt/β-catenin signaling pathway, thereby producing an anti-inflammatory effect (Wang et al., 2020). Experimental evidence suggests that resveratrol may be an effective agent for the prevention and inflammatory response of renal mesangial cells induced by LPS and the expression of fibronectin through the SphK1/S1P2/NF-κB signaling pathway, highlighting resveratrol's potential role in combating renal fibrosis in addition to its anti-hyperglycemic properties (Gong et al., 2020).

Astragalus membranaceus, known as Huangqi, is the dried root of Astragalus and a commonly used herb in traditional Chinese medicine. Increasing research demonstrates its beneficial effects on various diseases through activating immune mechanisms, alleviating oxidative stress, and reducing inflammation (Cho and Leung, 2007; Ko et al., 2005; Qin et al., 2012). Astragaloside IV (AS-IV), a saponin extracted from Astragalus membranaceus Bunge, is one of the main pharmacologically active components of Astragalus (Fu et al., 2014). Recent studies report that AS-IV possesses extensive pharmacological activities both *in vivo* and *in vitro*, including anti-oxidative stress, anti-inflammatory, anti-diabetic, and renal protective effects (Zhou et al., 2017; Chen et al., 2018). Further research utilizing advanced Western blotting techniques has found that the sharp decline and delayed recovery of SUMO1 activity are responsible for the initial increase and subsequent decrease in HIF-1α concentration. Enhanced expression of SUMO1 stabilizes HIF-1α within the nucleus, effectively reducing the extent of vascular

anomalies following hypoxia. Astragaloside IV (AS-IV) triggers sustained production of SUMO1 in vascular endothelial cells, consolidating the HIF-1α/VEGF axis and thus promoting angiogenesis under hypoxic conditions (Wang B. et al., 2021). Additionally, AS-IV seems to alleviate podocyte apoptosis by activating the PPARγ-Klotho-FoxO1 signaling pathway, offering therapeutic potential for DN (Xing et al., 2021). Peroxisome proliferator-activated receptor γ (PPARγ), a target of thiazolidinediones used to treat patients with type 2 diabetes mellitus (T2DM), plays a crucial role in enhancing insulin sensitivity, promoting adipogenesis and exerting anti-inflammatory effects (Sauer, 2015; Stechschulte et al., 2016; Rao et al., 2015). In cell-based studies, the SUMOylation of the N-terminal K107 on PPARγ has been shown to inhibit its transcriptional activity, suggesting that targeting this modification could be a strategic approach in drug therapy. This strategy aims to separate the beneficial insulin-sensitizing properties of PPARγ from its adverse effects on body weight (Katafuchi et al., 2018). Extensive research indicates that PTM of PPARγ regulate its transcriptional activity, with SUMOylation being a crucial negative regulatory mechanism (Ohshima et al., 2004). Studies have demonstrated that excessive SUMOylation of PPARγ, similar to the effects of HFD, induces endothelial insulin resistance (IR) and dysfunction by negatively regulating the eNOS-NO pathway. More importantly, it was discovered that excessive SUMOylation of PPARγ triggers an endogenous SUMOylation cascade, exacerbating endothelial IR and dysfunction (Yuan et al., 2019).

Gallic acid (GA) is a bio-active compound found, known for its antioxidant, anti-allergic, anti-cancer, anti-inflammatory, and anti-diabetic properties (Chen et al., 2009; D'Souza et al., 2014; Huang et al., 2012; Hidalgo et al., 2012; Ho et al., 2013; Habtemariam, 1997; Nabavi et al., 2013; Punithavathi et al., 2011; Liu K. Y. et al., 2013). In human diets, the primary sources of this compound are non-glycosylated esters of gallic acid (Wianowska and Olszowy-Tomczyk, 2023). Gallic acid is a potent phenolic substance involved in crucial deSUMOylation processes through SENP1, particularly targeting GATA2, NEMO, Pin1, SMAD4, and HIF-1α, thus having significant impacts on various diseases (Taghvaei et al., 2022). Studies have shown that gallic acid effectively inhibits the renal activation of p38 MAPK and nuclear NF-κB, ultimately alleviating renal dysfunction in diabetic rats induced by a combination of HFD and STZ through p38 MAPK-mediated pathways (Ahad et al., 2015). Toxicological investigations indicate that gallic acid exhibits minimal toxicity or adverse reactions across a range of animal experiments and clinical studies (Bai et al., 2021).

Ginsenosides, the primary active components extracted from the roots, stems, leaves, or fruits of ginseng, are extensively cultivated in Korea and Northeast China (Sui et al., 2023). Ginseng has been reported to have anti-diabetic properties due to its ability to induce insulin secretion, stimulate glucose uptake, inhibit intestinal glucose absorption, and reduce glycogen breakdown (Chen et al., 2012; Yuan et al., 2012). Ginsenoside Rg3 has been shown to effectively protect against hyperglycemia, obesity, and diabetes by preventing the death of pancreatic β-cell (Kim et al., 2016; Kim et al., 2009). In a supplementary study, an eight-week treatment with 20(R)-Rg3 not only lowered fasting blood glucose levels and harmful levels of advanced glycation end-products but also beneficially regulated insulin modulation, lipid profiles, oxidative markers, and overall kidney health. This was achieved by affecting the MAPK and NF-κB signaling cascades in DN mice, highlighting 20(R)-Rg3 as a promising therapeutic agent (Li et al., 2021). Additionally, the well-known ginsenoside Rg3 has been identified as up-regulating the phosphorylated RanBP2 (an E3 SUMO-protein ligase), thereby exerting inhibitory effects on the NF-κB signaling pathway and offering new therapeutic avenues for cancer treatment (Zou et al., 2018). This inhibitory effect may be related to the regulation of nucleocytoplasmic transport. The phosphorylation of RanBP2 might affect its interaction with Nup153, leading to the aggregation of these nucleoporins. This aggregation could prevent IκB from entering the nucleus, thereby prolonging its binding to the p65 subunit and inhibiting NF-κB activity (Liu Y. et al., 2020).

In addition to natural products, TAK-981 and ML-792 are small-molecule SUMO inhibitors with significant selectivity. Research has shown that the SUMOylation inhibitors TAK-981 and ML-792 stimulate cytotoxic NK cells, M1 macrophages, and CTLs, while also preventing the exhaustion of $CD8^+$ T cells, thereby enhancing antitumor activity (Wang et al., 2024). The SUMO E1 inhibitor TAK-981 is currently undergoing clinical trials for cancer treatment (Langston et al., 2021). The discovery of TAK-981 opens up possibilities for novel immunotherapies, offering new opportunities for the treatment of diseases such as cancer and sepsis (Zhang H. et al., 2022; Youssef et al., 2023).

Recent research indicates that the activation of the Notch, Wnt, and Hedgehog (Hh) signaling pathways plays a crucial role in the regeneration of damaged organs. However, excessive stimulation of these pathways can lead to fibrosis development. Therefore, reducing the activity of Notch, Wnt, or Hh signaling may represent a new therapeutic strategy for DN (Edeling et al., 2016). The Wnt pathway, particularly the canonical Wnt pathway, is considered a key regulator in the progression of DN. In DN, the TGF-β and Wnt signaling pathways mutually activate and regulate each other, promoting tissue fibrosis and damage repair, which exacerbates disease progression (Wang H. et al., 2021). At the same time, glucocorticoid receptors (GR) in endothelial cells play a key role in regulating renal fibrosis by inhibiting the canonical Wnt signaling pathway (Srivastava et al., 2021). Research on DN has shown that the Notch signaling pathway significantly affects podocytes and tubular epithelial cells (Yang and Liu, 2022; Ma et al., 2022). Additionally, fibroblast growth factor receptor 1 (FGFR1) signaling is involved in regulating kidney damage. Studies suggest that FGFR1 promotes the fibrotic response of renal tubular epithelial cells in the context of hypertension and elevated angiotensin II, indicating that FGFR1 is a potential target for preserving kidney function and integrity (Xu et al., 2022). Sirtuin 3 (SIRT3), a major mitochondrial deacetylase, plays a crucial role in the production and detoxification of reactive ROS. Experimental results show that regulating the SIRT3 pathway can improve diabetes-induced kidney damage. Specific activation of SIRT3 reduces oxidative stress induced by diabetes, protecting podocytes and glomeruli from damage (Locatelli et al., 2020). These signaling pathways and their interactions offer potential targets for future therapeutic strategies in treating DN.

5 Conclusion

To conclude, the present investigation provides an integrated review of SUMOylation's involvement in DN, alongside the medicinal actions of natural products that influence transcription factors and pivotal signaling molecules. Given the intricate nature of DN onset, which is characterized by an interplay of diverse factors and a spectrum of molecular dynamics, and is indicative of both systemic and renal-specific pathological conditions, efficacious therapeutic strategies are markedly lacking. The role of SUMOylation in DN is complex, as its effects depend on specific target proteins and pathological conditions. The application of SUMOylation regulation in DN requires further research and validation. Current studies indicate that SUMOylation levels are often elevated in DN, particularly in response to high glucose and oxidative stress. This process offers some benefits by enhancing the activity of antioxidant proteins, which temporarily protect the kidneys from further oxidative damage. However, excessive SUMOylation can also drive the activation of profibrotic and pro-inflammatory signaling pathways, such as TGF-β and NF-κB, exacerbating kidney fibrosis and chronic inflammation. Therefore, future research may need to focus on balancing SUMOylation regulation through precise therapeutic targeting. This approach would aim to prevent the excessive accumulation of SUMO modifications while preserving the necessary cellular stress responses.

Natural products are widespread and diverse in nature and may provide additional directions and options for the treatment of DN. The burgeoning research into natural products capable of modulating SUMOylation is poised to elucidate the role of SUMOylation in the progression of diabetic kidney ailments, spotlighting compounds like ginkgolic acid, ginkgolide B, astragaloside, resveratrol, gallic acid, and ginsenoside Rg3. It is important to note that the difference in specificity between natural products and modern synthetic inhibitors is significant, directly influencing their efficacy and safety in research and therapeutic applications. While the natural products mentioned in this paper can affect SUMOylation, they often exert their effects through multiple pathways that are not necessarily related to SUMO modification. In contrast, compounds like TAK981 are designed with high specificity, targeting the SUMOylation pathway by inhibiting the SUMO E1 enzyme, thereby blocking the SUMOylation process. This high specificity allows TAK981 to more precisely regulate SUMOylation. However, natural products, due to their broad availability and multi-target characteristics, can simultaneously influence multiple biological pathways, which is particularly advantageous for complex diseases. Moreover, they tend to have lower side effects and reduced risk of resistance, making them a crucial component in disease treatment. While investigations into natural products modulating SUMOylation are nascent, the pursuit of viable SUMOylation-based treatments is a considerable hurdle, necessitating extensive and detailed foundational studies.

Future research could focus on developing natural product-based drugs targeting SUMOylation pathways, with the aim of regulating specific SUMOylation processes to slow or prevent the progression of DN. Additionally, SUMOylation modulators could be combined with other DN treatments, such as SGLT2 inhibitors and RAAS inhibitors, to create multi-target therapeutic strategies. This approach could act on multiple pathological mechanisms simultaneously, improving treatment efficacy while reducing the side effects associated with monotherapies. In summary, regulating SUMOylation may open new avenues for DN treatment, especially by targeting various pathological processes to achieve more personalized and precise therapeutic interventions.

Author contributions

JW: Writing–original draft. RZ: Writing–original draft. CW: Writing–original draft. LW: Conceptualization, Writing–review and editing. PeL: Conceptualization, Supervision, Writing–review and editing. PiL: Conceptualization, 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. This study was supported by the National Natural Science Foundation of China (No. 82274489, 82174296, U23A20504), the Fifth Batch of National Training Program for Excellent Clinical Talents of Traditional Chinese Medicine [No.1 (2022) of People's Education of Traditional Chinese Medicine], Beijing Natural Science Foundation (No.7232326).

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.

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

Ahad, A., Ahsan, H., Mujeeb, M., and Siddiqui, W. A. (2015). Gallic acid ameliorates renal functions by inhibiting the activation of p38 MAPK in experimentally induced type 2 diabetic rats and cultured rat proximal tubular epithelial cells. *Chem. Biol. Interact.* 240, 292–303. doi:10.1016/j.cbi.2015.08.026

Arora, M. K., and Singh, U. K. (2013). Molecular mechanisms in the pathogenesis of diabetic nephropathy: an update. *Vasc. Pharmacol.* 58 (4), 259–271. doi:10.1016/j.vph.2013.01.001

Arvaniti, E., Vakrakou, A., Kaltezioti, V., Stergiopoulos, A., Prakoura, N., Politis, P. K., et al. (2016). Nuclear receptor NR5A2 is involved in the calreticulin gene regulation during renal fibrosis. *Biochim. Biophys. Acta* 1862 (9), 1774–1785. doi:10.1016/j.bbadis.2016.06.013

Asanuma, K. (2015). The role of podocyte injury in chronic kidney disease. *Gakkai Kaishi* 38 (1), 26–36. doi:10.2177/jsci.38.26

Attie, A. D. (2015). How do reducing equivalents increase insulin secretion? *J. Clin. Invest.* 125 (10), 3754–3756. doi:10.1172/JCI84011

Avula, B., Sagi, S., Gafner, S., Upton, R., Wang, Y. H., Wang, M., et al. (2015). Identification of Ginkgo biloba supplements adulteration using high performance thin layer chromatography and ultra high performance liquid chromatography-diode array detector-quadrupole time of flight-mass spectrometry. *Anal. Bioanal. Chem.* 407 (25), 7733–7746. doi:10.1007/s00216-015-8938-1

Bai, J., Zhang, Y., Tang, C., Hou, Y., Ai, X., Chen, X., et al. (2021). Gallic acid: pharmacological activities and molecular mechanisms involved in inflammation-related diseases. *Biomed. Pharmacother.* 133, 110985. doi:10.1016/j.biopha.2020.110985

Besnard, A., Galan-Rodriguez, B., Vanhoutte, P., and Caboche, J. (2011). Elk-1 a transcription factor with multiple facets in the brain. *Front. Neurosci.* 5, 35. doi:10.3389/fnins.2011.00035

Bessho, R., Takiyama, Y., Takiyama, T., Kitsunai, H., Takeda, Y., Sakagami, H., et al. (2019). Hypoxia-inducible factor-1α is the therapeutic target of the SGLT2 inhibitor for diabetic nephropathy. *Sci. Rep.* 9 (1), 14754. doi:10.1038/s41598-019-51343-1

Blahova, J., Martiniakova, M., Babikova, M., Kovacova, V., Mondockova, V., and Omelka, R. (2021). Pharmaceutical drugs and natural therapeutic products for the treatment of type 2 diabetes mellitus. *Pharm. (Basel)* 14 (8), 806. doi:10.3390/ph14080806

Bo, S., Ciccone, G., Castiglione, A., Gambino, R., De Michieli, F., Villois, P., et al. (2013). Anti-inflammatory and antioxidant effects of resveratrol in healthy smokers a randomized, double-blind, placebo-controlled, cross-over trial. *Curr. Med. Chem.* 20 (10), 1323–1331. doi:10.2174/0929867311320100009

Brackett, C. M., García-Casas, A., Castillo-Lluva, S., and Blagg, B. S. J. (2020). Synthesis and evaluation of ginkgolic acid derivatives as SUMOylation inhibitors. *ACS Med. Chem. Lett.* 11 (11), 2221–2226. doi:10.1021/acsmedchemlett.0c00353

Carrizzo, A., Forte, M., Damato, A., Trimarco, V., Salzano, F., Bartolo, M., et al. (2013). Antioxidant effects of resveratrol in cardiovascular, cerebral and metabolic diseases. *Food Chem. Toxicol.* 61, 215–226. doi:10.1016/j.fct.2013.07.021

Carter, L. G., D'Orazio, J. A., and Pearson, K. J. (2014). Resveratrol and cancer: focus on *in vivo* evidence. *Endocr. Relat. Cancer* 21 (3), R209–R225. doi:10.1530/ERC-13-0171

Chang, E., and Abe, J. I. (2016). Kinase-SUMO networks in diabetes-mediated cardiovascular disease. *Metabolism* 65 (5), 623–633. doi:10.1016/j.metabol.2016.01.007

Chang, H. M., and Yeh, E. (2020). SUMO: from bench to bedside. *Physiol. Rev.* 100 (4), 1599–1619. doi:10.1152/physrev.00025.2019

Chen, F., Chen, Y., Kang, X., Zhou, Z., Zhang, Z., and Liu, D. (2012). Anti-apoptotic function and mechanism of ginseng saponins in Rattus pancreatic β-cells. *Biol. Pharm. Bull.* 35 (9), 1568–1573. doi:10.1248/bpb.b12-00461

Chen, H. M., Wu, Y. C., Chia, Y. C., Chang, F. R., Hsu, H. K., Hsieh, Y. C., et al. (2009). Gallic acid, a major component of Toona sinensis leaf extracts, contains a ROS-mediated anti-cancer activity in human prostate cancer cells. *Cancer Lett.* 286 (2), 161–171. doi:10.1016/j.canlet.2009.05.040

Chen, L. C., Hsieh, Y. L., Tan, G. Y. T., Kuo, T. Y., Chou, Y. C., Hsu, P. H., et al. (2021). Differential effects of SUMO1 and SUMO2 on circadian protein PER2 stability and function. *Sci. Rep.* 11 (1), 14431. doi:10.1038/s41598-021-93933-y

Chen, Q., Su, Y., Ju, Y., Ma, K., Li, W., and Li, W. (2018). Astragalosides IV protected the renal tubular epithelial cells from free fatty acids-induced injury by reducing oxidative stress and apoptosis. *Biomed. Pharmacother.* 108, 679–686. doi:10.1016/j.biopha.2018.09.049

Chen, X., Lao, Y., Yi, J., Yang, J., He, S., and Chen, Y. (2020). SENP3 in monocytes/macrophages up-regulates tissue factor and mediates lipopolysaccharide-induced acute lung injury by enhancing JNK phosphorylation. *J. Cell. Mol. Med.* 24 (10), 5454–5462. doi:10.1111/jcmm.15199

Cheng, T. M., Chin, Y. T., Ho, Y., Chen, Y. R., Yang, Y. N., Yang, Y. C., et al. (2018). Resveratrol induces sumoylated COX-2-dependent anti-proliferation in human prostate cancer LNCaP cells. *Food Chem. Toxicol.* 112, 67–75. doi:10.1016/j.fct.2017.12.011

Cho, W. C., and Leung, K. N. (2007). *In vitro* and *in vivo* anti-tumor effects of Astragalus membranaceus. *Cancer Lett.* 252 (1), 43–54. doi:10.1016/j.canlet.2006.12.001

Choi, S. G., Kim, H., Jeong, E. I., Lee, H. J., Park, S., Lee, S. Y., et al. (2017). SUMO-modified FADD recruits cytosolic Drp1 and caspase-10 to mitochondria for regulated necrosis. *Mol. Cell. Biol.* 37 (2), 002544–e316. doi:10.1128/MCB.00254-16

Chung, S. S., Ahn, B. Y., Kim, M., Choi, H. H., Park, H. S., Kang, S., et al. (2010). Control of adipogenesis by the SUMO-specific protease SENP2. *Mol. Cell. Biol.* 30 (9), 2135–2146. doi:10.1128/MCB.00852-09

Claessens, L. A., and Vertegaal, A. (2024). SUMO proteases: from cellular functions to disease. *Trends Cell. Biol.* doi:10.1016/j.tcb.2024.01.002

Cubenas-Potts, C., and Matunis, M. J. (2013). SUMO: a multifaceted modifier of chromatin structure and function. *Dev. Cell.* 24 (1), 1–12. doi:10.1016/j.devcel.2012.11.020

Dai, X. Q., Plummer, G., Casimir, M., Kang, Y., Hajmrle, C., Gaisano, H. Y., et al. (2011). SUMOylation regulates insulin exocytosis downstream of secretory granule docking in rodents and humans. *Diabetes* 60 (3), 838–847. doi:10.2337/db10-0440

Deepa, B., and Venkatraman, A. C. (2013). Effects of linalool on inflammation, matrix accumulation and podocyte loss in kidney of streptozotocin-induced diabetic rats. *Toxicol. Mech. Methods* 23 (4), 223–234. doi:10.3109/15376516.2012.743638

Den Hartogh, D. J., and Tsiani, E. (2019). Health benefits of resveratrol in kidney disease: evidence from *in vitro* and *in vivo* studies. *Nutrients* 11 (7), 1624. doi:10.3390/nu11071624

Deretic, V. (2021). Autophagy in inflammation, infection, and immunometabolism. *Immunity* 54 (3), 437–453. doi:10.1016/j.immuni.2021.01.018

Derynck, R., Zhang, Y., and Feng, X. H. (1998). Smads: transcriptional activators of TGF-beta responses. *Cell.* 95 (6), 737–740. doi:10.1016/s0092-8674(00)81696-7

Di Bacco, A., Ouyang, J., Lee, H. Y., Catic, A., Ploegh, H., and Gill, G. (2006). The SUMO-specific protease SENP5 is required for cell division. *Mol. Cell. Biol.* 26 (12), 4489–4498. doi:10.1128/MCB.02301-05

Dou, X., Zhou, W. Y., Ding, M., Ma, Y. J., Yang, Q. Q., Qian, S. W., et al. (2022). The protease SENP2 controls hepatic gluconeogenesis by regulating the SUMOylation of the fuel sensor AMPKα. *J. Biol. Chem.* 298 (2), 101544. doi:10.1016/j.jbc.2021.101544

Drapeau, N., Lizotte, F., Denhez, B., Guay, A., Kennedy, C. R., and Geraldes, P. (2013). Expression of SHP-1 induced by hyperglycemia prevents insulin actions in podocytes. *Am. J. Physiol. Endocrinol. Metab.* 304 (11), E1188–E1198. doi:10.1152/ajpendo.00560.2012

Drucker, D. J., Philippe, J., Mojsov, S., Chick, W. L., and Habener, J. F. (1987). Glucagon-like peptide I stimulates insulin gene expression and increases cyclic AMP levels in a rat islet cell line. *Proc. Natl. Acad. Sci. U. S. A.* 84 (10), 3434–3438. doi:10.1073/pnas.84.10.3434

D'Souza, J. J., D'souza, P. P., Fazal, F., Kumar, A., Bhat, H. P., and Baliga, M. S. (2014). Anti-diabetic effects of the Indian indigenous fruit Emblica officinalis Gaertn: active constituents and modes of action. *Food Funct.* 5 (4), 635–644. doi:10.1039/c3fo60366k

Edeling, M., Ragi, G., Huang, S., Pavenstädt, H., and Susztak, K. (2016). Developmental signalling pathways in renal fibrosis: the roles of Notch, Wnt and Hedgehog. *Nat. Rev. Nephrol.* 12 (7), 426–439. doi:10.1038/nrneph.2016.54

Eifler, K., and Vertegaal, A. C. (2015). Mapping the SUMOylated landscape. *FEBS J.* 282 (19), 3669–3680. doi:10.1111/febs.13378

Evdokimov, E., Sharma, P., Lockett, S. J., Lualdi, M., and Kuehn, M. R. (2008). Loss of SUMO1 in mice affects RanGAP1 localization and formation of PML nuclear bodies, but is not lethal as it can be compensated by SUMO2 or SUMO3. *J. Cell. Sci.* 121 (Pt 24), 4106–4113. doi:10.1242/jcs.038570

Ferdaoussi, M., Dai, X., Jensen, M. V., Wang, R., Peterson, B. S., Huang, C., et al. (2015). Isocitrate-to-SENP1 signaling amplifies insulin secretion and rescues dysfunctional β cells. *J. Clin. Invest.* 125 (10), 3847–3860. doi:10.1172/JCI82498

Fu, J., Wang, Z., Huang, L., Zheng, S., Wang, D., Chen, S., et al. (2014). Review of the botanical characteristics, phytochemistry, and pharmacology of Astragalus membranaceus (Huangqi). *Phytother. Res.* 28 (9), 1275–1283. doi:10.1002/ptr.5188

Fujimoto, M., Maezawa, Y., Yokote, K., Joh, K., Kobayashi, K., Kawamura, H., et al. (2003). Mice lacking Smad3 are protected against streptozotocin-induced diabetic glomerulopathy. *Biochem. Biophys. Res. Commun.* 305 (4), 1002–1007. doi:10.1016/s0006-291x(03)00885-4

Fukuda, I., Ito, A., Hirai, G., Nishimura, S., Kawasaki, H., Saitoh, H., et al. (2009). Ginkgolic acid inhibits protein SUMOylation by blocking formation of the E1-SUMO intermediate. *Chem. Biol.* 16 (2), 133–140. doi:10.1016/j.chembiol.2009.01.009

Galkina, E., and Ley, K. (2006). Leukocyte recruitment and vascular injury in diabetic nephropathy. *J. Am. Soc. Nephrol.* 17 (2), 368–377. doi:10.1681/ASN.2005080859

Gao, C., Huang, W., Kanasaki, K., and Xu, Y. (2014). The role of ubiquitination and sumoylation in diabetic nephropathy. *Biomed. Res. Int.* 2014, 160692. doi:10.1155/2014/160692

Gareau, J. R., and Lima, C. D. (2010). The SUMO pathway: emerging mechanisms that shape specificity, conjugation and recognition. *Nat. Rev. Mol. Cell. Biol.* 11 (12), 861–871. doi:10.1038/nrm3011

Geiss-Friedlander, R., and Melchior, F. (2007). Concepts in sumoylation: a decade on. *Nat. Rev. Mol. Cell. Biol.* 8 (12), 947–956. doi:10.1038/nrm2293

Golebiowski, F., Szulc, A., Sakowicz, M., Szutowicz, A., and Pawełczyk, T. (2003). Expression level of Ubc9 protein in rat tissues. *Acta Biochim. Pol.* 50 (4), 1065–1073.

Gong, L., and Yeh, E. T. (2006). Characterization of a family of nucleolar SUMO-specific proteases with preference for SUMO-2 or SUMO-3. *J. Biol. Chem.* 281 (23), 15869–15877. doi:10.1074/jbc.M511658200

Gong, W., Li, J., Chen, W., Feng, F., and Deng, Y. (2020). Resveratrol inhibits lipopolysaccharide-induced extracellular matrix accumulation and inflammation in rat glomerular mesangial cells by SphK1/S1P2/NF-κB pathway. *Diabetes Metab. Syndr. Obes.* 13, 4495–4505. doi:10.2147/DMSO.S278267

Gong, X., Liao, Y., Ahner, A., Larsen, M. B., Wang, X., Bertrand, C. A., et al. (2019). Different SUMO paralogues determine the fate of wild-type and mutant CFTRs: biogenesis versus degradation. *Mol. Biol. Cell.* 30 (1), 4–16. doi:10.1091/mbc.E18-04-0252

Gross, J. L., de Azevedo, M. J., Silveiro, S. P., Canani, L. H., Caramori, M. L., and Zelmanovitz, T. (2005). Diabetic nephropathy: diagnosis, prevention, and treatment. *Diabetes Care* 28 (1), 164–176. doi:10.2337/diacare.28.1.164

Guo, B., Yang, S. H., Witty, J., and Sharrocks, A. D. (2007). Signalling pathways and the regulation of SUMO modification. *Biochem. Soc. Trans.* 35 (Pt 6), 1414–1418. doi:10.1042/BST0351414

Guo, D., Li, M., Zhang, Y., Yang, P., Eckenrode, S., Hopkins, D., et al. (2004). A functional variant of SUMO4, a new I kappa B alpha modifier, is associated with type 1 diabetes. *Nat. Genet.* 36 (8), 837–841. doi:10.1038/ng1391

Guo, F., Song, Y., Wu, L., Zhao, Y., Ma, X., Wang, J., et al. (2023). SUMO specific peptidase 6 regulates the crosstalk between podocytes and glomerular endothelial cells in diabetic kidney disease. *Biochim. Biophys. Acta Mol. Basis Dis.* 1869 (5), 166685. doi:10.1016/j.bbadis.2023.166685

Habtemariam, S. (1997). Flavonoids as inhibitors or enhancers of the cytotoxicity of tumor necrosis factor-alpha in L-929 tumor cells. *J. Nat. Prod.* 60 (8), 775–778. doi:10.1021/np960581z

Hajmrle, C., Ferdaoussi, M., Plummer, G., Spigelman, A. F., Lai, K., Manning Fox, J. E., et al. (2014). SUMOylation protects against IL-1β-induced apoptosis in INS-1 832/13 cells and human islets. *Am. J. Physiol. Endocrinol. Metab.* 307 (8), E664–E673. doi:10.1152/ajpendo.00168.2014

Hamdoun, S., and Efferth, T. (2017). Ginkgolic acids inhibit migration in breast cancer cells by inhibition of NEMO sumoylation and NF-κB activity. *Oncotarget* 8 (21), 35103–35115. doi:10.18632/oncotarget.16626

Han, X., Wang, X. L., Li, Q., Dong, X. X., Zhang, J. S., and Yan, Q. C. (2015). HIF-1α SUMOylation affects the stability and transcriptional activity of HIF-1α in human lens epithelial cells. *Graefes Arch. Clin. Exp. Ophthalmol.* 253 (8), 1279–1290. doi:10.1007/s00417-015-2999-x

Han, Z. J., Feng, Y. H., Gu, B. H., Li, Y. M., and Chen, H. (2018). The post-translational modification, SUMOylation, and cancer (Review). *Int. J. Oncol.* 52 (4), 1081–1094. doi:10.3892/ijo.2018.4280

Harlan, S. M., Heinz-Taheny, K. M., Sullivan, J. M., Wei, T., Baker, H. E., Jaqua, D. L., et al. (2018). Progressive renal disease established by renin-coding adeno-associated virus-driven hypertension in diverse diabetic models. *J. Am. Soc. Nephrol.* 29 (2), 477–491. doi:10.1681/ASN.2017040385

He, X., Lai, Q., Chen, C., Li, N., Sun, F., Huang, W., et al. (2018). Both conditional ablation and overexpression of E2 SUMO-conjugating enzyme (UBC9) in mouse pancreatic beta cells result in impaired beta cell function. *Diabetologia* 61 (4), 881–895. doi:10.1007/s00125-017-4523-9

Heo, K. S., Chang, E., Le, N. T., Cushman, H., Yeh, E. T. H., Fujiwara, K., et al. (2013). De-SUMOylation enzyme of sentrin/SUMO-specific protease 2 regulates disturbed flow-induced SUMOylation of ERK5 and p53 that leads to endothelial dysfunction and atherosclerosis. *Circ. Res.* 112 (6), 911–923. doi:10.1161/CIRCRESAHA.111.300179

Hidalgo, M., Martin-Santamaria, S., Recio, I., Sanchez-Moreno, C., de Pascual-Teresa, B., Rimbach, G., et al. (2012). Potential anti-inflammatory, anti-adhesive, anti-estrogenic, and angiotensin-converting enzyme inhibitory activities of anthocyanins and their gut metabolites. *Genes. Nutr.* 7 (2), 295–306. doi:10.1007/s12263-011-0263-5

Ho, H. H., Chang, C. S., Ho, W. C., Liao, S. Y., Lin, W. L., and Wang, C. J. (2013). Gallic acid inhibits gastric cancer cells metastasis and invasive growth via increased expression of RhoB, downregulation of AKT/small GTPase signals and inhibition of NF-κB activity. *Toxicol. Appl. Pharmacol.* 266 (1), 76–85. doi:10.1016/j.taap.2012.10.019

Howes, M. J., and Houghton, P. J. (2012). Ethnobotanical treatment strategies against Alzheimer's disease. *Curr. Alzheimer Res.* 9 (1), 67–85. doi:10.2174/156720512799015046

Hu, Q., Chen, Y., Deng, X., Li, Y., Ma, X., Zeng, J., et al. (2023a). Diabetic nephropathy: focusing on pathological signals, clinical treatment, and dietary regulation. *Biomed. Pharmacother.* 159, 114252. doi:10.1016/j.biopha.2023.114252

Hu, Q., Jiang, L., Yan, Q., Zeng, J., Ma, X., and Zhao, Y. (2023b). A natural products solution to diabetic nephropathy therapy. *Pharmacol. Ther.* 241, 108314. doi:10.1016/j.pharmthera.2022.108314

Huang, H. L., Lin, C. C., Jeng, K. C. G., Yao, P. W., Chuang, L. T., Kuo, S. L., et al. (2012). Fresh green tea and gallic acid ameliorate oxidative stress in kainic acid-induced status epilepticus. *J. Agric. Food Chem.* 60 (9), 2328–2336. doi:10.1021/jf203709q

Huang, W., Liang, Y., Dong, J., Zhou, L., Gao, C., Jiang, C., et al. (2017). SUMO E3 ligase PIASy mediates high glucose-induced activation of NF-κB inflammatory signaling in rat mesangial cells. *Mediat. Inflamm.* 2017, 1685194. doi:10.1155/2017/1685194

Huang, W., Xu, L., Zhou, X., Gao, C., Yang, M., Chen, G., et al. (2013). High glucose induces activation of NF-κB inflammatory signaling through IκBα sumoylation in rat mesangial cells. *Biochem. Biophys. Res. Commun.* 438 (3), 568–574. doi:10.1016/j.bbrc.2013.07.065

Hwang, J. Y., Kan, W. C., Liu, Y. B., Chuang, L. Y., Guh, J. Y., Yang, Y. L., et al. (2019). Angiotensin-converting enzyme inhibitors attenuated advanced glycation end products-induced renal tubular hypertrophy via enhancing nitric oxide signaling. *J. Cell. Physiol.* 234 (10), 17473–17481. doi:10.1002/jcp.28369

Imoto, S., Ohbayashi, N., Ikeda, O., Kamitani, S., Muromoto, R., Sekine, Y., et al. (2008). Sumoylation of Smad3 stimulates its nuclear export during PIASy-mediated suppression of TGF-beta signaling. *Biochem. Biophys. Res. Commun.* 370 (2), 359–365. doi:10.1016/j.bbrc.2008.03.116

Isah, T. (2015). Rethinking Ginkgo biloba L.: medicinal uses and conservation. *Pharmacogn. Rev.* 9 (18), 140–148. doi:10.4103/0973-7847.162137

Jia, Y., Claessens, L. A., Vertegaal, A. C. O., and Ovaa, H. (2019). Chemical tools and biochemical assays for SUMO specific proteases (SENPs). *ACS Chem. Biol.* 14 (11), 2389–2395. doi:10.1021/acschembio.9b00402

Jiang, Y., Liang, M., Chen, L., Wang, J., Huang, Y., Huo, H., et al. (2023). Myeloid SENP3 deficiency protects mice from diet and age-induced obesity via regulation of YAP1 SUMOylation. *Cell. Mol. Life Sci.* 81 (1), 4. doi:10.1007/s00018-023-05050-w

Jung, H. S., Kang, Y. M., Park, H. S., Ahn, B. Y., Lee, H., Kim, M. J., et al. (2016). Senp2 expression was induced by chronic glucose stimulation in INS1 cells, and it was required for the associated induction of Ccnd1 and Mafa. *Islets* 8 (6), 207–216. doi:10.1080/19382014.2016.1235677

Kabir, M. T., Tabassum, N., Uddin, M. S., Aziz, F., Behl, T., Mathew, B., et al. (2021). Therapeutic potential of polyphenols in the management of diabetic neuropathy. *Evid. Based Complement. Altern. Med.* 2021, 9940169. doi:10.1155/2021/9940169

Kamynina, E., and Stover, P. J. (2017). The roles of SUMO in metabolic regulation. *Adv. Exp. Med. Biol.* 963, 143–168. doi:10.1007/978-3-319-50044-7_9

Katafuchi, T., Holland, W. L., Kollipara, R. K., Kittler, R., Mangelsdorf, D. J., and Kliewer, S. A. (2018). PPARγ-K107 SUMOylation regulates insulin sensitivity but not adiposity in mice. *Proc. Natl. Acad. Sci. U. S. A.* 115 (48), 12102–12111. doi:10.1073/pnas.1814522115

Khatoon, E., Banik, K., Harsha, C., Sailo, B. L., Thakur, K. K., Khwairakpam, A. D., et al. (2022). Phytochemicals in cancer cell chemosensitization: current knowledge and future perspectives. *Semin. Cancer Biol.* 80, 306–339. doi:10.1016/j.semcancer.2020.06.014

Kim, K. I., and Baek, S. H. (2009). Small ubiquitin-like modifiers in cellular malignancy and metastasis. *Int. Rev. Cell. Mol. Biol.* 273, 265–311. doi:10.1016/S1937-6448(08)01807-8

Kim, M., Ahn, B. Y., Lee, J. S., Chung, S. S., Lim, S., Park, S. G., et al. (2009). The ginsenoside Rg3 has a stimulatory effect on insulin signaling in L6 myotubes. *Biochem. Biophys. Res. Commun.* 389 (1), 70–73. doi:10.1016/j.bbrc.2009.08.088

Kim, Y. J., Park, S. M., Jung, H. S., Lee, E. J., Kim, T. K., Kim, T. N., et al. (2016). Ginsenoside Rg3 prevents INS-1 cell death from intermittent high glucose stress. *Islets* 8 (3), 57–64. doi:10.1080/19382014.2016.1161874

Ko, J. K., Lam, F. Y., and Cheung, A. P. (2005). Amelioration of experimental colitis by Astragalus membranaceus through anti-oxidation and inhibition of adhesion molecule synthesis. *World J. Gastroenterol.* 11 (37), 5787–5794. doi:10.3748/wjg.v11.i37.5787

Koch, E. (2005). Inhibition of platelet activating factor (PAF)-induced aggregation of human thrombocytes by ginkgolides: considerations on possible bleeding complications after oral intake of Ginkgo biloba extracts. *Phytomedicine* 12 (1-2), 10–16. doi:10.1016/j.phymed.2004.02.002

Kumar, A., and Zhang, K. Y. (2015). Advances in the development of SUMO specific protease (SENP) inhibitors. *Comput. Struct. Biotechnol. J.* 13, 204–211. doi:10.1016/j.csbj.2015.03.001

Kunz, K., Piller, T., and Muller, S. (2018). SUMO-specific proteases and isopeptidases of the SENP family at a glance. *J. Cell. Sci.* 131 (6), jcs211904. doi:10.1242/jcs.211904

Kushwaha, K., Sharma, S., and Gupta, J. (2020a). Metabolic memory and diabetic nephropathy: beneficial effects of natural epigenetic modifiers. *Biochimie* 170, 140–151. doi:10.1016/j.biochi.2020.01.007

Kushwaha, K., Sharma, S., and Gupta, J. (2020b). Metabolic memory and diabetic nephropathy: beneficial effects of natural epigenetic modifiers. *Biochimie* 170, 140–151. doi:10.1016/j.biochi.2020.01.007

Langston, S. P., Grossman, S., England, D., Afroze, R., Bence, N., Bowman, D., et al. (2021). Discovery of TAK-981, a first-in-class inhibitor of SUMO-activating enzyme for the treatment of cancer. *J. Med. Chem.* 64 (5), 2501–2520. doi:10.1021/acs.jmedchem.0c01491

Lao, Y., Yang, K., Wang, Z., Sun, X., Zou, Q., Yu, X., et al. (2018). DeSUMOylation of MKK7 kinase by the SUMO2/3 protease SENP3 potentiates lipopolysaccharide-induced inflammatory signaling in macrophages. *J. Biol. Chem.* 293 (11), 3965–3980. doi:10.1074/jbc.M117.816769

Li, M., Guo, D., Isales, C. M., Eizirik, D. L., Atkinson, M., She, J. X., et al. (2005). SUMO wrestling with type 1 diabetes. *J. Mol. Med. Berl.* 83 (7), 504–513. doi:10.1007/s00109-005-0645-5

Li, O., Ma, Q., Li, F., Cai, G. Y., Chen, X. M., and Hong, Q. (2019). Progress of small ubiquitin-related modifiers in kidney diseases. *Chin. Med. J. Engl.* 132 (4), 466–473. doi:10.1097/CM9.0000000000000094

Li, S. J., and Hochstrasser, M. (1999). A new protease required for cell-cycle progression in yeast. *Nature* 398 (6724), 246–251. doi:10.1038/18457

Li, S. J., and Hochstrasser, M. (2000). The yeast ULP2 (SMT4) gene encodes a novel protease specific for the ubiquitin-like Smt3 protein. *Mol. Cell. Biol.* 20 (7), 2367–2377. doi:10.1128/mcb.20.7.2367-2377.2000

Li, X., Luo, Y., Yu, L., Lin, Y., Luo, D., Zhang, H., et al. (2008). SENP1 mediates TNF-induced desumoylation and cytoplasmic translocation of HIPK1 to enhance ASK1-dependent apoptosis. *Cell. Death Differ.* 15 (4), 739–750. doi:10.1038/sj.cdd.4402303

Li, Y., Hou, J. G., Liu, Z., Gong, X. J., Hu, J. N., Wang, Y. P., et al. (2021). Alleviative effects of 20(R)-Rg3 on HFD/STZ-induced diabetic nephropathy via MAPK/NF-κB signaling pathways in C57BL/6 mice. *J. Ethnopharmacol.* 267, 113500. doi:10.1016/j.jep.2020.113500

Li, Y. Y., Wang, H., Yang, X. X., Geng, H. Y., Gong, G., Kim, H. J., et al. (2017). Small ubiquitin-like modifier 4 (SUMO4) gene M55V polymorphism and type 2 diabetes mellitus: a meta-analysis including 6,823 subjects. *Front. Endocrinol. (Lausanne)* 8, 303. doi:10.3389/fendo.2017.00303

Liang, Q., Zheng, Q., Zuo, Y., Chen, Y., Ma, J., Ni, P., et al. (2019). SENP2 suppresses necdin expression to promote Brown adipocyte differentiation. *Cell. Rep.* 28 (8), 2004–2011. doi:10.1016/j.celrep.2019.07.083

Liang, X., Su, Y., and Huo, Y. (2021). Forkhead box protein O1 (FoxO1)/SERPINB1 ameliorates ROS production in diabetic nephropathy. *Food Sci. Nutr.* 9 (1), 44–51. doi:10.1002/fsn3.1859

Liang, Y. C., Lee, C. C., Yao, Y. L., Lai, C. C., Schmitz, M. L., and Yang, W. M. (2016). SUMO5, a novel poly-SUMO isoform, regulates PML nuclear bodies. *Sci. Rep.* 6, 26509. doi:10.1038/srep26509

Lin, H. Y., Wang, C. L., Hsiao, P. J., Lu, Y. C., Chen, S. Y., Lin, K. D., et al. (2007). SUMO4 M55V variant is associated with diabetic nephropathy in type 2 diabetes. *Diabetes* 56 (4), 1177–1180. doi:10.2337/db06-1283

Lin, Q., Chen, O., Wise, J. P., Jr, Shi, H., Wintergerst, K. A., Cai, L., et al. (2022). $FGF1^{\Delta HBS}$ delays the progression of diabetic nephropathy in late-stage type 2 diabetes mouse model by alleviating renal inflammation, fibrosis, and apoptosis. *Biochim. Biophys. Acta Mol. Basis Dis.* 1868 (8), 166414. doi:10.1016/j.bbadis.2022.166414

Liu, J., Tao, X., Zhang, J., Wang, P., Sha, M., Ma, Y., et al. (2016). Small ubiquitin-related modifier 1 is involved in hepatocellular carcinoma progression via mediating p65 nuclear translocation. *Oncotarget* 7 (16), 22206–22218. doi:10.18632/oncotarget.8066

Liu, K. Y., Hu, S., Chan, B. C. L., Wat, E. C. L., Lau, C. B. S., Hon, K. L., et al. (2013b). Anti-inflammatory and anti-allergic activities of Pentaherb formula, Moutan Cortex (Danpi) and gallic acid. *Molecules* 18 (3), 2483–2500. doi:10.3390/molecules18032483

Liu, M., Yan, M., Lv, H., Wang, B., Lv, X., Zhang, H., et al. (2020a). Macrophage K63-linked ubiquitination of YAP promotes its nuclear localization and exacerbates atherosclerosis. *Cell. Rep.* 32 (5), 107990. doi:10.1016/j.celrep.2020.107990

Liu, P., Zhang, J., Wang, Y., Wang, C., Qiu, X., and Chen, D. Q. (2022a). Natural products against renal fibrosis via modulation of SUMOylation. *Front. Pharmacol.* 13, 800810. doi:10.3389/fphar.2022.800810

Liu, X., Chen, L., Zhang, C., Dong, W., Liu, H., Xiao, Z., et al. (2022b). Ginkgolic acid promotes inflammation and macrophage apoptosis *via* SUMOylation and NF-κB pathways in sepsis. *Front. Med. (Lausanne)* 9, 1108882. doi:10.3389/fmed.2022.1108882

Liu, X., Chen, W., Wang, Q., Li, L., and Wang, C. (2013a). Negative regulation of TLR inflammatory signaling by the SUMO-deconjugating enzyme SENP6. *PLoS Pathog.* 9 (6), e1003480. doi:10.1371/journal.ppat.1003480

Liu, Y., Dou, X., Zhou, W. Y., Ding, M., Liu, L., Du, R. Q., et al. (2021). Hepatic small ubiquitin-related modifier (SUMO)-Specific protease 2 controls systemic metabolism through SUMOylation-dependent regulation of liver-adipose tissue crosstalk. *Hepatology* 74 (4), 1864–1883. doi:10.1002/hep.31881

Liu, Y., Trnka, M. J., Guan, S., Kwon, D., Kim, D. H., Chen, J. J., et al. (2020b). A novel mechanism for NF-κB-activation via IκB-aggregation: implications for hepatic mallory-denk-body induced inflammation. *Mol. Cell. Proteomics* 19 (12), 1968–1986. doi:10.1074/mcp.RA120.002316

Lizotte, F., Denhez, B., Guay, A., Gévry, N., Côté, A. M., and Geraldes, P. (2016). Persistent insulin resistance in podocytes caused by epigenetic changes of SHP-1 in diabetes. *Diabetes* 65 (12), 3705–3717. doi:10.2337/db16-0254

Lizotte, F., Rousseau, M., Denhez, B., Lévesque, D., Guay, A., Liu, H., et al. (2023). Deletion of protein tyrosine phosphatase SHP-1 restores SUMOylation of podocin and reverses the progression of diabetic kidney disease. *Kidney Int.* 104 (4), 787–802. doi:10.1016/j.kint.2023.06.038

Locatelli, M., Zoja, C., Zanchi, C., Corna, D., Villa, S., Bolognini, S., et al. (2020). Manipulating Sirtuin 3 pathway ameliorates renal damage in experimental diabetes. *Sci. Rep.* 10 (1), 8418. doi:10.1038/s41598-020-65423-0

Long, J., Wang, G., He, D., and Liu, F. (2004). Repression of Smad4 transcriptional activity by SUMO modification. *Biochem. J.* 379 (Pt 1), 23–29. doi:10.1042/BJ20031867

Luciani, A., Villella, V. R., Vasaturo, A., Giardino, I., Raia, V., Pettoello-Mantovani, M., et al. (2009). SUMOylation of tissue transglutaminase as link between oxidative stress and inflammation. *J. Immunol.* 183 (4), 2775–2784. doi:10.4049/jimmunol.0900993

Ma, L., Wu, F., Shao, Q., Chen, G., Xu, L., and Lu, F. (2021). Baicalin alleviates oxidative stress and inflammation in diabetic nephropathy via Nrf2 and MAPK signaling pathway. *Drug Des. Devel Ther.* 15, 3207–3221. doi:10.2147/DDDT.S319260

Ma, T., Li, X., Zhu, Y., Yu, S., Liu, T., Zhang, X., et al. (2022). Excessive activation of Notch signaling in macrophages promote kidney inflammation, fibrosis, and necroptosis. *Front. Immunol.* 13, 835879. doi:10.3389/fimmu.2022.835879

Mabb, A. M., and Miyamoto, S. (2007). SUMO and NF-kappaB ties. *Cell. Mol. Life Sci.* 64 (15), 1979–1996. doi:10.1007/s00018-007-7005-2

Magee, C., Grieve, D. J., Watson, C. J., and Brazil, D. P. (2017). Diabetic nephropathy: a tangled web to unweave. *Cardiovasc Drugs Ther.* 31 (5-6), 579–592. doi:10.1007/s10557-017-6755-9

Manning, F. J., Hajmrle, C., and Macdonald, P. E. (2012). Novel roles of SUMO in pancreatic β-cells: thinking outside the nucleus. *Can. J. Physiol. Pharmacol.* 90 (6), 765–770. doi:10.1139/y11-134

Meinecke, I., Cinski, A., Baier, A., Peters, M. A., Dankbar, B., Wille, A., et al. (2007). Modification of nuclear PML protein by SUMO-1 regulates Fas-induced apoptosis in rheumatoid arthritis synovial fibroblasts. *Proc. Natl. Acad. Sci. U. S. A.* 104 (12), 5073–5078. doi:10.1073/pnas.0608773104

Melchior, F., Schergaut, M., and Pichler, A. (2003). SUMO: ligases, isopeptidases and nuclear pores. *Trends Biochem. Sci.* 28 (11), 612–618. doi:10.1016/j.tibs.2003.09.002

Meng, X. M., Huang, X. R., Xiao, J., Chung, A. C. K., Qin, W., Chen, H. y., et al. (2012). Disruption of Smad4 impairs TGF-β/Smad3 and Smad7 transcriptional regulation during renal inflammation and fibrosis *in vivo* and *in vitro*. *Kidney Int.* 81 (3), 266–279. doi:10.1038/ki.2011.327

Miyata, T., and de Strihou, C. (2010). Diabetic nephropathy: a disorder of oxygen metabolism? *Nat. Rev. Nephrol.* 6 (2), 83–95. doi:10.1038/nrneph.2009.211

Mohanta, T. K., Tamboli, Y., and Zubaidha, P. K. (2014). Phytochemical and medicinal importance of Ginkgo biloba L. *Nat. Prod. Res.* 28 (10), 746–752. doi:10.1080/14786419.2013.879303

Muller, S., Ledl, A., and Schmidt, D. (2004). SUMO: a regulator of gene expression and genome integrity. *Oncogene* 23 (11), 1998–2008. doi:10.1038/sj.onc.1207415

Nabavi, S. M., Habtemariam, S., Nabavi, S. F., Sureda, A., Daglia, M., Moghaddam, A. H., et al. (2013). Protective effect of gallic acid isolated from Peltiphyllum peltatum against sodium fluoride-induced oxidative stress in rat's kidney. *Mol. Cell. Biochem.* 372 (1-2), 233–239. doi:10.1007/s11010-012-1464-y

Nagata, M. (2016). Podocyte injury and its consequences. *Kidney Int.* 89 (6), 1221–1230. doi:10.1016/j.kint.2016.01.012

Nan, J., Lee, J. S., Moon, J. H., Lee, S. A., Park, Y. J., Lee, D. S., et al. (2022). SENP2 regulates mitochondrial function and insulin secretion in pancreatic β cells. *Exp. Mol. Med.* 54 (1), 72–80. doi:10.1038/s12276-021-00723-7

Nanjan, M. J., and Betz, J. (2014). Resveratrol for the management of diabetes and its downstream pathologies. *Eur. Endocrinol.* 10 (1), 31–35. doi:10.17925/EE.2014.10.01.31

Nash, K. M., and Shah, Z. A. (2015). Current perspectives on the beneficial role of ginkgo biloba in neurological and cerebrovascular disorders. *Integr. Med. Insights* 10, 1–9. doi:10.4137/IMI.S25054

Nyirimigabo, E., Jin, M., Yang, Z., Wang, J., Zhai, K., Mao, Y., et al. (2019). The role of ERp44 in glucose and lipid metabolism. *Arch. Biochem. Biophys.* 671, 175–184. doi:10.1016/j.abb.2019.06.011

Ohshima, T., Koga, H., and Shimotohno, K. (2004). Transcriptional activity of peroxisome proliferator-activated receptor gamma is modulated by SUMO-1 modification. *J. Biol. Chem.* 279 (28), 29551–29557. doi:10.1074/jbc.M403866200

Owerbach, D., McKay, E. M., Yeh, E. T. H., Gabbay, K. H., and Bohren, K. M. (2005). A proline-90 residue unique to SUMO-4 prevents maturation and sumoylation. *Biochem. Biophys. Res. Commun.* 337 (2), 517–520. doi:10.1016/j.bbrc.2005.09.090

Pandey, K. B., and Rizvi, S. I. (2009). Plant polyphenols as dietary antioxidants in human health and disease. *Oxid. Med. Cell. Longev.* 2 (5), 270–278. doi:10.4161/oxim.2.5.9498

Peiyuan, H., Zhiping, H., Chengjun, S., Chunqing, W., Bingqing, L., and Imam, M. U. (2017). Resveratrol ameliorates experimental alcoholic liver disease by modulating oxidative stress. *Evid. Based Complement. Altern. Med.* 2017, 4287890. doi:10.1155/2017/4287890

Perez-Morales, R. E., Del Pino, M. D., Valdivielso, J. M., Ortiz, A., Mora-Fernández, C., and Navarro-González, J. F. (2019). Inflammation in diabetic kidney disease. *Nephron* 143 (1), 12–16. doi:10.1159/000493278

Podolsky, R., Prasad, L. M., She, J. X., and Type I Diabetes Genetics Consortium (2009). Analyses of multiple single-nucleotide polymorphisms in the SUMO4/IDDM5 region in affected sib-pair families with type I diabetes. *Genes. Immun.* 10 (Suppl. 1), S16–S20. doi:10.1038/gene.2009.86

Pop-Busui, R., Ang, L., Boulton, A., Feldman, E., Marcus, R., Mizokami-Stout, K., et al. (2022). Diagnosis and treatment of painful diabetic peripheral neuropathy. *Arlingt. (VA) Am. Diabetes Assoc.* 2022, 1–32. doi:10.2337/db2022-01

Prasad, K. (2012). Resveratrol, wine, and atherosclerosis. *Int. J. Angiol.* 21 (1), 7–18. doi:10.1055/s-0032-1306417

Punithavathi, V. R., Stanely Mainzen Prince, P., Kumar, M. R., and Selvakumari, C. J. (2011). Protective effects of gallic acid on hepatic lipid peroxide metabolism, glycoprotein components and lipids in streptozotocin-induced type II diabetic Wistar rats. *J. Biochem. Mol. Toxicol.* 25 (2), 68–76. doi:10.1002/jbt.20360

Qin, Q., Niu, J., Wang, Z., Xu, W., Qiao, Z., and Gu, Y. (2012). Astragalus membranaceus inhibits inflammation via phospho-P38 mitogen-activated protein kinase (MAPK) and nuclear factor (NF)-κB pathways in advanced glycation end product-stimulated macrophages. *Int. J. Mol. Sci.* 13 (7), 8379–8387. doi:10.3390/ijms13078379

Qiu, J. Y., Chen, X., Zheng, X. x., Jiang, X. l., Yang, D. z., Yu, Y. y., et al. (2015). Target cell extraction coupled with LC-MS/MS analysis for screening potential bioactive components in Ginkgo biloba extract with preventive effect against diabetic nephropathy. *Biomed. Chromatogr.* 29 (2), 226–232. doi:10.1002/bmc.3264

Rajan, S., Torres, J., Thompson, M. S., and Philipson, L. H. (2012). SUMO downregulates GLP-1-stimulated cAMP generation and insulin secretion. *Am. J. Physiol. Endocrinol. Metab.* 302 (6), E714–E723. doi:10.1152/ajpendo.00486.2011

Rao, A., Pandya, V., and Whaley-Connell, A. (2015). Obesity and insulin resistance in resistant hypertension: implications for the kidney. *Adv. Chronic Kidney Dis.* 22 (3), 211–217. doi:10.1053/j.ackd.2014.12.004

Reutens, A. T., and Atkins, R. C. (2011). Epidemiology of diabetic nephropathy. *Contrib. Nephrol.* 170, 1–7. doi:10.1159/000324934

Roborel, D. C. A., Pain, E., Boss, A., and Shaunik, A. (2020). Understanding reasons for treatment discontinuation, attitudes and education needs among People who discontinue type 2 diabetes treatment: results from an online patient survey in the USA and UK. *Diabetes Ther.* 11 (8), 1873–1881. doi:10.1007/s13300-020-00843-9

Sakai, N., Wada, T., Furuichi, K., Iwata, Y., Yoshimoto, K., Kitagawa, K., et al. (2005). Involvement of extracellular signal-regulated kinase and p38 in human diabetic nephropathy. *Am. J. Kidney Dis.* 45 (1), 54–65. doi:10.1053/j.ajkd.2004.08.039

Sato, M., Muragaki, Y., Saika, S., Roberts, A. B., and Ooshima, A. (2003). Targeted disruption of TGF-beta1/Smad3 signaling protects against renal tubulointerstitial fibrosis induced by unilateral ureteral obstruction. *J. Clin. Invest.* 112 (10), 1486–1494. doi:10.1172/JCI19270

Sauer, S. (2015). Ligands for the nuclear peroxisome proliferator-activated receptor gamma. *Trends Pharmacol. Sci.* 36 (10), 688–704. doi:10.1016/j.tips.2015.06.010

Schulz, S., Chachami, G., Kozaczkiewicz, L., Winter, U., Stankovic-Valentin, N., Haas, P., et al. (2012). Ubiquitin-specific protease-like 1 (USPL1) is a SUMO isopeptidase with essential, non-catalytic functions. *EMBO Rep.* 13 (10), 930–938. doi:10.1038/embor.2012.125

Sheng, Z., Zhu, J., Deng, Y. N., Gao, S., and Liang, S. (2021). SUMOylation modification-mediated cell death. *Open Biol.* 11 (7), 210050. doi:10.1098/rsob.210050

Shin, E. J., Shin, H. M., Nam, E., Kim, W. S., Kim, J. H., Oh, B. H., et al. (2012). DeSUMOylating isopeptidase: a second class of SUMO protease. *EMBO Rep.* 13 (4), 339–346. doi:10.1038/embor.2012.3

Shoeib, H. M., Keshk, W. A., Al-Ghazaly, G. M., Wagih, A. A., and El-Dardiry, S. A. (2023). Interplay between long non-coding RNA MALAT1 and pyroptosis in diabetic nephropathy patients. *Gene* 851, 146978. doi:10.1016/j.gene.2022.146978

Sinha, N., Yadav, A. K., Kumar, V., Dutta, P., Bhansali, A., and Jha, V. (2016). SUMO4 163 G>A variation is associated with kidney disease in Indian subjects with type 2 diabetes. *Mol. Biol. Rep.* 43 (5), 345–348. doi:10.1007/s11033-016-3979-x

Skov, J., Christiansen, J. S., and Poulsen, P. L. (2016). Hypertension and diabetic nephropathy. *Endocr. Dev.* 31, 97–107. doi:10.1159/000439393

Smith, S., Normahani, P., Lane, T., Hohenschurz-Schmidt, D., Oliver, N., and Davies, A. H. (2022). Prevention and management strategies for diabetic neuropathy. *Life (Basel)* 12 (8), 1185. doi:10.3390/life12081185

Srivastava, S. P., Zhou, H., Setia, O., Liu, B., Kanasaki, K., Koya, D., et al. (2021). Loss of endothelial glucocorticoid receptor accelerates diabetic nephropathy. *Nat. Commun.* 12 (1), 2368. doi:10.1038/s41467-021-22617-y

Stechschulte, L. A., Czernik, P. J., Rotter, Z. C., Tausif, F. N., Corzo, C. A., Marciano, D. P., et al. (2016). PPARG post-translational modifications regulate bone formation and bone resorption. *EBioMedicine* 10, 174–184. doi:10.1016/j.ebiom.2016.06.040

Sui, Z., Sui, D., Li, M., Yu, Q., Li, H., and Jiang, Y. (2023). Ginsenoside Rg3 has effects comparable to those of ginsenos*ide re* on diabetic kidney disease prevention in db/db mice by regulating inflammation, fibrosis and PPARγ. *Mol. Med. Rep.* 27 (4), 84. doi:10.3892/mmr.2023.12971

Taghvaei, S., Sabouni, F., Minuchehr, Z., and Taghvaei, A. (2022). Identification of novel anti-cancer agents, applying *in silico* method for SENP1 protease inhibition. *J. Biomol. Struct. Dyn.* 40 (14), 6228–6242. doi:10.1080/07391102.2021.1880480

Tan, X., Liu, Y., Liu, D., Tang, X., Xia, M., Chen, G., et al. (2022). SUMO1 promotes mesangial cell proliferation through inhibiting autophagy in a cell model of IgA nephropathy. *Front. Med. (Lausanne)* 9, 834164. doi:10.3389/fmed.2022.834164

Tang, D., Zhang, Z., Gao, Y., Wei, Y., and Han, L. (2009). Protective effects of serum containing Ginkgo biloba extract on glomerulosclerosis in rat mesangial cells. *J. Ethnopharmacol.* 124 (1), 26–33. doi:10.1016/j.jep.2009.04.017

Tang, G., Li, S., Zhang, C., Chen, H., Wang, N., and Feng, Y. (2021). Clinical efficacies, underlying mechanisms and molecular targets of Chinese medicines for diabetic nephropathy treatment and management. *Acta Pharm. Sin. B* 11 (9), 2749–2767. doi:10.1016/j.apsb.2020.12.020

Tokarz, P., and Wozniak, K. (2021). SENP proteases as potential targets for cancer therapy. *Cancers (Basel)* 13 (9), 2059. doi:10.3390/cancers13092059

Tossidou, I., Himmelseher, E., Teng, B., Haller, H., and Schiffer, M. (2014). SUMOylation determines turnover and localization of nephrin at the plasma membrane. *Kidney Int.* 86 (6), 1161–1173. doi:10.1038/ki.2014.198

Tossidou, I., Niedenthal, R., Klaus, M., Teng, B., Worthmann, K., King, B. L., et al. (2012). CD2AP regulates SUMOylation of CIN85 in podocytes. *Mol. Cell. Biol.* 32 (6), 1068–1079. doi:10.1128/MCB.06106-11

Vertegaal, A. (2022). Signalling mechanisms and cellular functions of SUMO. *Nat. Rev. Mol. Cell. Biol.* 23 (11), 715–731. doi:10.1038/s41580-022-00500-y

Wada, T., Pippin, J. W., Marshall, C. B., Griffin, S. V., and Shankland, S. J. (2005). Dexamethasone prevents podocyte apoptosis induced by puromycin aminonucleoside: role of p53 and Bcl-2-related family proteins. *J. Am. Soc. Nephrol.* 16 (9), 2615–2625. doi:10.1681/ASN.2005020142

Wang, A., Ziyadeh, F. N., Lee, E. Y., Pyagay, P. E., Sung, S. H., Sheardown, S. A., et al. (2007). Interference with TGF-beta signaling by Smad3-knockout in mice limits diabetic glomerulosclerosis without affecting albuminuria. *Am. J. Physiol. Ren. Physiol.* 293 (5), F1657–F1665. doi:10.1152/ajprenal.00274.2007

Wang, B., Zhang, C., Chu, D., Ma, X., Yu, T., Liu, X., et al. (2021c). Astragaloside IV improves angiogenesis under hypoxic conditions by enhancing hypoxia-inducible factor-1α SUMOylation. *Mol. Med. Rep.* 23 (4), 244. doi:10.3892/mmr.2021.11883

Wang, C. Y., and She, J. X. (2008). SUMO4 and its role in type 1 diabetes pathogenesis. *Diabetes Metab. Res. Rev.* 24 (2), 93–102. doi:10.1002/dmrr.797

Wang, H., Zhang, R., Wu, X., Chen, Y., Ji, W., Wang, J., et al. (2021a). The Wnt signaling pathway in diabetic nephropathy. *Front. Cell. Dev. Biol.* 9, 701547. doi:10.3389/fcell.2021.701547

Wang, J., Zhang, Z., Fang, A., Wu, K., Chen, X., Wang, G., et al. (2020). Resveratrol attenuates inflammatory bowel disease in mice by regulating SUMO1. *Biol. Pharm. Bull.* 43 (3), 450–457. doi:10.1248/bpb.b19-00786

Wang, L., Wang, H. L., Liu, T. T., and Lan, H. Y. (2021b). TGF-beta as a master regulator of diabetic nephropathy. *Int. J. Mol. Sci.* 22 (15), 7881. doi:10.3390/ijms22157881

Wang, L., Wansleeben, C., Zhao, S., Miao, P., Paschen, W., and Yang, W. (2014a). SUMO2 is essential while SUMO3 is dispensable for mouse embryonic development. *EMBO Rep.* 15 (8), 878–885. doi:10.15252/embr.201438534

Wang, L., Zhang, T., Fang, M., Shen, N., Wang, D., Teng, J., et al. (2015a). Podocytes protect glomerular endothelial cells from hypoxic injury via deSUMOylation of HIF-1α signaling. *Int. J. Biochem. Cell. Biol.* 58, 17–27. doi:10.1016/j.biocel.2014.10.030

Wang, L., Zhu, J., Fang, M., Zhang, T., Xie, H., Wang, N., et al. (2014b). Inhibition of p53 deSUMOylation exacerbates puromycin aminonucleoside-induced apoptosis in podocytes. *Int. J. Mol. Sci.* 15 (11), 21314–21330. doi:10.3390/ijms151121314

Wang, W., and Matunis, M. J. (2023). Paralogue-specific roles of SUMO1 and SUMO2/3 in protein quality control and associated diseases. *Cells* 13 (1), 8. doi:10.3390/cells13010008

Wang, Y., Jiang, Y., Fan, X., Tan, H., Zeng, H., Wang, Y., et al. (2015b). Hepatoprotective effect of resveratrol against acetaminophen-induced liver injury is associated with inhibition of CYP-mediated bioactivation and regulation of SIRT1-p53 signaling pathways. *Toxicol. Lett.* 236 (2), 82–89. doi:10.1016/j.toxlet.2015.05.001

Wang, Z., Pan, B., Su, L., Yu, H., Wu, X., Yao, Y., et al. (2024). SUMOylation inhibitors activate anti-tumor immunity by reshaping the immune microenvironment in a preclinical model of hepatocellular carcinoma. *Cell. Oncol. (Dordr)* 47 (2), 513–532. doi:10.1007/s13402-023-00880-z

Wei, W., Yang, P., Pang, J., Zhang, S., Wang, Y., Wang, M. H., et al. (2008). A stress-dependent SUMO4 sumoylation of its substrate proteins. *Biochem. Biophys. Res. Commun.* 375 (3), 454–459. doi:10.1016/j.bbrc.2008.08.028

Wianowska, D., and Olszowy-Tomczyk, M. (2023). A concise profile of gallic acid-from its natural sources through biological properties and chemical methods of determination. *Molecules* 28 (3), 1186. doi:10.3390/molecules28031186

Wu, T., Ding, L., Andoh, V., Zhang, J., and Chen, L. (2023). The mechanism of hyperglycemia-induced renal cell injury in diabetic nephropathy disease: an update. *Life (Basel)* 13 (2), 539. doi:10.3390/life13020539

Wu, W., and Huang, C. (2023). SUMOylation and DeSUMOylation: prospective therapeutic targets in cancer. *Life Sci.* 332, 122085. doi:10.1016/j.lfs.2023.122085

Wu, X. M., Gao, Y. B., Cui, F. Q., and Zhang, N. (2016). Exosomes from high glucose-treated glomerular endothelial cells activate mesangial cells to promote renal fibrosis. *Biol. Open* 5 (4), 484–491. doi:10.1242/bio.015990

Xie, H., Wang, Y. H., Liu, X., Gao, J., Yang, C., Huang, T., et al. (2023). SUMOylation of ERp44 enhances Ero1α ER retention contributing to the pathogenesis of obesity and insulin resistance. *Metabolism* 139, 155351. doi:10.1016/j.metabol.2022.155351

Xing, L., Fang, J., Zhu, B., Wang, L., Chen, J., Wang, Y., et al. (2021). Astragaloside IV protects against podocyte apoptosis by inhibiting oxidative stress via activating PPARγ-Klotho-FoxO1 axis in diabetic nephropathy. *Life Sci.* 269, 119068. doi:10.1016/j.lfs.2021.119068

Xiong, Y., and Zhou, L. (2019). The signaling of cellular senescence in diabetic nephropathy. *Oxid. Med. Cell. Longev.* 2019, 7495629. doi:10.1155/2019/7495629

Xu, J., Deng, Y., Wang, Y., Sun, X., Chen, S., and Fu, G. (2020). SPAG5-AS1 inhibited autophagy and aggravated apoptosis of podocytes via SPAG5/AKT/mTOR pathway. *Cell. Prolif.* 53 (2), e12738. doi:10.1111/cpr.12738

Xu, Z., Luo, W., Chen, L., Zhuang, Z., Yang, D., Qian, J., et al. (2022). Ang II (angiotensin II)-Induced FGFR1 (fibroblast growth factor receptor 1) activation in tubular epithelial cells promotes hypertensive kidney fibrosis and injury. *Hypertension* 79 (9), 2028–2041. doi:10.1161/HYPERTENSIONAHA.122.18657

Yang, J., and Liu, Z. (2022). Mechanistic pathogenesis of endothelial dysfunction in diabetic nephropathy and retinopathy. *Front. Endocrinol. (Lausanne)* 13, 816400. doi:10.3389/fendo.2022.816400

Yang, Z., Zhang, Y., and Sun, S. (2019). Deciphering the SUMO code in the kidney. *J. Cell. Mol. Med.* 23 (2), 711–719. doi:10.1111/jcmm.14021

Yeh, E. T. (2009). SUMOylation and De-SUMOylation: wrestling with life's processes. *J. Biol. Chem.* 284 (13), 8223–8227. doi:10.1074/jbc.R800050200

Yong, H. Y., Koh, M. S., and Moon, A. (2009). The p38 MAPK inhibitors for the treatment of inflammatory diseases and cancer. *Expert Opin. Investig. Drugs* 18 (12), 1893–1905. doi:10.1517/13543780903321490

Youssef, A., Mohammed, B. K., Prasad, A., Del Aguila, A., Bassi, G., Yang, W., et al. (2023). Splenic SUMO1 controls systemic inflammation in experimental sepsis. *Front. Immunol.* 14, 1200939. doi:10.3389/fimmu.2023.1200939

Yu, L., Wang, Y., Guo, Y. H., Wang, L., Yang, Z., Zhai, Z. H., et al. (2021). HIF-1α alleviates high-glucose-induced renal tubular cell injury by promoting Parkin/PINK1-mediated mitophagy. *Front. Med. (Lausanne)* 8, 803874. doi:10.3389/fmed.2021.803874

Yuan, H. D., Kim, J. T., Kim, S. H., and Chung, S. H. (2012). Ginseng and diabetes: the evidences from *in vitro*, animal and human studies. *J. Ginseng Res.* 36 (1), 27–39. doi:10.5142/jgr.2012.36.1.27

Yuan, W., Zhou, Y., Wang, M., Zeng, G., and Huang, Q. (2019). Negative regulation of eNOS-NO signaling by over-SUMOylation of PPARγ contributes to insulin resistance and dysfunction of vascular endothelium in rats. *Vasc. Pharmacol.* 122-123, 106597. doi:10.1016/j.vph.2019.106597

Yun, C., Wang, Y., Mukhopadhyay, D., Backlund, P., Kolli, N., Yergey, A., et al. (2008). Nucleolar protein B23/nucleophosmin regulates the vertebrate SUMO pathway through SENP3 and SENP5 proteases. *J. Cell. Biol.* 183 (4), 589–595. doi:10.1083/jcb.200807185

Zhang, H., Tomar, V. S., Li, J., Basavaraja, R., Yan, F., Gui, J., et al. (2022b). Protection of regulatory T cells from fragility and inactivation in the tumor microenvironment. *Cancer Immunol. Res.* 10 (12), 1490–1505. doi:10.1158/2326-6066.CIR-22-0295

Zhang, J., and Yan, J. (2019). Protective effect of ginkgolic acid in attenuating LDL induced inflammation human peripheral blood mononuclear cells via altering the NF-κB signaling pathway. *Front. Pharmacol.* 10, 1241. doi:10.3389/fphar.2019.01241

Zhang, Z., Sun, Y., Xue, J., Jin, D., Li, X., Zhao, D., et al. (2022a). The critical role of dysregulated autophagy in the progression of diabetic kidney disease. *Front. Pharmacol.* 13, 977410. doi:10.3389/fphar.2022.977410

Zheng, Q., Cao, Y., Chen, Y., Wang, J., Fan, Q., Huang, X., et al. (2018). Senp2 regulates adipose lipid storage by de-SUMOylation of Setdb1. *J. Mol. Cell. Biol.* 10 (3), 258–266. doi:10.1093/jmcb/mjx055

Zhou, X., Gao, C., Huang, W., Yang, M., Chen, G., Jiang, L., et al. (2014). High glucose induces sumoylation of Smad4 via SUMO2/3 in mesangial cells. *Biomed. Res. Int.* 2014, 782625. doi:10.1155/2014/782625

Zhou, X., Li, W., Wang, S., Zhang, P., Wang, Q., Xiao, J., et al. (2019). YAP aggravates inflammatory bowel disease by regulating M1/M2 macrophage polarization and gut microbial homeostasis. *Cell. Rep.* 27 (4), 1176–1189. doi:10.1016/j.celrep.2019.03.028

Zhou, X., Sun, X., Gong, X., Yang, Y., Chen, C., Shan, G., et al. (2017). Astragaloside IV from Astragalus membranaceus ameliorates renal interstitial fibrosis by inhibiting inflammation via TLR4/NF-κB *in vivo* and *in vitro*. *Int. Immunopharmacol.* 42, 18–24. doi:10.1016/j.intimp.2016.11.006

Zou, M., Wang, J., Gao, J., Han, H., and Fang, Y. (2018). Phosphoproteomic analysis of the antitumor effects of ginsenoside Rg3 in human breast cancer cells. *Oncol. Lett.* 15 (3), 2889–2898. doi:10.3892/ol.2017.7654

Zuo, W., Yan, F., Zhang, B., Li, J., and Mei, D. (2017). Advances in the studies of ginkgo biloba leaves extract on aging-related diseases. *Aging Dis.* 8 (6), 812–826. doi:10.14336/AD.2017.0615

frontiers | Frontiers in Pharmacology

TYPE Original Research
PUBLISHED 22 October 2024
DOI 10.3389/fphar.2024.1464463

Check for updates

OPEN ACCESS

EDITED BY
Swayam Prakash Srivastava,
University of Michigan, United States

REVIEWED BY
Amany Mohammed Gad,
Sinai University, Egypt
Mmamosheledi Elsie Mothibe,
Rhodes University, South Africa

*CORRESPONDENCE
Phatchawan Arjinajarn,
arjinajarn_jin@hotmail.com,
phatchawan.ar@cmu.ac.th

RECEIVED 14 July 2024
ACCEPTED 10 October 2024
PUBLISHED 22 October 2024

CITATION
Laorodphun P, Chaisen S, Amattat S, Maphet P, Printrakul N, Pandith H, Panya A, Kongmali B, Swe MT and Arjinajarn P (2024) *Sphagnum cuspidatulum* extract prevents acute kidney injury induced by high-fat diet and streptozotocin via alleviation of oxidative stress and apoptosis in pre-diabetic rats. *Front. Pharmacol.* 15:1464463. doi: 10.3389/fphar.2024.1464463

COPYRIGHT
© 2024 Laorodphun, Chaisen, Amattat, Maphet, Printrakul, Pandith, Panya, Kongmali, Swe and Arjinajarn. 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.

Sphagnum cuspidatulum extract prevents acute kidney injury induced by high-fat diet and streptozotocin via alleviation of oxidative stress and apoptosis in pre-diabetic rats

Pongrapee Laorodphun[1], Sutheera Chaisen[1], Sarocha Amattat[1], Pornchita Maphet[1], Narin Printrakul[1], Hataichanok Pandith[1], Aussara Panya[1], Burit Kongmali[2], Myat Theingi Swe[3] and Phatchawan Arjinajarn[1]*

[1]Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand, [2]Interdisciplinary Program in Biotechnology, Faculty of Graduate School, Chiang Mai University, Chiang Mai, Thailand, [3]Department of Physiology, University of Medicine 2, Yangon, Myanmar

Context: Obesity and pre-diabetes are associated with renal dysfunction via elevated oxidative stress. Peat moss, or *Sphagnum cuspidatulum* Müll. Hal., Sphagnaceae (SC), are rich in phenolic compounds that enhance antioxidant activity.

Objective: SC might show beneficial effects in pre-diabetes-associated renal dysfunction.

Materials and methods: Male Wistar rats, after 4 weeks on a high-fat diet, received low-dose streptozotocin to induce pre-diabetes. Then, the pre-diabetic rats were randomly divided into 4 groups: untreated pre-diabetic rats (P-DM), pre-diabetic rats treated with SC 50 or 100 mg/kg/day (P-DM50 or P-DM100), and pre-diabetic rats treated with metformin 100 mg/kg/day (MET). The drugs were fed by gavage for 4 weeks.

Results: Treatment with SC100 dramatically lowered serum creatinine (S.Cr.), blood urea nitrogen (BUN), and augmented creatinine clearance in pre-diabetic rats. Additionally, SC100 significantly decreased the malondialdehyde level. Furthermore, pre-diabetic rats treated with SC100 significantly upregulated the expression of nuclear factor erythroid 2-related factor 2 (Nrf2) and its downstream mediators, with downregulated apoptotic markers.

Discussion and conclusion: Our findings provide a scientific basis for the clinical application of SC and a new strategy for the prevention of nephrotoxicity and other kidney disease in the future.

KEYWORDS

peat moss, phenolic compounds, pre-diabetic, renal injury, obesity

Introduction

Obesity is considered a worldwide health burden as it increases the risk of various chronic diseases, including type 2 diabetes (T2D), cardiovascular disease, liver disease, and chronic kidney disease (CKD) (Ahmed et al., 2021). CKD increases the risk of end-stage renal function if left untreated. From a metabolic perspective, the imbalance between the body's energy intake and expenditure is one of the primary variables contributing to obesity. Excessive consumption of a high-fat diet, obesity, and obesity-related kidney diseases are strongly associated with systemic oxidative stress, both in humans and animals (Savini et al., 2013). Reactive oxygen species (ROS) are overproduced in obesity and have a crucial role in the development of T2D and associated consequences (Jaikumkao et al., 2018). Elevated levels of malonaldehyde (MDA) have been seen in patients with obesity, demonstrating a significant association with body mass index (BMI), body fat percentage, low-density lipoprotein (LDL) levels, and triglyceride (TG) levels (Islayem et al., 2022).

Diabetic nephropathy and diabetes associated renal dysfunction are a common complication in T2D. The glomerular filtration barrier, a three-part system consisting of fenestrated endothelial cells, the glomerular basement membrane, and podocytes, deteriorates in response to oxidative stress. Additionally, nephrin, a protein biomarker that reflects podocyte function, becomes downregulated (Drummond and Mauer, 2002; Ponchiardi et al., 2013) whereas excessive energy intake leads to renal injury by promoting abnormal lipid accumulation and triggering epigenetic activation of pro-lipogenic and pro-fibrotic signaling pathways within the renal tissue (Chen et al., 2019). Furthermore, epithelial mesenchymal transition (EMT) and endothelial mesenchymal transition (EndMT) have been well documented to play a crucial role in diabetic renal fibrosis (Liu et al., 2022). Several responsible signaling pathway, i.e., endothelial fibroblast growth factor receptor 1 (FGFR1) (Srivastava et al., 2020; Wu et al., 2018), endothelial Sirtuin3 (SIRT3)-mediated signaling mechanism (Quan et al., 2020), dipeptidyl peptidase-4 (DPP-4)-mediated signaling mechanism (Daza-Arnedo et al., 2021; Hsu et al., 2022), glucocorticoid receptor signaling (Srivastava et al., 2021a) therefore becomes as key target to inhibit renal fibrosis and related kidney injury. Protein kinase C (PKC) plays a pivotal role in the process of signal transduction and the regulation of gene expression (Singh et al., 2017). Its involvement in oxidative stress has been reported recently. In the presence of oxidative stress, the PKC becomes excessively active, leading to the translocation of nuclear factor erythroid 2-related factor 2 (Nrf-2). Nrf-2 subsequently triggers the activation of several antioxidant enzymes, including glutamate-cysteine ligase (GCLC), superoxide dismutase (SOD), and heme oxygenase-1 (HO-1) (Ma, 2013). Moreover, previous studies have indicated that increased production of reactive ROS within cells might exert an impact on the B-cell lymphoma 2 (Bcl-2) protein family and the caspase cascade, hence leading to cellular apoptosis (Arjinajarn et al., 2016).

The landscape of diabetes management is rapidly evolving as traditional treatments have not fully addressed the underlying causes of the disease and are often associated with significant side effects. Currently, the treatment of T2D includes insulin injections and oral hypoglycemic agents, which play key roles in managing the disease but are also associated with adverse effects. Despite advances, several challenges remain, including optimizing treatments for effective glycemic, lipid, and blood pressure control, and addressing safety. Specifically, certain treatments have been employed to manage T2D and mitigate associated kidney injury. Therapies such as SIRT3 activators, glycolysis inhibitors, DPP-4 inhibitors (e.g., linagliptin), and the peptide AcSDKP are increasingly utilized in both clinical practice and research to prevent kidney damage. For example, DPP-4 inhibitors, such as vildagliptin, are approved for the treatment of type 2 diabetes. By inhibiting DPP-4, vildagliptin increases and prolongs GLP-1 levels, enhancing insulin secretion and reducing blood glucose levels (D'Alessio et al., 2009). Previous studies reported that vildagliptin treated in type 1 and 2 diabetic rats showed significant attenuated blood glucose, improved lipid profiles, and also decreased oxidative stress (Ali et al., 2015). A recent meta-analysis reported the beneficial effect of DPP-4 inhibitors including vildagliptin and alogliptin on total cholesterol and triglyceride levels compared to placebo (Monami et al., 2012).

In recent years, natural compounds have become a new strategy to counteract metabolic diseases, and plant-extracted phenolic compounds have become a central position in nutrition research. *Sphagnum cuspidatulum* Müll. Hal., Sphagnaceae (SC) extract has been used as a folk medicine to treat certain diseases in several Asian countries, including Thailand. SC is a semi-aquatic moss that can be found in Aug Ka Doi Inthanon, Chiang Mai, Thailand. It contains many flavonoids and phenolic compounds, which have been traditionally used to treat stroke and chest pain (Chen and Zhang, 2021). Apparently, SC contained high levels of phenolic compounds that can act as an anticancer agent by ameliorating oxidative stress and inhibiting cell division (Sharifi-Rad et al., 2023). Numerous studies have shown that phenolic compounds can prevent and improve several chronic illnesses as they have anti-atherosclerotic, antitumor, antidiabetic, anti-inflammatory, and anticancer properties (Rahman et al., 2021). However, the effects of SC extract on obesity and pre-diabetic-associated renal dysfunction have not been reported yet. Therefore, the present study is designed to investigate the effects and related underlying mechanisms of SC extract on the renal function of obese pre-diabetic rats.

Materials and methods

Sample collection and preparation

Gametophores of *Sphagnum cuspidatulum* were harvested on 5 September 2021 from Ang Ka swamp under Rhododendron (Ericaceae) at about 2,550 m elevation near the summit of Doi Inthanon National Park (Permit number: TS0907.4/29044), Chiang Mai Province, Northern Thailand, 18° 35′ 20″ N and 98° 29′ 10″ E. The voucher specimens (Printarakul 05092021_5) were housed in the CMUB herbarium, Biology Department, Faculty of Science, Chiang Mai University, Thailand. The sample was extracted using absolute methanol (AR grade, RCI Labscan, Bangkok, Thailand) for 24 h at room temperature three times, at a ratio of 1:10 w/v (Salih et al., 2021). The solution was purified prior to being used in combination with Whatman No. 1 (Cytiva, Marlborough,

Massachusetts, United States) filter paper. Using a rotatory evaporator, the solvent was eliminated, and it was then dried in a fume hood. Then, until use, the crude extract was kept in an amber bottle at −20°C.

Preparation of caffeine from *Sphagnum cuspidatulum*

To prepare the peat moss sample exhibiting the highest quantity of caffeine, the procedure performed by Antioxidant Activities (AAs) was utilized. The first step was an optimization process to determine the most effective extraction conditions for caffeine from peat moss. Once these ideal conditions were established, they were then utilized in the subsequent experiments. The extraction method was carried out over a period of 3 h at room temperature, with constant agitation at a rate of 150 rpm. Following that, the mixture was filtered and evaporated under decreased pressure at a temperature of 40°C. The procedure led to the extraction of the aqueous residual fraction from the whole-stem peat moss. Subsequently, the fraction underwent evaporation to remove all liquid content and was subjected to fractionation using solid-phase extraction. This process involved utilizing a 75 × 8 cm Amberlite XAD7HP column. Subsequently, the fraction was subjected to defatting by partitioning with hexane, leading to the formation of a fraction enriched in caffeine.

Determination of total polyphenolic compounds and identification of bioactive compounds in crude extract

The Folin-Ciocalteu method, was used for the quantitative measurement of polyphenolic chemicals. The crude extract was solubilized in a solution consisting of 80% methanol. Subsequently, a mixture was prepared by combining 60 µL of the crude extract with 2.5 mL of Folin-Ciocalteu's phenol reagent. Following a period of incubation in the absence of light for a duration of 10 min, a 2 mL volume of sodium carbonate (Na_2CO_3) solution with a concentration of 7.5% was introduced to the solution. Subsequently, the solution was subjected to incubation at a temperature of 50°C for a duration of 15 min, until the completion of the reaction. The measurement of absorbance was determined using a spectrophotometer set at a wavelength of 760 nm. The gallic acid was produced as a standard reagent at concentrations of 25, 50, 100, 200, and 400 µg/mL.

The bioactive chemicals were analyzed using the gas chromatography-mass spectrometry (GC-MS) technique, which involved the utilization of an Agilent Technologies 7890b gas chromatograph and an MSD 5977B mass spectrometer. Initially, a 1 µL sample dissolved in 80% methanol was introduced into a GC-MS system. The injection was performed using the split (10:1) inlet mode. The sample was then separated using a non-polar phenyl arylene polymer column (DB-5MS, 30 m × 0.25 mm, 0.5 µm film thickness) with a temperature gradient. The gradient started at 0°C and increased at a rate of 4°C/min until reaching 100°C, where it was held for 10 min. Subsequently, the temperature was further increased at a rate of 4°C/min from 100°C to 270°C and held for 20 min. Finally, the temperature was raised from 270°C to 280°C at a rate of 4°C/min and held for 20 min. Helium gas was used as the carrier gas at a flow rate of 1.9 mL/min. The identification of bioactive chemicals was conducted using a mass spectrometry (MS) instrument operating in electron impact mode. The mass range analyzed was m/z 25–700, and an electron energy of 70 eV was utilized. The mass spectra were discovered using a comparative analysis with the Wiley and NIST libraries.

The samples that underwent sialylation were introduced into a GC-MS system consisting of an MSD 5977B mass spectrometer and a 7890b gas chromatograph, both manufactured by Agilent Technologies in Shanghai, China. The configuration of the system involved operating in the Electron Impact (EI) mode, utilizing a mass range spanning from m/z 25 to 700 with an electron energy of 70 electron volts (eV). The experiment utilized a capillary column DB-5MS with dimensions of 30 m × 0.25 mm (i.d.) and a coating substance with a film thickness of 0.5 µm. Both the detector and injector were set to a temperature of 280°C. GC was performed in splitless mode with a 1-min splitless duration. The temperature protocol involved a gradual increase in temperature from 50°C to 100°C at a rate of 4°C per min. This temperature was then maintained for a duration of 10 min. Subsequently, the temperature was further increased from 100°C to 270°C at the same rate of 4°C per min and held at 270°C for a period of 20 min. Finally, the temperature was raised from 270°C to 280°C at a rate of 4°C per min and maintained at 280°C for an additional 20 min. A post-run duration of 10 min at a temperature of 50°C was found to be enough for the second injection. The carrier gas, helium, maintained a constant flow rate of 1.9 mL/min. Compounds were identified by means of comparing their retention periods with those of authentic compounds and utilizing the spectrum information available in the Wiley and NIST libraries. The process of decision-making was replicated.

Animals

The male Wistar rats, with an average weight ranging from 180 to 200 g, were obtained from Nomura Siam International, a supplier based in Bangkok, Thailand. The animals require 7 days to adapt to their unusual surroundings. The rats utilized as experimental subjects were housed in a controlled environment with certain conditions. These conditions included a constant temperature of 25°C, a humidity level of 55%, and a 12 h light/dark cycle. During the acclimatization phase, the rats were given full access to a regular pellet diet and distilled water. The Laboratory Animal Center at Chiang Mai University in Chiang Mai, Thailand, has provided approval (Permit Number: 2565/RT-0025) for the animal facilities and procedures used during this work.

Experimental design

The rats were randomly allocated to two dietary groups: the normal diet (ND) group and the high-fat diet (HFD) group. In the normal diet group, a total of 16 rats were administered the ordinary chow diet (C.P. Mice Feed Food No. 082), which included 19.77% of

calories derived from fat. The rats in the high-fat diet groups (n = 32) were administered a food with a high-fat content, comprising 57.60% of their calorie intake. Prior to inducing diabetic impairment, rats were maintained on a high-fat diet for a duration of 4 weeks. To induce T2D, rats were administered a high-fat meal and afterwards received an intraperitoneal injection of nicotinamide (120 mg/kg), followed by a low dosage of streptozotocin (STZ) (40 mg/kg). In contrast, the control group of animals received citrate buffer instead. The rats that were fed with ND were divided into two groups consisting of 8 rats each. One group received a vehicle, while the other group received a SC extract (ND100) at a dosage of 100 mg/kg/day. This division was done after conducting an oral glucose tolerance test (OGTT) to confirm the validity of the model. The rats that were given a high-fat diet were then divided into four groups consisting of 8 rats each. These groups were as follows: pre-diabetic rats treated with normal saline (NSS) (referred to as P-DM), pre-diabetic rats treated with an extract from SC (referred to as P-DM50; administered at a dosage of 50 mg/kg/day), pre-diabetic rats treated with an extract from SC (referred to as P-DM100; administered at a dosage of 100 mg/kg/day), and pre-diabetic rats treated with metformin (referred to as MET). The dosage of SC extract was chosen based on a previous study (Afolabi et al., 2023) in which the caffeine levels used were either equal to or lower than those in our study. The study demonstrated that such caffeine dosages provided beneficial effects without causing any toxicity effect in a rat model of aluminium chloride (AlCl3) induced nephrotoxicity. Oral administration of SC extract and metformin was conducted for a duration of 4 weeks using a gavage technique, after the immediate dissolution in NSS.

The energy intake was calculated using equations:

$$\text{Energy intake of ND (Kcal/day)} = \text{food intake (g/day)} \times \text{constant of standard chow (4.2 Kcal/g)}$$

$$\text{Energy intake of HF (Kcal/day)} = \text{food intake (g/day)} \times \text{constant of high} - \text{fat diet (5.35 Kcal/g)}$$

Oral glucose tolerance test (OGTT)

The administration of the OGTT took place in the fourth and eighth weeks. The rats were subjected to gavage administration of a glucose solution (2 g/kg) after a period of fasting overnight. Blood samples were obtained from the distal end of the tail at various time intervals, encompassing the pre-glucose loading phase (0 min) as well as the post-glucose injection periods of 30, 60, and 120 min, during which the subjects were anesthetized. Colorimetric test kits (Erba Diagnostics Mannheim GmbH, Mannheim, Germany) were utilized to measure blood glucose levels. Following that, the evaluation of impaired glucose tolerance was performed using the trapezoidal formula, which entailed calculating the area under the curve for glucose levels during the OGTT, generally known as TAUCg.

Blood and renal tissue sampling

The collection of 24 h urine samples was conducted before each OGTT trial, utilizing individual metabolic cages. The animals were subjected to euthanasia by the administration of an excessive amount of the anesthetic 2% isoflurane (Hawkley et al., 2024), and thereafter blood samples were obtained from the abdominal aorta. The serum and plasma samples underwent separation prior to use and were then kept at a temperature of −20°C. The kidneys were promptly removed, devoid of their capsules, and assessed in terms of their weight. For histological study, the kidney was partitioned into two segments. The initial portion of the experiment involved submerging the sample in a 4% solution of recently made paraformaldehyde at a pH level of 7.4 to achieve fixation. The last portion of the experiment involved meticulous partitioning to separate the renal cortex, which was then preserved at a temperature of −80°C. The preserved cortical tissue will be employed in future research that encompasses Western blotting as well as the examination of tissue MDA levels, glutathione (GSH), and reducing glutathione (GSSG) content.

Blood parameter assessment

A colorimetric test kit was used to quantify the concentrations of triglyceride, cholesterol, and fasting plasma glucose (Erba Diagnostics Mannheim GmbH, Mannheim, Germany). Plasma HDL was determined by using an automated chemical analyzer (Sysmex BX-3010, Gobe, Japan). The LDL was calculated using the standard formula.

$$\text{LDL} - \text{c} = \text{total cholesterol} - \text{HDL} - (\text{triglyceride}/5)$$

Renal function assessment

Serum creatinine and blood urea nitrogen (BUN) levels were measured using an automated analyzer (Sysmex BX-3010, Gobe, Japan). The creatinine clearance was calculated using the standard formula.

$$C = UV/P$$

where C was creatinine clearance, U was the urine concentration of creatinine in mg/dL, V was the urine flow rate per min in mL/min, and P was the plasma concentration of creatinine in mg/dL.

Determination of renal oxidative stress

A thiobarbituric acid reactive substances (TBARS) test kit was used to measure the amount of MDA in the renal cortical tissue (Cayman Chemical Company, Ann Arbor, Michigan, United States). A commercial assay kit was used to measure the level of GSH/GSSG in the renal cortex (EGTT-100, Bioassay Systems, Hayward, California, United States) according to the manufacturer's instructions.

Western blotting

Renal cortical tissues were subjected to the normal procedure for Western blotting. Kidney cortex samples were cut into slices and

mixed with mammalian lysis solution from Sigma-Aldrich (Sigma-Aldrich, St. Louis, Missouri, United States) to get whole-cell kidney lysate that also contained a protease inhibitor (Roche, Indianapolis, Indiana, United States). This procedure was conducted in a centrifuge at 10,000 *g* for 15 min at 4°C. Equal volumes of protein samples were loaded into 10 or 12% of sodium dodecyl sulfate-polyacrylamide gels (SDS-PAGE), then electrophoresed and transferred to 0.2 μm polyvinylidene fluoride (PVDF) membranes (Bio-Rad, Pennsylvania, United States). Following this, membranes were blocked with 5% BSA in PBS or TBS for an hour at room temperature. The primary antibodies used were anti-Bax (Cat# ab182733), Bcl-2 (Cat# ab194583), HO-1 (Cat# ab13248), GCLC (Cat# 13475), and nephrin (Cat# ab216341), antibodies originated from Abcam (Abcam, Cambridge, United Kingdom). The antibodies targeting PKC-α (Cat# sc-208) and Nrf-2 (Cat# 33649) were acquired from Santa Cruz Biotechnology (Santa Cruz, California, United States). The SOD2 (Cat# 13149), α-SMA (Cat# 19245), Cytochrome C (Cat# 4272) and, TGF-β (Cat# 3711) antibodies were purchased from Cell Signaling Technology (Cell Signaling Technology, Massachusetts, United States). The membranes were probed with these primary antibodies at 4°C overnight. After that, membranes were rinsed and incubated with an HRP-conjugated anti-mouse or anti-rabbit secondary antibody (Amersham, Illinois, United States) for an hour at room temperature. Thereafter, proteins were detected by enhanced chemiluminescence agent (ECL) and Chemi Doc imaging systems (Image Quant LAS500, GE Healthcare Limited, Buckinghamshire, United Kingdom). The densities of the bands were analyzed using ImageJ software (National Institutes of Health, Bethesda, Maryland, United States). As a loading control, the anti-β-actin (Cat# 5125) antibody was utilized.

Histological examination

The kidneys were prepared for preservation by immersing them in a 4% solution of fresh paraformaldehyde (pH 7.4) for a duration of 24 h. Subsequently, the preserved kidneys were embedded in paraffin to perform histological analyses. The kidneys were sectioned into slices with a thickness of 5 micrometers. Subsequently, these slices were subjected to counterstaining using hematoxylin and eosin (H&E) and examined using an Olympus light microscope (Olympus America Inc., New York, United States). (40 × magnification) by an observer, who was blinded to the treatment groups. The evaluation of the kidney injury score was conducted based on the subsequent semi-quantitative scoring system (Laorodphun et al., 2021). The staining techniques utilized in this study were periodic acid-Schiff (PAS) and Masson's trichrome. These stains were applied to kidney slides to assess the presence of glycogen buildup, determine the glomerulosclerotic index, and examine the deposition of collagenous connective tissue fibers.

Statistical analysis

The statistical analysis was conducted with GraphPad Prism version 8 (Graph Pad Software, La Jolla, California, United States). The data were presented using the mean ± standard error of the mean (SEM) format. The statistical methods applied in this study encompassed the application of an independent sample t-test was used to compare the differences between two groups and analysis of variance (ANOVA) with Fisher's Least Significant Difference (LSD) *post hoc* tests. These approaches were performed to evaluate the statistical significance of the observed disparities. Statistical significance was determined using a significance threshold of $p < 0.05$.

Results

Bioactive compounds in crude extract

The crude extract of *Sphagnum cuspidatulum* was characterized to determine the total phenolic content and identified the bioactive compounds. The result showed that *S. cuspidatulum* exhibited a high concentration of polyphenolic chemicals, with a measured content of 18.1083 mg GAE/mL using the Folin-Ciocalteu technique. The gas chromatography/mass spectrometry (GC-MS) approach successfully detected six substances, namely, propanoic acid, 2-oxo-methylester, 3,4-dimethyl-3-pyrrolin-2-one, 2-hydroxymethyl-2-methyl-pyrrolidine-1-carboxaldehyde, 1,1,3,3-tetramethy-1,3-disilaphenalane, and 1,3,7-trimethylpurine-2,6-dione, which is also known as caffeine (Figure 1). The primary bioactive molecule, caffeine, was shown to have the greatest peak height on the chromatogram, as evidenced by its detection at a retention time of 37.92 min.

The effect of HF diet and low does STZ on metabolic and renal parameters at 4th week

Table 1 displays the metabolic parameters observed at the fourth week. Before the initiation of the SC extract therapy, the HF groups exhibited substantially elevated levels of body weight, energy intake, plasma glucose, total area under the curve for glucose (TAUCg), plasma cholesterol, and plasma LDL compared to the ND ($p < 0.05$) (Table 1). Significant reductions in HDL levels, urine creatinine and creatinine clearance were seen in HF group in comparison to the ND group ($p < 0.05$). It was also observed that the HF groups demonstrated significantly increased levels of serum creatinine and urine protein than the ND group ($p < 0.05$). However, there was no observable disparity among the experimental groups with regards to the blood urea nitrogen (BUN) level and urine volume. These findings indicate that the administration of a high-fat diet alongside a moderate dose of streptozotocin (STZ) resulted in the manifestation of pre-diabetic condition and a deterioration in renal function.

Effect of SC extract on metabolic status

After 8 weeks, it was observed that the P-DM group exhibited notably elevated levels of body weight, energy intake, fasting plasma glucose, total area under the curve for glucose (TAUCg), plasma cholesterol, plasma triglyceride, low-density lipoprotein (LDL), and visceral fat weight. The statistical significance ($p < 0.05$) was seen in comparison to the ND and ND100 groups (Table 2). When P-DM

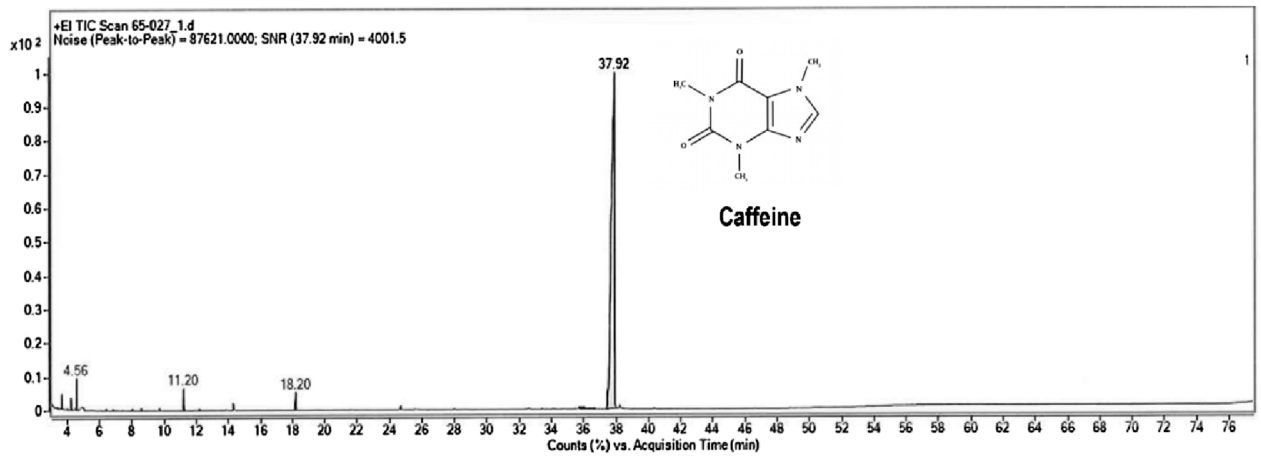


FIGURE 1
Gas chromatogram with mass-spectrometric detection of the total lipid extract of *Sphagnum cuspidatulum* (SC).

TABLE 1 Metabolic parameters in male Wistar rats at 4 weeks of high-fat diet consumption (Before treatment).

	ND	HF
Body weight (g)	441.42 ± 4.98	482.08 ± 5.18*
Energy intake (Kcal/day)	88.84 ± 2.73	108.20 ± 2.47*
Fasting blood glucose (mg/dL)	113.38 ± 1.58	145.12 ± 3.41*
TAUCg (mg/dL x min x 10^3)	16.57 ± 0.20	20.28 ± 0.25*
Cholesterol (mg/dL)	55.20 ± 2.04	114.47 ± 3.14*
Triglyceride (mg/dL)	18.70 ± 1.38	24.50 ± 1.61*
HDL (mg/dL)	48.10 ± 1.06	33.50 ± 0.57*
LDL (mg/dL)	16.34 ± 2.51	66.64 ± 3.37*
S.Cr (mg/dL)	0.550 ± 0.005	0.617 ± 0.005*
BUN (mg/dL)	12.91 ± 0.50	15.00 ± 0.77
U.Cr (mg/dL)	88.73 ± 2.41	68.49 ± 4.85*
Creatinine clearance (mL/min)	1.85 ± 0.07	1.21 ± 0.08*
U.Protein (g/dL)	111.79 ± 4.11	170.83 ± 6.06*
24-h U.Volume (mL/day)	19.60 ± 0.72	19.40 ± 1.14

Data are presented as mean ± standard error of mean (SEM) ND, normal diet rats treated with vehicle, n = 10 rats/group (t-test, $p < 0.05$). HF, high-fat diet rats, n = 20 rats/group (t-test, $p < 0.05$).

was contrasted with the ND and ND100 groups, HDL was lower in P-DM ($p < 0.05$). Interestingly, the P-DM100 rats that received SC extract at doses of 100 mg/kg/day showed a significant improvement in metabolic parameters as measured by fasting plasma glucose, TAUCg, and HDL levels. Additionally, in P-DM100 groups, visceral fat weight, plasma cholesterol, and plasma LDL exhibited a declining tendency. Rats that were fed a high-fat diet exhibited reduced 24 h urine volume and creatinine clearance, along with increased kidney weight and elevated levels of serum creatinine and BUN in comparison to ND and ND100 ($p < 0.05$). After receiving SC treatment, serum creatinine was considerably lower in the P-DM50 and P-DM100 groups compared to the P-DM group. In contrast, when compared to P-DM, the P-DM100 group showed a considerably greater increase in creatinine clearance ($p < 0.05$) (Table 2). The 24 h urine volume was improved in the SC treated groups when compared with pre-diabetic rats ($p < 0.05$).

Effects of SC extract on renal oxidative stress

Malondialdehyde (MDA) is a byproduct of lipid peroxidation, serving as a biomarker for oxidative stress in obese rats subjected to a high-fat diet. The MDA in our P-DM group exhibited a statistically significant increase in comparison to both the ND and

TABLE 2 Metabolic parameters and renal functions in male Wistar rats at 8 weeks (After treatment).

	ND	ND100	P-DM	P-DM50	P-DM100	MET
Body weight (g)	474.53 ± 30.45	473.71 ± 5.09	474.12 ± 25.35*	537.26 ± 16.96*	521.02 ± 17.03	551.01 ± 15.23*
Energy intake (Kcal/day)	78.13 ± 4.46*	74.54 ± 2.52	122.29 ± 0.84*	112.50 ± 2.13*	104.90 ± 1.74*	116.40 ± 0.91*
Fasting blood glucose (mg/dL)	101.2 ± 1.69	99.0 ± 1.52	188.6 ± 31.56*	133.0 ± 1.22†	126.2 ± 2.29†	127.46 ± 4.04†
TAUCg (mg/dL × min × 10^3)	15.25 ± 0.15	15.29 ± 0.24	21.17 ± 0.73*	18.79 ± 0.55*,†	17.29 ± 0.46*,†,‡	16.79 ± 0.64*,†,‡
Cholesterol (mg/dL)	63.03 ± 5.84	60.28 ± 9.97	122.52 ± 19.02*	98.51 ± 9.06*	85.29 ± 9.54†	60.72 ± 4.40†
Triglyceride (mg/dL)	24.92 ± 1.78	27.4 ± 1.48	66.63 ± 4.49*	54.51 ± 5.28*	49.16 ± 6.26*	47.92 ± 11.43*,†
HDL (mg/dL)	46.0 ± 2.41	49.4 ± 2.96	39.4 ± 1.77	45.4 ± 2.75	53.4 ± 6.24†	42.8 ± 3.60†
LDL (mg/dL)	22.21 ± 5.09	19.30 ± 3.04	66.34 ± 14.62*	38.05 ± 8.06†	32.35 ± 12.66†	19.49 ± 1.21†
S.Cr (mg/dL)	0.65 ± 0.002	0.67 ± 0.002	0.80 ± 0.03*	0.73 ± 0.02†	0.71 ± 0.03*,†	0.66 ± 0.01†,‡
BUN (mg/dL)	13.46 ± 0.56	14.66 ± 1.14	26.54 ± 2.25*	13.62 ± 0.89†	14.02 ± 2.16†	13.92 ± 1.0†
U.Cr (mg/dL)	113.59 ± 4.59	113.47 ± 4.03	52.14 ± 17.76*	88.81 ± 4.87†	103.50 ± 13.82†	111.60 ± 10.81†
Creatinine clearance (mL/min)	1.85 ± 0.23	1.90 ± 0.35	1.07 ± 0.03*	1.35 ± 0.12	1.58 ± 0.05†	1.51 ± 0.04
U.Protein (g/dL)	0.137 ± 0.008	0.129 ± 0.021	0.196 ± 0.019*	0.172 ± 0.012	0.148 ± 0.010	0.144 ± 0.015
24-h U.Volume (mL/day)	28.8 ± 2.58	27.4 ± 3.31	13.2 ± 1.66*	18.2 ± 2.06*	16.0 ± 2.07*	21.8 ± 4.09†
Kidney weight (g)	1.37 ± 0.04	1.36 ± 0.01	1.52 ± 0.06*	1.39 ± 0.02†	1.28 ± 0.02†,‡	1.36 ± 0.02†
Kidney weight/Body weight ratio	2.75 ± 0.05	2.85 ± 0.04	3.19 ± 0.43	2.35 ± 0.03†	2.36 ± 0.13†	2.54 ± 0.03†
Visceral fat (g)	10.76 ± 2.73	8.69 ± 1.11	24.22 ± 3.27*	19.93 ± 0.37*,†	16.47 ± 2.06*,†	19.32 ± 0.86*

Data are presented as mean ± standard error of mean (SEM, n = 5 rats/group) ND, normal diet rats treated with vehicle; ND100, normal diet rats treated with peat moss extract 100 mg/kg/day; P-DM, pre-diabetic rats treated with vehicle. P-DM50, pre-diabetic rats treated with peat moss extract 50 mg/kg/day; P-DM100, pre-diabetic diet rats treated with peat moss extract 100 mg/kg/day; MET, pre-diabetic diet rats treated with metformin 100 mg/kg/day. Values with different superscript letters *$p < 0.05$ vs. ND, and ND100; †$p < 0.05$ vs. P-DM; ‡$p < 0.05$ vs. P-DM50 (LSD, test, $p < 0.05$).

ND100 groups ($p < 0.05$) (Figure 2A). Following treatment with SC extract, the concentrations of MDA exhibited a statistically significant reduction in both the P-DM50 and P-DM100 groups ($p < 0.05$). Renal cortical level of antioxidant GSH was significantly lower in the P-DM group compared to the ND and ND100 groups ($p < 0.05$) (Figure 2B). In contrast, the GSSG level was substantially increased in the P-DM group compared to the ND and ND100 groups ($p < 0.05$) (Figure 2C). The GSH level increased and GSSG levels decreased in the P-DM100 group after SC treatment ($p < 0.05$) which indicated that SC have the potential to scavenge ROS and reduce oxidative stress. The ratio of GSH/GSSG in the P-DM condition exhibited a significant decrease compared to the ND and ND100 groups ($p < 0.05$). Interestingly, these parameters were reversed following the administration of SC therapy (Figure 2D). The findings of our study indicate that there is a disparity in the effectiveness of SC on metabolic profiles when comparing the dosage of 50 mg/kg BW to that of 100 mg/kg BW. In the next experiments, our research will concentrate on investigating the advantageous impacts of SC extract administered at a dosage of 100 mg/kg body weight in the context of obesity.

Effects of SC extract on renal oxidative defense mechanism

A significant elevation in PKC-α protein expression was noted in the renal cortical tissue of rats subjected to a high-fat diet in comparison to rats on a normal diet that were in the ND and ND100 groups ($p < 0.05$), as depicted in Figure 2E. In contrast, the levels of Nrf2, SOD, HO1, and GCLC expression in the obese rats that did not receive any treatment were found to be considerably lower compared to the groups of rats that were given a regular diet ($p < 0.05$), as seen in Figures 2F–I. The results of this study indicate that the consumption of a high-fat diet leads to the activation of oxidative stress through the Nrf-2 pathway. The levels of Nrf-2, SOD, and GCLC expression, but not HO-1, were found to be considerably increased in the P-DM100 group compared to the P-DM group ($p < 0.05$).

Effects of SC extract on renal morphology

The representative images of H&E staining (Figure 3) demonstrate the infiltration of macrophages, renal tubular dilation, the area of Bowman's capsule, periglomerular fibrosis, and interstitial fibrosis. SC extract significantly attenuated renal histopathological injuries and the semi-quantitative kidney injury score in P-DM100 when compared with the P-DM group (Figures 3A, B) ($p < 0.05$). In addition, the P-DM group showed significantly reduced nephrin and podocin expression compared to the ND group (Figures 3C, D). These findings suggested that the oral administration of SC extract could improve the renal histopathological injuries induced by pre-diabetic conditions.

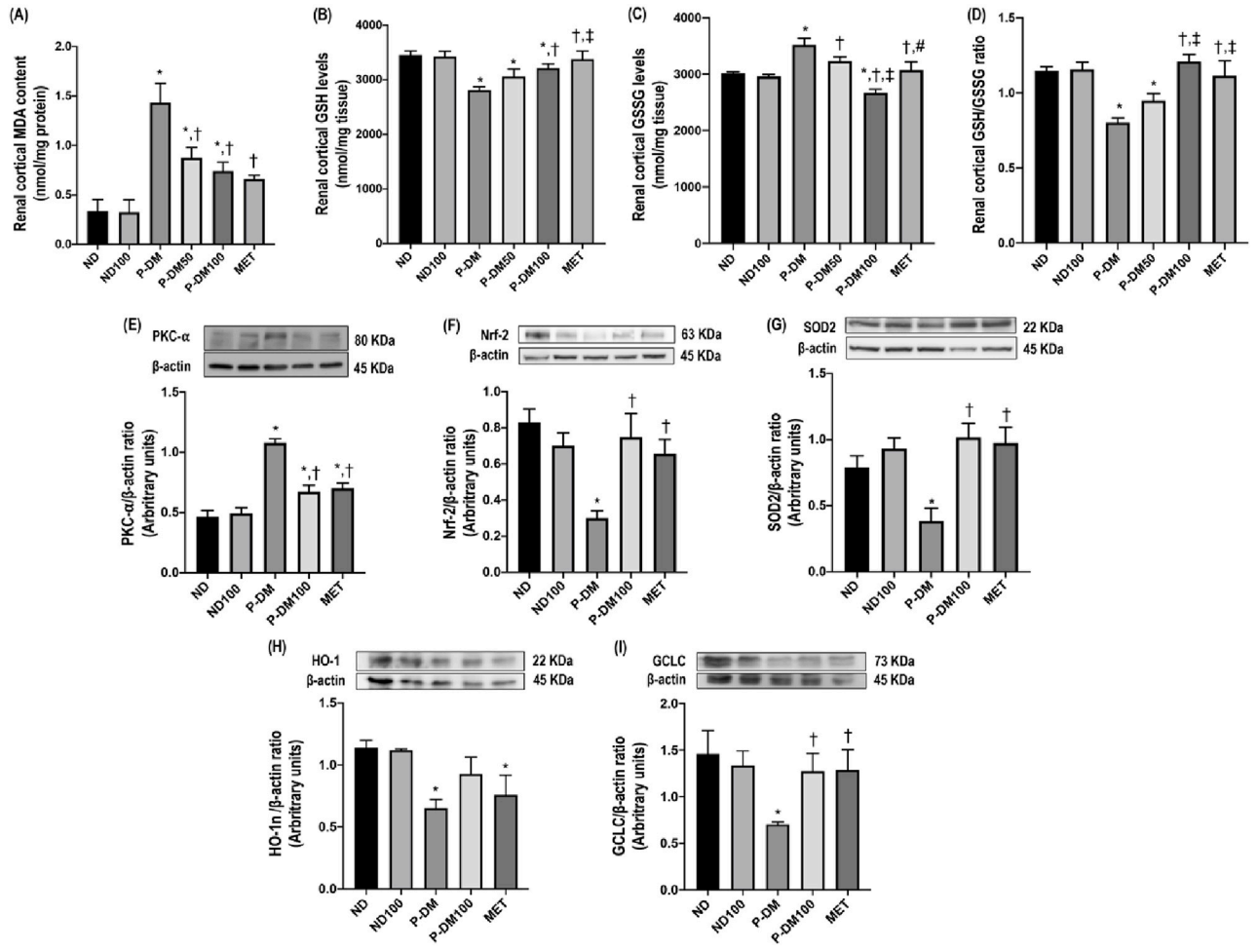


FIGURE 2
Effect of SC extract on renal oxidative stress. Renal cortical MDA content **(A)**, renal cortical GSH levels **(B)**, renal cortical GSSG levels **(C)**, renal cortical GSH/GSSG ratio **(D)**, renal cortical expression of PKC-α **(E)**, Nrf-2 **(F)**, SOD2 **(G)**, HO-1 **(H)**, and GCLC **(I)**. ND, normal diet rats treated with vehicle; ND100, normal diet rats treated with peat moss extract 100 mg/kg/day; P-DM, pre-diabetic rats treated with vehicle; P-DM50, pre-diabetic rats treated with peat moss extract 50 mg/kg/day; P-DM100, pre-diabetic diet rats treated with peat moss extract 100 mg/kg/day; MET, pre-diabetic diet rats treated with metformin 100 mg/kg/day. Values with different superscript letters *$p < 0.05$ vs. ND and ND100; †$p < 0.05$ vs. P-DM; ‡$p < 0.05$ vs. P-DM50 indicate significant differences between treatments (LSD test, $p < 0.05$).

Effects of SC extract on renal fibrosis

The kidney sections taken from P-DM rats had significantly elevated levels of glomerulosclerosis, as proven by the presence of collagenous connective tissue deposits in both the glomerular and tubular regions, as observed by staining with PAS and Masson's trichrome. This observation contrasted with the kidney sections collected from ND rats (Figure 4A). The assessment of glomerulosclerotic index and fibrotic area were significantly increased in the P-DM group compared to the ND group ($p < 0.05$) (Figures 4B, C). In addition, the P-DM rats showed significant increases in the expression of TGF-β and α-SMA in comparison to the ND rats ($p < 0.05$) (Figures 4D, E). Rats in the P-DM100 group had a significantly lower glomerulosclerotic index and fibrotic area, together with reduced TGF-β and α-SMA expression, than HF rats ($p < 0.05$).

Effects of SC extract on renal apoptotic pathway

As shown in Figures 5A–C, high-fat diet consumption significantly increased Bax and cytochrome C expressions and markedly reduced Bcl-2 expression in renal tissues in P-DM when compared to the ND group ($p < 0.05$). SC treatment significantly downregulated the expression of Bax and cytochrome C while upregulating the expression of Bcl-2 in comparison with P-DM rats ($p < 0.05$).

Discussion

To our knowledge, this is the first study to suggest that peat moss (*Sphagnum cuspidatulum*) extract mitigates renal impairment

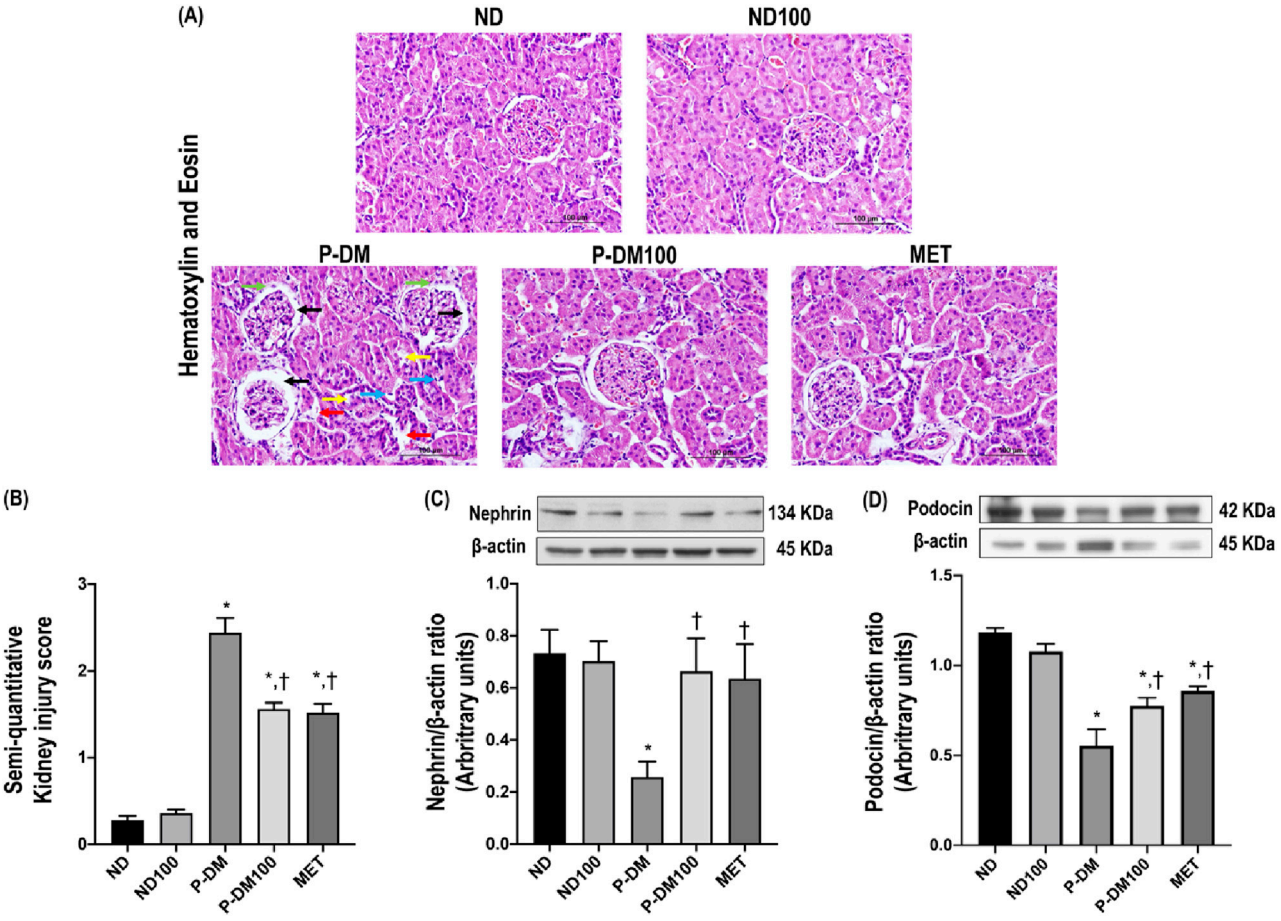


FIGURE 3
Effect of SC extract on renal morphology. Photomicrograph histological section of kidney using hematoxylin and eosin (H&E) stain (×40) in obese rats **(A)**: Dilation of Bowman's capsule (black arrow), interstitial fibrosis (light blue arrow), macrophage infiltration (red arrow), periglomerular fibrosis (green arrow) and tubular dilation (yellow arrow), Semi-quantitative kidney injury score **(B)**, renal cortical expression of nephrin **(C)**, podocin **(D)**. ND, normal diet rats treated with vehicle; ND100, normal diet rats treated with peat moss extract 100 mg/kg/day; P-DM, pre-diabetic rats treated with vehicle; P-DM50, pre-diabetic rats treated with peat moss extract 50 mg/kg/day; P-DM100, pre-diabetic diet rats treated with peat moss extract 100 mg/kg/day; MET, pre-diabetic diet rats treated with metformin 100 mg/kg/day. Values with different superscript letters *$p < 0.05$ vs. ND and ND100; †$p < 0.05$ vs. P-DM; ‡$p < 0.05$ vs. P-DM50 indicate significant differences between treatments (LSD test, $p < 0.05$).

induced by pre-diabetes via antioxidative and anti-apoptotic capabilities. The traditional use of SC extract as a remedy for various ailments has been observed in multiple Asian countries, including Thailand. SC is a semi-aquatic moss that can be found in Aug Ka Doi Inthanon, Chiang Mai, Thailand. It contains plenty of caffeine, flavonoids, and phenolic compounds. Caffeine is a phenolic phytochemical that acts as a powerful natural antioxidant, offering various beneficial effects for humans.

This study demonstrated that the administration of a high-fat meal to rats, along with a low dosage of STZ injection, leads to several detrimental effects. These effects include increased body weight and visceral fat weight, impaired glucose tolerance, dyslipidemia, heightened oxidative stress, renal cell apoptosis, and renal dysfunction. Impaired glucose and fat metabolism elicits oxidative stress and the suppression of the oxidative defense mechanism via the Nrf-2 pathway. This imbalance between oxidant and antioxidant machinery, in turn, contributes to the development of renal tissue damage. In addition, pre-diabetic conditions might lead to renal cell death by upregulating the apoptotic proteins Bax and cytochrome C while simultaneously downregulating the expression of the anti-apoptotic protein Bcl-2.

The administration of phenolic compounds to rats subjected to a high-fat diet leads to a decrease in plasma glucose levels and an improvement in lipid metabolism (Hanhineva et al., 2010). The results from our study also demonstrate that phenolic compounds obtained from SC extract effectively reduce plasma glucose levels in P-DM rats.

The visceral fat accumulates around the kidneys in obese pre-diabetic rats. The physical compression of the kidney via perirenal adipose mass may reduce renal tubular flow in the distensible loop of Henle, which leads to an increase in glomerular filtration rate (GFR) (Kataoka et al., 2023). Similar to a previous study, this study also demonstrates that the accumulation of visceral fat is associated with elevated GFR and blood creatinine levels (Gerchman et al., 2009). Nephrin and podocin are important proteins of the glomerular filtration barrier. In our study, P-DM rats had decreased expression

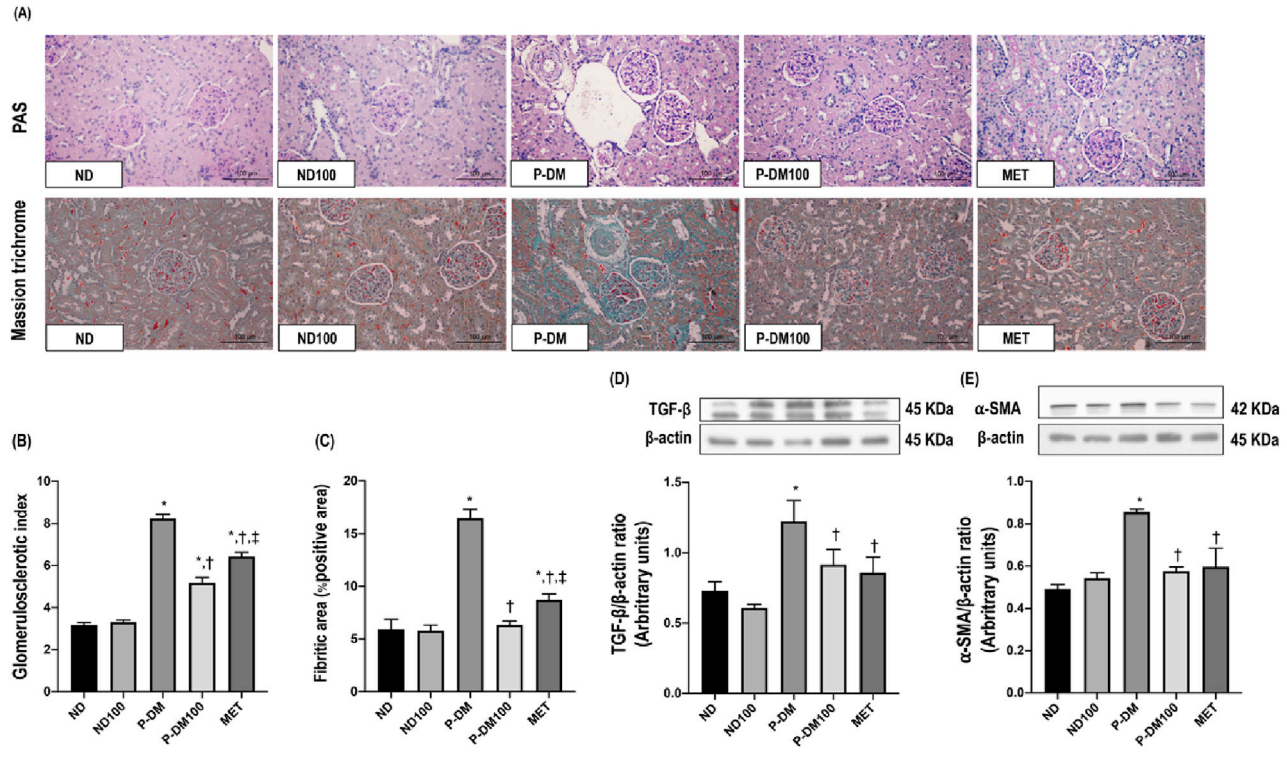


FIGURE 4
Effect of SC extract on renal fibrosis. Histological sections of kidneys stained with PAS and Masson's trichrome (×40) **(A)**, Glomerulosclerotic index **(B)**, Fibrotic area **(C)**, Western blot analysis showing the renal expression of TGF-β **(D)**, and α-SMA **(E)**. ND, normal diet rats treated with vehicle; ND100, normal diet rats treated with peat moss extract 100 mg/kg/day; P-DM, pre-diabetic rats treated with vehicle; P-DM50, pre-diabetic rats treated with peat moss extract 50 mg/kg/day; P-DM100, pre-diabetic diet rats treated with peat moss extract 100 mg/kg/day; MET, pre-diabetic diet rats treated with metformin 100 mg/kg/day. Values with different superscript letters $^{*}p < 0.05$ vs. ND and ND100; $^{\dagger}p < 0.05$ vs. P-DM; $^{\ddagger}p < 0.05$ vs. P-DM50 indicate significant differences between treatments (LSD test, $p < 0.05$).

of renal cortical nephrin and podocin, which was reversed by the administration of SC extract.

Hyperlipidemia contributes to the gradual decline of renal function as a result of oxidative stress, endoplasmic reticulum stress, inflammatory reactions, and the restricted proliferative capacity of glomerular and mesangial cells (Chen et al., 2023). In this study, we observed the histological changes in the renal tissues of pre-diabetic rats, including macrophage infiltration, dilatation of Bowman's capsule, and fibrosis in both the glomerular and tubular regions. TGFβ and αSMA are the important proteins involved in the processes of glomerulosclerosis and tubulointerstitial fibrosis, which are the common pathological features of kidney diseases. We observed that SC extract reduced the expression of TGFβ and αSMA in the P-DM rats. Our study was confirmed by another experiment, caffeine can activate AMPK and SIRT3 that inhibits several fibrogenic pathways (Xu et al., 2020), including those mediated by TGF-β, a key player in kidney fibrosis can cause partial epithelial to EMT and EndMT. This inhibition can reduce the production of fibrotic extracellular matrix components (Srivastava et al., 2021b).

The presence of heightened oxidative stress has a pivotal role in the development of nephropathy in individuals with pre-diabetes (Vodošek Hojs et al., 2020). A chronic state of hyperglycemia augments the generation of ROS, inducing oxidative stress (González et al., 2023). The elevation in MDA levels, which is regarded as a marker of oxidative stress (Jaikumkao et al., 2018; Swe et al., 2019), seen in pre-diabetic rats in our study. To mitigate the effects of oxidative stress, cells employ a detoxification mechanism, which includes the Nrf-2 pathway. This system serves as a key regulator of cytoprotective responses in the face of oxidative stress (Ngo and Duennwald, 2022). Under physiological circumstances, Nrf-2 is typically confined to the cytosol, where it forms a complex with the Kelch-like ECH-associated protein 1 (Keap1). The translocation of Nrf-2 to the nucleus is induced by the ubiquitination and proteasomal degradation of Keap1 in the presence of ROS. This translocation facilitates the translation of enzymes associated with antioxidant activity (Baird et al., 2020) such as SOD, GCLC, HO-1 and, GSH. The GSH and GSSG exhibit exceptional sensitivity to variations in ROS flux caused by alterations in the glutathione reservoir in reaction to elevated levels of oxidative stress (Mailloux and Treberg, 2016). In our study, renal cortical Nrf-2 levels decreased which may be due to long-term oxidative stress from pre-diabetic conditions (Figure 4D). This result was supported by previous studies in which Nrf2 expression was reduced in obese condition (Cheng et al., 2011; Tan et al., 2011).

SC have high level of caffein can enhance the expression of the catalytic subunit of α-glutamyl cysteine synthase, which governs the

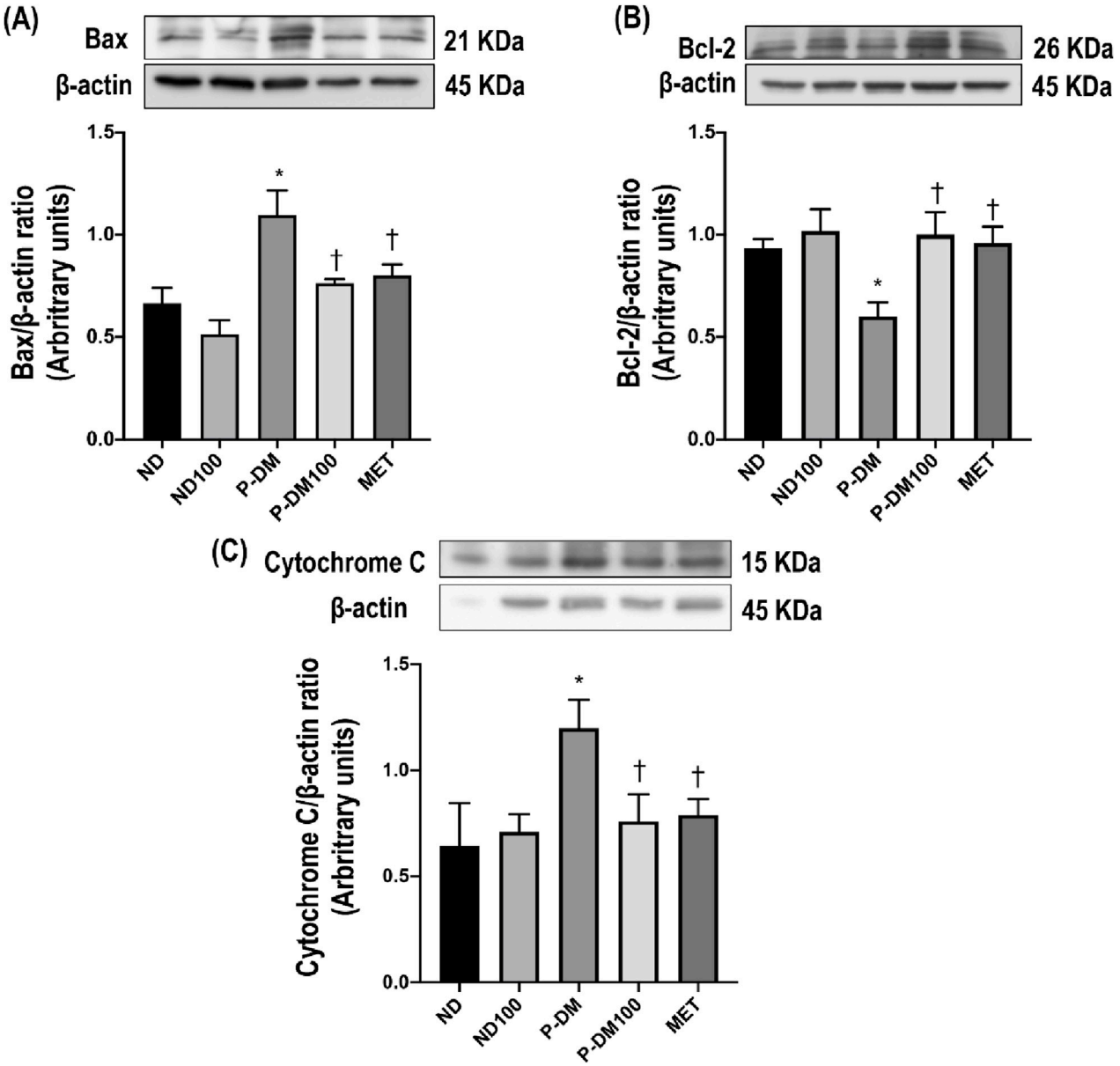


FIGURE 5
Effect of SC extract on renal apoptosis. Renal cortical expression of Bax **(A)**, Bcl-2 **(B)**, and Cytochrome C **(C)**. ND, normal diet rats treated with vehicle; ND100, normal diet rats treated with peat moss extract 100 mg/kg/day; P-DM, pre-diabetic rats treated with vehicle; P-DM50, pre-diabetic rats treated with peat moss extract 50 mg/kg/day; P-DM100, pre-diabetic diet rats treated with peat moss extract 100 mg/kg/day; MET, pre-diabetic diet rats treated with metformin 100 mg/kg/day. Values with different superscript letters $^{*}p < 0.05$ vs. ND and ND100; $^{\dagger}p < 0.05$ vs. P-DM; $^{\dagger}p < 0.05$ vs. P-DM50 indicate significant differences between treatments (LSD test, $p < 0.05$).

rate at which glutathione is produced (Myhrstad et al., 2002). According to our research, the phenolic compounds obtained from the SC extract exhibit a stimulatory impact on the concentration of glutathione while simultaneously decreases the levels of MDA in the renal tissue. Furthermore, our investigation reveals that the administration of SC extract increases the expression of Nrf-2 and its associated signaling molecules, such as GCLC, HO-1, SOD, and GSH as a defensive mechanism in response to renal oxidative injury (Figure 6).

In our previous study, we found that a high-fat diet induced renal injury via oxidative stress and apoptosis (Laorodphun et al., 2021). The present study also provides evidence for the activation of the apoptotic pathway in P-DM rats. The expression of the pro-apoptotic protein Bax is increased, which is accompanied by a decrease in the levels of the anti-apoptotic protein Bcl-2. Furthermore, this apoptotic activation is shown to be related to an upregulation of cytochrome C. SC extract can reverse these changes. Various studies have demonstrated the diverse health benefits attributed to phenolic compounds, encompassing their antioxidative, antihyperglycemic, antihyperlipidemic, and anti-inflammatory capabilities (El-Hadary and Ramadan, 2019; Singh and Yadav, 2022). In our study, we demonstrated that SC extract

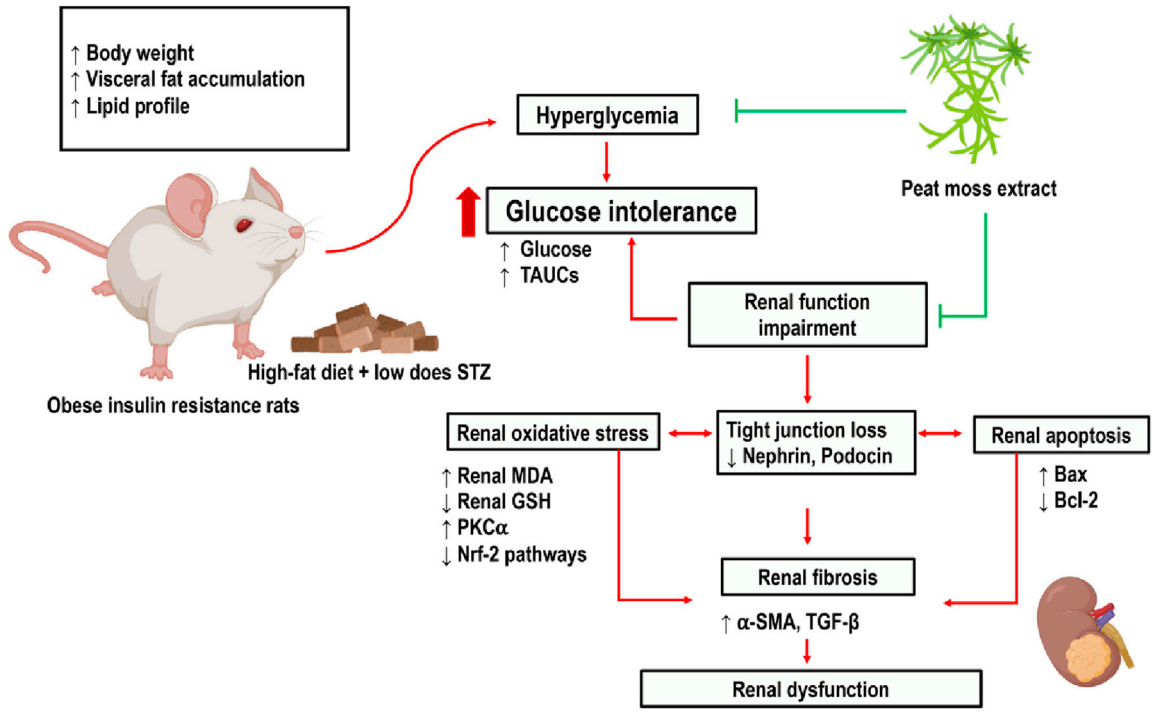


FIGURE 6
The pathway figure summarizing the findings of the study.

also possesses anti-apoptotic effects. In conclusion, our investigation has revealed that the SC extract has various therapeutic effects in a pre-diabetic rat model. The mechanisms of the beneficial effects of SC extract include the mitigation of hyperglycemia, hyperlipidemia, oxidative stress, apoptosis, and renal injury.

Conclusion

Our study shows the potential of SC extract in alleviating hyperglycemia, dyslipidemia, renal morphological changes, and renal dysfunction in pre-diabetic rats induced with a high-fat diet and streptozotocin. We demonstrate that the renoprotective effect of SC extract is due in part to the antioxidant and anti-apoptotic properties of the extract. However, further clinical trials in humans are required to provide important details about the efficacy and safety before use of SC extract as an alternative treatment for renoprotection in patients with diabetes.

Study limitation

Our study is limited to the precise exploration of how the kidney responds to ROS signaling, a crucial regulator of renal function. We would like to address the limitation related to the study of renal cortical cell lysates as recent research has revealed that the kidney is composed of over 20 distinct cell groups, many of which exhibit gradual variations in their gene expression patterns, as determined by single-cell and single-nucleus RNA sequencing techniques. Future research should focus on exploring the intricate variations in gene expression patterns across distinct renal cell types.

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 animal study was approved by the Laboratory Animal Center at Chiang Mai University in Chiang Mai, Thailand, has provided approval (Permit Number: 2565/RT-0025). The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

PL: Data curation, Formal Analysis, Investigation, Methodology, Software, Validation, Visualization, Writing–original draft, Writing–review and editing. SC: Investigation, Methodology, Writing–original draft. SA: Investigation, Methodology, Writing–original draft. PM: Investigation, Methodology, Writing–original draft. NP: Investigation, Methodology, Visualization, Writing–original draft. HP: Investigation,

Methodology, Visualization, Writing–original draft. AP: Supervision, Writing–review and editing. BK: Formal Analysis, Validation, Writing–original draft. MS: Writing–review and editing. PA: Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Validation, Writing–review and editing, Writing–original draft.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. This research project was supported by Fundamental Fund 2022, Chiang Mai University.

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.

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

Afolabi, O. B., Olasehinde, O. R., Olaoye, O. A., Jaiyesimi, K. F., Ekakitie, I. L., and Oloyede, O. I. (2023). Nephroprotective effects of caffeine, vanillin, and their combination against experimental AlCl3-induced renal toxicity in adult male wistar rats. *Biochem. Res. Int.* 2023 (1), 6615863. doi:10.1155/2023/6615863

Ahmed, B., Sultana, R., and Greene, M. W. (2021). Adipose tissue and insulin resistance in obese. *Biomed. Pharmacother.* 137, 111315. doi:10.1016/j.biopha.2021.111315

Ali, M. A., El-Abhar, H. S., Kamel, M. A., and Attia, A. S. (2015). Antidiabetic effect of galantamine: novel effect for a known centrally acting drug. *PLoS One* 10 (8), e0134648. doi:10.1371/journal.pone.0134648

Arjinajarn, P., Pongchaidecha, A., Chueakula, N., Jaikumkao, K., Chatsudthipong, V., Mahatheeranont, S., et al. (2016). Riceberry bran extract prevents renal dysfunction and impaired renal organic anion transporter 3 (Oat3) function by modulating the PKC/Nrf2 pathway in gentamicin-induced nephrotoxicity in rats. *Phytomedicine* 23 (14), 1753–1763. doi:10.1016/j.phymed.2016.10.014

Baird, L., Yamamoto, M., Takahashi, Y., Hishinuma, E., and Saigusa, D. (2020). Geldanamycin-derived HSP90 inhibitors are synthetic lethal with NRF2. *Mol. Cell. Biol.* 40 (13), e00377. doi:10.1128/MCB.00377-20

Chen, J. S., Xie, P. F., and Feng, H. (2023). The role of exercise in improving hyperlipidemia-renal injuries induced by a high-fat diet: a literature review. *PeerJ* 11, e15435. doi:10.7717/peerj.15435

Chen, Y., Deb, D. K., Fu, X., Yi, B., Liang, Y., Du, J., et al. (2019). ATP-citrate lyase is an epigenetic regulator to promote obesity-related kidney injury. *Faseb J.* 33 (8), 9602–9615. doi:10.1096/fj.201900213R

Chen, Z., and Zhang, S.-L. (2021). The role of flavonoids in the prevention and management of cardiovascular complications: a narrative review. *Ann. Palliat. Med.* 10 (7), 8254–8263. doi:10.21037/apm-21-1343

Cheng, X., Siow, R. C., and Mann, G. E. (2011). Impaired redox signaling and antioxidant gene expression in endothelial cells in diabetes: a role for mitochondria and the nuclear factor-E2-related factor 2-Kelch-like ECH-associated protein 1 defense pathway. *Antioxid. Redox Signal* 14 (3), 469–487. doi:10.1089/ars.2010.3283

D'Alessio, D. A., Denney, A. M., Hermiller, L. M., Prigeon, R. L., Martin, J. M., Tharp, W. G., et al. (2009). Treatment with the dipeptidyl peptidase-4 inhibitor vildagliptin improves fasting islet-cell function in subjects with type 2 diabetes. *J. Clin. Endocrinol. Metab.* 94 (1), 81–88. doi:10.1210/jc.2008-1135

Daza-Arnedo, R., Rico-Fontalvo, J. E., Pájaro-Galvis, N., Leal-Martínez, V., Abuabara-Franco, E., Raad-Sarabia, M., et al. (2021). Dipeptidyl peptidase-4 inhibitors and diabetic kidney disease: a narrative review. *Kidney Med.* 3 (6), 1065–1073. doi:10.1016/j.xkme.2021.07.007

Drummond, K., and Mauer, M. (2002). The early natural history of nephropathy in type 1 diabetes: II. Early renal structural changes in type 1 diabetes. *Diabetes* 51 (5), 1580–1587. doi:10.2337/diabetes.51.5.1580

El-Hadary, A. E., and Ramadan, M. F. (2019). Phenolic profiles, antihyperglycemic, antihyperlipidemic, and antioxidant properties of pomegranate (Punica granatum) peel extract. *J. Food Biochem.* 43 (4), e12803. doi:10.1111/jfbc.12803

Gerchman, F., Tong, J., Utzschneider, K. M., Zraika, S., Udayasankar, J., McNeely, M. J., et al. (2009). Body mass index is associated with increased creatinine clearance by a mechanism independent of body fat distribution. *J. Clin. Endocrinol. Metab.* 94 (10), 3781–3788. doi:10.1210/jc.2008-2508

González, P., Lozano, P., Ros, G., and Solano, F. (2023). Hyperglycemia and oxidative stress: an integral, updated and critical overview of their metabolic interconnections. *Int. J. Mol. Sci.* 24 (11), 9352. doi:10.3390/ijms24119352

Hanhineva, K., Törrönen, R., Bondia-Pons, I., Pekkinen, J., Kolehmainen, M., Mykkänen, H., et al. (2010). Impact of dietary polyphenols on carbohydrate metabolism. *Int. J. Mol. Sci.* 11 (4), 1365–1402. doi:10.3390/ijms11041365

Hawkley, T. F., Preston, M., and Maani, C. V. (2024). "Isoflurane. StatPearls. Treasure Island (FL) ineligible companies. Disclosure: matthew Preston declares no relevant financial relationships with ineligible companies," in *Disclosure: christopher Maani declares no relevant financial relationships with ineligible companies* (StatPearls).

Hsu, W.-C., Lin, C.-S., Chen, J.-F., and Chang, C.-M. (2022). The effects of dipeptidyl peptidase 4 inhibitors on renal function in patients with type 2 diabetes mellitus. *J. Clin. Med.* 11 (9), 2653. doi:10.3390/jcm11092653

Islayem, D., Fakih, F. B., and Lee, S. (2022). Comparison of colorimetric methods to detect malondialdehyde, A biomarker of reactive oxygen species. *ChemistrySelect* 7 (11), e202103627. doi:10.1002/slct.202103627

Jaikumkao, K., Pongchaidecha, A., Chueakula, N., Thongnak, L., Wanchai, K., Chatsudthipong, V., et al. (2018). Renal outcomes with sodium glucose cotransporter 2 (SGLT2) inhibitor, dapagliflozin, in obese insulin-resistant model. *Biochim. Biophys. Acta Mol. Basis Dis.* 1864 (6), 2021–2033. doi:10.1016/j.bbadis.2018.03.017

Kataoka, H., Nitta, K., and Hoshino, J. (2023). Visceral fat and attribute-based medicine in chronic kidney disease. *Front. Endocrinol. (Lausanne)* 14, 1097596. doi:10.3389/fendo.2023.1097596

Laorodphun, P., Arjinajarn, P., Thongnak, L., Promsan, S., Swe, M. T., Thitisut, P., et al. (2021). Anthocyanin-rich fraction from black rice, Oryza sativa L. var. indica "Luem Pua," bran extract attenuates kidney injury induced by high-fat diet involving oxidative stress and apoptosis in obese rats. *Phytother. Res.* 35 (9), 5189–5202. doi:10.1002/ptr.7188

Liu, Y., Zheng, J.-Y., Wei, Z.-T., Liu, S.-K., Sun, J.-L., Mao, Y.-H., et al. (2022). Determination of HMGB1 in hepatitis B virus-related acute-on-chronic liver failure patients with acute kidney injury: early prediction and prognostic implications. *Front. Pharmacol.* 13, 1031790. doi:10.3389/fphar.2022.1031790

Ma, Q. (2013). Role of nrf2 in oxidative stress and toxicity. *Annu. Rev. Pharmacol. Toxicol.* 53, 401–426. doi:10.1146/annurev-pharmtox-011112-140320

Mailloux, R. J., and Treberg, J. R. (2016). Protein S-glutathionlyation links energy metabolism to redox signaling in mitochondria. *Redox Biol.* 8, 110–118. doi:10.1016/j.redox.2015.12.010

Monami, M., Lamanna, C., Desideri, C. M., and Mannucci, E. (2012). DPP-4 inhibitors and lipids: systematic review and meta-analysis. *Adv. Ther.* 29 (1), 14–25. doi:10.1007/s12325-011-0088-z

Myhrstad, M., Carlsen, H., Nordström, O., Blomhoff, R., and Moskaug, J. (2002). Flavonoids increase the intracellular glutathione level by transactivation of the gamma-glutamylcysteine synthetase catalytical subunit promoter. *Free Radic. Biol. Med.* 32, 386–393. doi:10.1016/s0891-5849(01)00812-7

Ngo, V., and Duennwald, M. L. (2022). Nrf2 and oxidative stress: a general overview of mechanisms and implications in human disease. *Antioxidants (Basel)* 11 (12), 2345. doi:10.3390/antiox11122345

Ponchiardi, C., Mauer, M., and Najafian, B. (2013). Temporal profile of diabetic nephropathy pathologic changes. *Curr. Diab Rep.* 13 (4), 592–599. doi:10.1007/s11892-013-0395-7

Quan, Y., Park, W., Jin, J., Kim, W., Park, S. K., and Kang, K. P. (2020). Sirtuin 3 activation by honokiol decreases unilateral ureteral obstruction-induced renal inflammation and fibrosis via regulation of mitochondrial dynamics and the renal NF-κBTGF-β1/Smad signaling pathway. *Int. J. Mol. Sci.* 21 (2), 402. doi:10.3390/ijms21020402

Rahman, M. M., Rahaman, M. S., Islam, M. R., Rahman, F., Mithi, F. M., Alqahtani, T., et al. (2021). Role of phenolic compounds in human disease: current knowledge and future prospects. *Molecules* 27 (1), 233. doi:10.3390/molecules27010233

Salih, A. M., Al-Qurainy, F., Nadeem, M., Tarroum, M., Khan, S., Shaikhaldein, H. O., et al. (2021). Optimization method for phenolic compounds extraction from medicinal plant (juniperus procera) and phytochemicals screening. *Molecules* 26 (24), 7454. doi:10.3390/molecules26247454

Savini, I., Catani, M. V., Evangelista, D., Gasperi, V., and Avigliano, L. (2013). Obesity-associated oxidative stress: strategies finalized to improve redox state. *Int. J. Mol. Sci.* 14 (5), 10497–10538. doi:10.3390/ijms140510497

Sharifi-Rad, J., Seidel, V., Izabela, M., Monserrat-Mequida, M., Sureda, A., Ormazabal, V., et al. (2023). Phenolic compounds as Nrf2 inhibitors: potential applications in cancer therapy. *Cell. Commun. Signal* 21 (1), 89. doi:10.1186/s12964-023-01109-0

Singh, N., and Yadav, S. S. (2022). A review on health benefits of phenolics derived from dietary spices. *Curr. Res. Food Sci.* 5, 1508–1523. doi:10.1016/j.crfs.2022.09.009

Singh, R. K., Kumar, S., Gautam, P. K., Tomar, M. S., Verma, P. K., Singh, S. P., et al. (2017). Protein kinase C-α and the regulation of diverse cell responses. *Biomol. Concepts* 8 (3-4), 143–153. doi:10.1515/bmc-2017-0005

Srivastava, S. P., Kanasaki, K., Goodwin, J. E., and Koya, D. (2020). Inhibition of angiotensin-converting enzyme ameliorates renal fibrosis by mitigating DPP-4 level and restoring antifibrotic MicroRNAs. *Front. Pharmacol.* 11, 211. doi:10.3390/genes11020211

Srivastava, S. P., Li, J., Takagaki, Y., Kitada, M., Goodwin, J. E., Kanasaki, K., et al. (2021b). Endothelial SIRT3 regulates myofibroblast metabolic shifts in diabetic kidneys. *iScience* 24 (5), 102390. doi:10.1016/j.isci.2021.102390

Srivastava, S. P., Zhou, H., Setia, O., Liu, B., Kanasaki, K., Koya, D., et al. (2021a). Loss of endothelial glucocorticoid receptor accelerates diabetic nephropathy. *Nat. Commun.* 12 (1), 2368. doi:10.1038/s41467-021-22617-y

Swe, M. T., Thongnak, L., Jaikumkao, K., Pongchaidecha, A., Chatsudthipong, V., and Lungkaphin, A. (2019). Dapagliflozin not only improves hepatic injury and pancreatic endoplasmic reticulum stress, but also induces hepatic gluconeogenic enzymes expression in obese rats. *Clin. Sci.* 133 (23), 2415–2430. doi:10.1042/CS20190863

Tan, Y., Ichikawa, T., Li, J., Si, Q., Yang, H., Chen, X., et al. (2011). Diabetic downregulation of Nrf2 activity via ERK contributes to oxidative stress-induced insulin resistance in cardiac cells *in vitro* and *in vivo*. *Diabetes* 60 (2), 625–633. doi:10.2337/db10-1164

Vodošek Hojs, N., Bevc, S., Ekart, R., and Hojs, R. (2020). Oxidative stress markers in chronic kidney disease with emphasis on diabetic nephropathy. *Antioxidants (Basel)* 9 (10), 925. doi:10.3390/antiox9100925

Wu, Y., Li, Y., Jiang, T., Yuan, Y., Li, R., Xu, Z., et al. (2018). Reduction of cellular stress is essential for Fibroblast growth factor 1 treatment for diabetic nephropathy. *J. Cell. Mol. Med.* 22 (12), 6294–6303. doi:10.1111/jcmm.13921

Xu, H., Gan, C., Gao, Z., Huang, Y., Wu, S., Zhang, D., et al. (2020). Caffeine targets SIRT3 to enhance SOD2 activity in mitochondria. *Front. Cell. Dev. Biol.* 8, 822. doi:10.3389/fcell.2020.00822

frontiers | Frontiers in Pharmacology

TYPE Original Research
PUBLISHED 28 October 2024
DOI 10.3389/fphar.2024.1424968

Check for updates

OPEN ACCESS

EDITED BY
Swayam Prakash Srivastava,
NewYork-Presbyterian, United States

REVIEWED BY
Fang Zhong,
Icahn School of Medicine at Mount Sinai, United States
Dan Li,
Chengdu University of Traditional Chinese Medicine, China

*CORRESPONDENCE
Harvest F. Gu,
feng.gu@cpu.edu.cn
Wei Tang,
drtangwei@njmu.edu.cn

RECEIVED 29 April 2024
ACCEPTED 30 September 2024
PUBLISHED 28 October 2024

CITATION
Yu H, Tang H, Saxu R, Song Y, Cui X, Xu J, Li N, Cui S, Ge H, Tang W and Gu HF (2024) Effects of *Abelmoschus manihot* (L.) and its combination with irbesartan in the treatment of diabetic nephropathy via the gut–kidney axis. *Front. Pharmacol.* 15:1424968. doi: 10.3389/fphar.2024.1424968

COPYRIGHT
© 2024 Yu, Tang, Saxu, Song, Cui, Xu, Li, Cui, Ge, Tang and Gu. 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.

Effects of *Abelmoschus manihot* (L.) and its combination with irbesartan in the treatment of diabetic nephropathy via the gut–kidney axis

Hongmei Yu[1], Haitao Tang[2], Rengui Saxu[1], Yuhui Song[1], Xu Cui[1], Jingjing Xu[1], Nan Li[3], Siyuan Cui[4], Haitao Ge[2], Wei Tang[5]* and Harvest F. Gu [1]*

[1]Laboratory of Molecular Medicine, School of Basic Medicine and Clinical Pharmacy, China Pharmaceutical University, Nanjing, Jiangsu Province, China, [2]Suzhong Pharmaceutical Group Co. Ltd., Suzhong Pharmaceutical Research Institute, Nanjing, Jiangsu Province, China, [3]Department of Endocrinology, Jiangsu Province Hospital of Traditional Chinese Medicine, The Affiliated Hospital of Nanjing University of Chinese Medicine, Nanjing, China, [4]Department of Endocrinology, Wuxi Second People's Hospital, Jiangnan University Medical Center, Wuxi, Jiangsu Province, China, [5]Islet Cell Senescence and Function Research Laboratory, Department of Endocrinology, Nanjing Medical University Affiliated Geriatric Hospital/Jiangsu Province Geriatric Hospital, Nanjing, Jiangsu Province, China

Background: Clinical observations have recently shown that *Abelmoschus manihot* (L.) in the form of Huangkui capsule (HKC) and in combination with irbesartan (EB) is an effective therapy for diabetic nephropathy (DN) in patients with type 2 diabetes (T2D). The present study aims to explore the mechanisms underlying the therapeutic efficacies of HKC and its combination with EB in DN via the gut-kidney axis.

Methods: HKC, EB, and their combination or vehicle were administered in db/db mice, which is an animal model for the study of T2D and DN. Comparative analyses of the gut microbiota, serum metabolites, and kidney transcriptomics before and after drug administration were performed.

Results: After treatment with HKC, EB, and their combination for 4 weeks, the urinary albumin-to-creatinine ratios decreased significantly in the db/db mice with DN. In terms of the gut microbiota, the abundances of Faecalitalea, Blautia, and Streptococcus increased but those of Bacteroidetes, Firmicutes, Enterobacteriaceae, and Desulfovibrio decreased. Parallelly, serum metabolites, mainly including quercetin 3′-glucuronide and L-dopa, were elevated while cortisol and cytochalasin B were reduced. Furthermore, the *S100a8*, *S100a9*, *Trem1*, and *Mmp7* genes in the kidneys were downregulated. These altered elements were associated with proteinuria/albuminuria reduction. However, EB had no effects on the changes in blood pressure and specific differentially expressed genes in the kidneys.

Abbreviations: DN, diabetic nephropathy; DCT, distal convoluted tubule; DTC, distinct tubule cell; EB, irbesartan; ESRD, end-stage renal disease; GFR, glomerular filtration rate; HKC, Huangkui capsule; PCT, proximal convoluted tubule; PTC, proximal tubule cell; SLC, solute carrier; T2D, type 2 diabetes.

Conclusion: The present study provides experimental evidence that HKC regulates the gut microbiota, circulating metabolites, and renal gene activities, which are useful for better understanding of the action mechanisms of *A. manihot* in the treatment of DN through the gut-kidney axis.

KEYWORDS

Abelmoschus manihot **L., diabetic nephropathy, Huangkui capsule, irbesartan, type 2 diabetes**

Introduction

Abelmoschus manihot (L.) is called Huangkui in Chinese ("Huang" means yellow and "Kui" means sunflower). Similar to the discovery of Artemisinin (Tu, 2016), the medical applications of *A. manihot* were first recorded in the Handbook of Prescriptions for Emergencies by Mr. Hong Ge during the Eastern Jin Dynasty (317–420 AD) in China. As a traditional Chinese medicine, the Huangkui capsule (HKC) is made from the ethanolic extract of the flowers of *A. manihot* and received approval from the Chinese Food and Drug Administration (Z19990040) in 1999 (Chen et al., 2016; Li et al., 2021). Currently, HKC is used in China for treating patients with kidney diseases, including diabetic nephropathy (DN). The main active chemical constituents of HKC are the flavones of *A. manihot* (L.); according to liquid chromatography quadrupole time-of-flight mass spectrometry (LC-Q-TOF/MS) analysis, we identified seven flavonoids as the components of HKC, namely rutin, hyperoside, isoquercitrin, gossypetin-8-O-β-D-glucuronide, myricetin, quercetin-3-O-β-D-glucuronide, and quercetin (Diao et al., 2024).

Clinical observations have demonstrated the efficacy and safety of HKC in the treatment of primary glomerular diseases and IgA nephropathy that mainly present as albuminuria and proteinuria (Zhang et al., 2014; Li et al., 2017; Li et al., 2020). Irbesartan (EB) is an angiotensin receptor blocker and that independently has renoprotective blood pressure lowering effects in patients with type 2 diabetes (T2D) and microalbuminuria (Parving et al., 2001; Lewis et al., 2001). When used in combination with EB, a multicenter randomized double-blind parallel controlled clinical trial recently reported HKC as an effective therapy for DN in T2D patients for reducing albuminuria and proteinuria (Zhao et al., 2022). However, the mechanisms underlying the effects of HKC and its combination with EB in the treatment of DN are unknown.

Gut microbiota and their metabolites may have pathogenic or beneficial effects on the onset and progression of DN. The theory of the gut-kidney axis was proposed by Meijers and Evenepoel (2011) based on their finding that changes in the intestinal microecology could affect the progression of chronic kidney disease by regulating metabolites (Meijers et al., 2019; Krautkramer et al., 2021). In recent years, increasing evidence has shown that gut microbiota and their metabolites play essential roles in the pathophysiological processes of DN through the gut-kidney axis (Zhao et al., 2023; Tao et al., 2019). Our research group has carried out an experimental study using non-obese diabetic (NOD) mice as a model for the study of type 1 diabetes and DN; the results suggest that HKC may modulate gut microbiota and subsequently ameliorate the metabolite levels in DN (Shi et al., 2023). Both HKC and EB are orally administered drugs that may exert their pharmaceutical effects in the treatment of DN by regulating the gut-kidney axis.

In the present work, we designed an experimental study using db/db mice as the animal model to study T2D and DN (Sharma et al., 2003; Wang et al., 2014). First, we investigated the changes in gut microbiota before and after administration of HKC, EB, and their combination. Then, we identified the altered metabolites in the serum. Finally, we analyzed the transcriptomics of the kidneys. This study provides novel information for understanding the efficacy of HKC and its combination with EB in the treatment of T2D-related DN with a focus on the gut-kidney axis.

Methods

Animals

Ten-week-old db/db (BKS.Cg-Dock7m +/+ Leprdb/J) and C57BL/KsJ mice were purchased from the animal experimental center of Nanjing University (Nanjing, China). All mice were males and housed in a specific pathogen-free barrier environment at the animal experimental center of Xuanwu campus, China Pharmaceutical University. The animal room was maintained at a temperature of 24°C ± 2°C and humidity of 50% ± 10% with 12-h light/dark cycles. After 1 week of adaptation, the bodyweights and blood glucose levels of all mice were measured weekly. The urine samples were collected using metabolic cages (DXL-XS, Fengshi, Suzhou, China) for 6 h. Microalbuminuria and creatinine were measured using ELISA quantitative kits (Elabscience Biotechnology, China). When the blood glucose level was ≥16.7 mmol/L and urinary albumin-to-creatinine ratio (UACR) was ≥200 ng/μg for two consecutive days, the db/db mice were diagnosed to have DN. The db/db mice with DN were randomly divided into five groups as DN without treatment (n = 13), HKC (receiving HKC treatment, n = 11), EB (receiving EB treatment, n = 6), HKCEB (receiving HKC with EB as treatment, n = 8), and C57BL/KsJ mice as the non-diabetic control group (WT, n = 16).

Drugs and administration

HKC was produced by Suzhong Pharmaceutical Group Co., Ltd. (Taizhou, China). Each HKC contains 0.43 g of *A. manihot* (L.) extract. The quality of the HKC was examined through fingerprint analysis using high-performance liquid chromatography, as

reported previously (Lai et al., 2009; Guo et al., 2015). EB was produced by Sanofi Shengdelabao Minsheng Pharmaceutical Co., Ltd. (Hangzhou, China). Based on the conversion of human and mouse body surface areas, HKC (0.84 g/kg/d) and EB (0.0195 g/kg/d) or vehicle were administered daily via oral gavage for 4 weeks in the db/db mice. The administration period was determined based on clinical trials (Zhao et al., 2022) and our previous experimental studies (Yu et al., 2023a; Yu et al., 2023b; Shi et al., 2023). The experiments were performed according to the guidelines of the Declaration of Helsinki and approved by the ethics committee of China Pharmaceutical University (Approval Code: 2019-08-0003 and Approval Date: 26-08-2019).

16S ribosomal DNA sequence analysis

As reported previously, our research group used the intestinal contents as samples to analyze the gut microbiota in mice (Shi et al., 2023). In the present study, mice colon were retrieved by surgical double ligation. Total g.DNA from the colon microbiota was extracted using the CTAB/SDS method and amplified by polymerase chain reaction (PCR) using 341F (5′-CCTAYGGGRBGCASCAG-3′) and 806R (5′-GGACTACNNGGGTATCTAAT-3′) as the primers belonging to the V3–V4 variable region of 16S rDNA. Each sample was repeated thrice, and the mixed PCR products were detected through 2% agarose gel electrophoresis. The samples with main band brightness between 400 and 450 bp were selected for further experiments. The amplicons were purified using the AxyPrep DNA gel extraction kit (Axygen Biosciences, Union City, CA, United States). After the DNA libraries were built using the NEB Ultra DNA Library Prep Kit (NEB), the 16S RNA sequences were aligned and analyzed using the NovaSeq 6000 (Illumina, San Diego, CA, United States) platform and Silva database.

Serum metabolomics

Serum samples stored at −80°C were removed from the refrigerator and thawed on ice until there were no ice cubes in the samples. The samples were then mixed by vortexing for 10 s; approximately 50 μL of each sample was transferred to the corresponding numbered centrifuge tube, and 300 μL of a mixture of acetonitrile and methanol (ACN: methanol = 1:4, V/V) was added. The sample plates were vortexed for 3 min and centrifuged at 12,000 r/min for 10 min at 4°C. After centrifugation, approximately 200 μL of the supernatant was removed into another corresponding numbered centrifuge tube and placed in a refrigerator at −20°C for 30 min. Qualitative analysis of the serum metabolites was initially performed by untargeted metabolomics in the LC-Q-TOF/MS platform and further adapted to widely targeted metabolomics on the LC-ESI-MS/MS system. Acquisition conditions for the untargeted assays included ultraperformance liquid chromatography (UPLC) and quadrupole time-of-flight (TripleTOF 6600, AB SCIEX) data acquisition systems, and the targeted acquisition conditions included UPLC and tandem mass spectrometry (Xing et al., 2024a).

Kidney transcriptomics

The total RNAs in the kidneys were extracted using a TRIzol regent kit (Ambion, Shanghai Yubo Biological Technology Co., Ltd., China). The concentration of the total RNAs was detected with the Qubit®RNA assay kit (Life Technologies, CA, United States). The integrity of the total RNAs was analyzed with the Nano6000 bioanalyzer 2100 system (Agilent Technologies, CA, United States). Library construction for the total RNAs >1 μg was performed with the NEBNext® Ultra RNA library prep kit (Illumina, NEB, United States). Oligo (dT) beads were enriched using mRNA with polyA tail, and the mRNA were randomly interrupted in the NEB fragmentation buffer. The cDNA was synthesized using the M-MuLV reverse transcriptase system (Illumina, NEB, United States) with fragmented mRNA as the template and random oligonucleotides as the primers. The cDNAs of approximately 200 bp were screened using AMPure XP beads (Beckman Coulter, Beverly, United States). The library was quantitated using the Qubit2.0 Fluorometer, and the insert size was detected using the Agilent 5400 bioanalyzer (Agilent, United States). Once qualified, the library was sequenced on the Illumina platform (Illumina, Novo Geno Bioinformatics Co., Ltd., China).

Statistical analysis

All analyses and graphics were obtained using GraphPad Prism software (version 8.0), SPSS software (version 23.0), and R software (version 3.6.0). The principal coordinates analysis (PCoA) was used for Adonis's multivariate analysis of variance; linear discriminant analysis effect size (LEfSe) differences between pairs of groups were tested for significance using the Kruskal–Wallis sum-rank test. Biological significance was subsequently analyzed through pairwise tests among the groups using the Wilcoxon rank-sum test. Furthermore, the genus levels of the gut microbiota were used in the Metastats statistical analysis. Metabolic pathway analysis that integrated pathway enrichment and pathway topology analyses were visualized using MetaboAnalyst (Chong and Xia, 2020; Pang et al., 2022). The correlations among the gut microbiota, metabolites, and differentially expressed genes (DEGs) were analyzed using the Spearman correlation test. The significance comparisons between the groups were calculated using Student's t-test. All values are presented in terms of mean ± standard error of the mean (SEM) unless otherwise noted. A p-value < 0.05 was used as the threshold for statistical significance.

Results

UACR after treatment with HKC, EB, and their combination

The blood glucose, bodyweight, and UACR values of the db/db mice were higher than those of the mice in the WT group. After treatment with HKC, EB, and HKCEB in the db/db mice, the UACR decreased significantly (Figures 1J–L). As reported recently, several biomarkers such as col4a3, slc5a2, slc34a1, slc12a3, and slc4a1in the

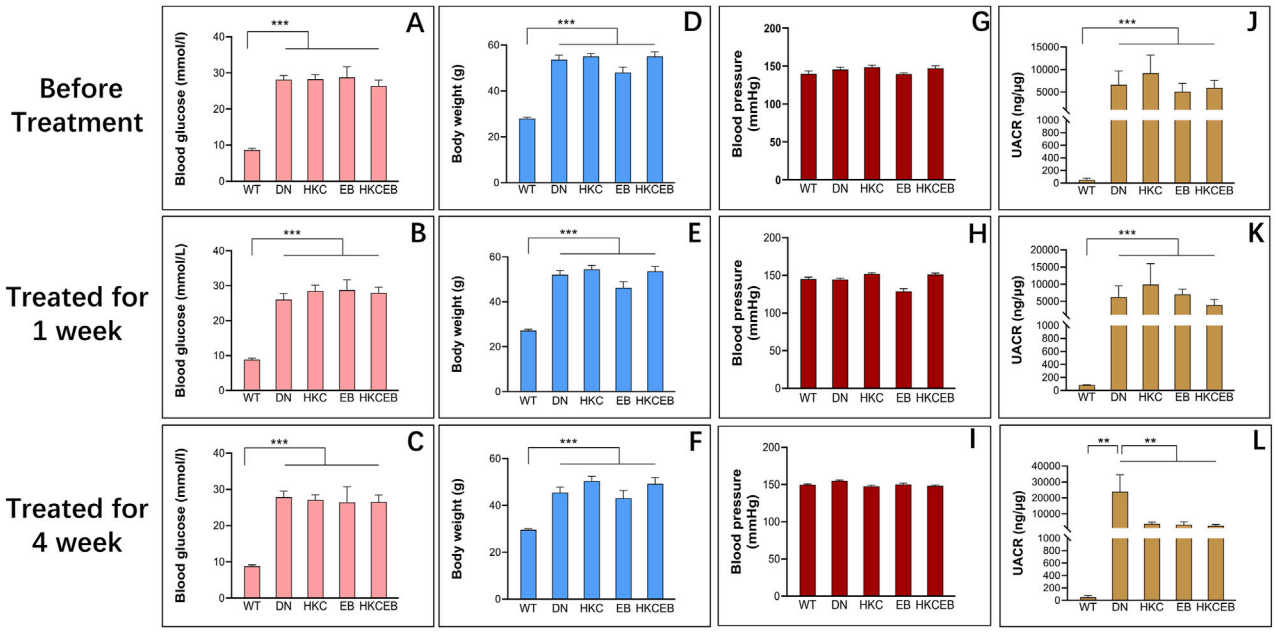


FIGURE 1
Blood glucose levels, bodyweights, UACRs, and blood pressure values of db/db mice before and after treatment with *Abelmoschus manihot*, irbesartan, and their combination. **(A–C)** Blood glucose, **(D–F)** bodyweight, **(G–I)** blood pressure, and **(J–L)** UACR of the DN group compared to the WT group and after treatment with HKC, EB, or HKCEB for 1 and 4 weeks. DN, diabetic nephropathy; WT, non-diabetic control; HKC, Huangkui capsule of *A. manihot*; EB, irbesartan; HKCEB, HKC combined with EB; UACR, urinary albumin-to-creatinine ratio; **P < 0.01 and ***P < 0.001.

glomerulus as well as the proximal and distal convoluted tubules of the kidneys in db/db mice treated with HKC were observed to have improved (Yu et al., 2023a). However, changes in the blood glucose levels and bodyweights were not statistically significant (Figures 1A–F). The blood pressures of all mice were examined, and no significant changes were observed (Figures 1G–I).

Changes in intestinal flora after treatment with HKC, EB, and their combination

Comparative analyses of the intestinal flora in the WT, DN, HKC, EB, and HKCEB groups were conducted. A plot of the PCoA results is shown in Supplementary Figure S1A. Bacteroidetes and Firmicutes were the dominant phyla (Supplementary Figure S1B), while Ligilactobacillus and Limosilactobacillus were dominant at the genus level (Supplementary Figure S1F). LEfSe demonstrated that f._Enterobacteriaceae and s._Bacteroides_acidifaciens increased, while g._Streptococcus and g._Blautia decreased in DN compared to the WT group (Figure 2A). After HKC treatment, g._Anaeroplasma, g._unidentified_Lachnospiraceae, and s._Clostridiales_bacterium_CIEAF_020 increased (Figure 2B). In the EB group, g._Akkermansia was found to have increased (Figure 2C). s._Streptococcus_pneumoniae and s._Staphylococcus_epidermidis had increased in the HKCEB group (Figure 2D). A heatmap of the flora abundance of the gut microbiota in the WT, DN, HKC, EB, and HKCEB groups is shown in Figure 2E. The data demonstrate that Bacteroidetes, Firmicutes, Weissella, Alloprevotella, Mailhella, Treponema, Enterobacteriaceae, Rikenellaceae, Enterococcus, and Desulfovibrio increased in DN compared to WT but decreased after HKC, EB, and HKCEB treatments (Figures 2F–O). Conversely, Muribaculaceae, Anaerovibrio, Dietzia, Faecalitalea, Anaerofustis, *Limosilactobacillus*, *Ligilactobacillus*, *Arthrobacter*, *Streptococcus*, and Blautia were lower in DN but increased upon treatment with HKC, EB, and HKCEB (Figures 2P–Y).

Alteration of serum metabolites after treatment with HKC, EB, and their combination

The numbers of metabolites detected in the sera of the WT, DN, HKC, EB, and HKCEB groups are summarized in Supplementary Figures S2A–C. Principal component analysis (PCA) shows the altered serum metabolites in the db/db mice after treatment with HKC, EB, and their combination (Figure 3A). The cluster heatmaps of the top-50 metabolites in DN and the remaining groups are shown in Figure 3B and Supplementary Figures S3A–C, respectively. Compared to the WT group, cortisol, cytochalasin B, acetoxy-8-gingerol, (3-ethenylphenyl) oxidanesulfonic acid, 3,5-dinitrocatechol, and 2,7-dichlorodihydrofluorescein diacetate were higher in the DN group (red boxes indicate upregulation), while kaempferide, quercetin, Thr-Asp-Phe-Glu, N-cinnamylglycine, ganoderiol I, L-dopa, and acrylamide were lower (blue boxes indicate downregulation). After HKC and HKCEB treatments, cortisol, cytochalasin B, 3,5-dinitrocatechol, (3-ethenylphenyl) oxidanesulfonic acid, 2,7-dichlorodihydrofluorescein, and acetoxy-8-gingerol were found to be downregulated, while kaempferide, ganoderiol I, Thr-Asp-Phe-Glu, N-cinnamylglycine, and acrylamide were upregulated (Figures 3C–E). In the EB group,

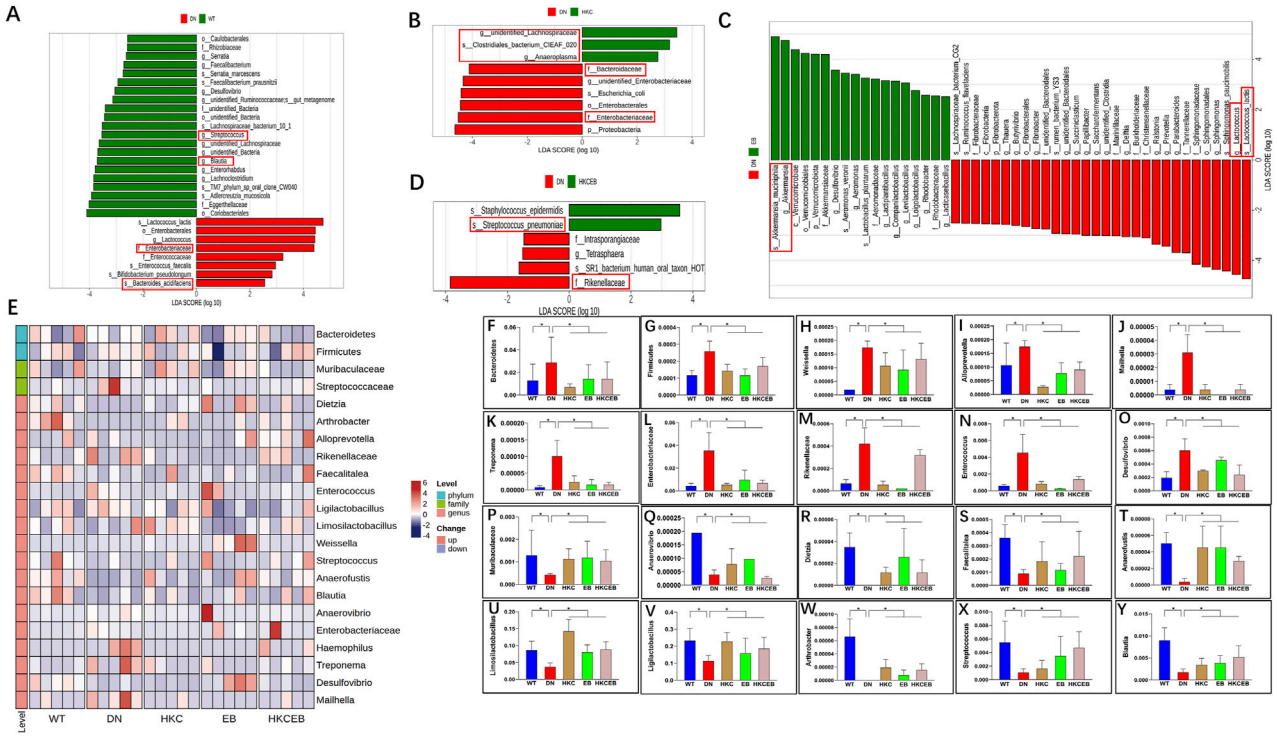


FIGURE 2
Changes in the intestinal flora after treatment with *A. manihot*, irbesartan, and their combination. LEfSe analyses demonstrate changes in the intestinal flora **(A)** in DN compared to the WT group and after the treatment with **(B)** HKC, **(C)** EB, and **(D)** HKCEB. **(E)** Clustering heatmap of the abundance of intestinal flora. The heatmap indicates that **(F–O)** 10 gut microbiota types were decreased while **(P–Y)** 10 other types of intestinal flora were increased after treatment with HKC, EB, or HKCEB. DN, diabetic nephropathy; WT, non-diabetic control; HKC, Huangkui capsule of *A. manihot*; EB, irbesartan; HKCEB, HKC combined with EB; *P< 0.05.

only cortisol, cytochalasin B, 3,5-dinitrocatechol, and (3-ethenylphenyl) oxidanesulfonic acid were found to be downregulated, while kaempferide, ganoderiol I, Thr-Asp-Phe-Glu, and N-cinnamylglycine were upregulated (Figure 3E).

KEGG pathway enrichment analyses of the metabolites

The Kyoto encyclopedia of genes and genomes (KEGG) is a database for systematic analysis of the gene functions and genome information; KEGG also provides integrated metabolic pathways for carbohydrates, nucleosides, and amino acids. The annotation results of the significantly altered metabolites in the DN, HKC, EB, and HKCEB groups are shown in Supplementary Figure S4A–D and subsequently classified into four major KEGG pathways, including organismal systems, metabolism, human disease, and environmental information processing and cellular processing (Supplementary Figure S5A–D). In the DN group, cortisol was found in the steroid hormone biosynthesis, cortisol synthesis, and secretion pathways; 3-hydroxypyruvic acid was enriched in the carbon metabolism, glyoxylate, and dicarboxylate metabolism pathways (Figure 4A). In the HKC group, cortisol was downregulated and enriched in the steroid hormone biosynthesis as well as cortisol synthesis and secretion pathways (Figure 4B). In the EB group, hypoxanthine, xanthine, erucic acid, L-arginine, and urea were found to be enriched in the nucleotide and purine metabolism pathways. Moreover, erucic acid was downregulated in the nucleotide metabolism pathway, while L-arginine was upregulated and enriched in the arginine and proline metabolism pathways. Urea was downregulated in the pyrimidine metabolism pathway (Figure 4C). In the HKCEB group, 11β-hydroxyprogesterone, 17α-hydroxyprogesterone, and xanthosine were found in the steroid hormone biosynthesis and purine metabolism pathways (Figure 4D).

Correlation between gut microbiota changes and serum metabolite alterations

The correlations between metabolites and gut microbiota at the phylum, family, and genus levels in DN compared to WT and after treatment with HKC, EB, and HKCEB are shown in Figures 5A–D. At the phylum level, Firmicutes was positively (indicated by red line) correlated with cytochalasin B and quercetin 3′-glucuronide but negatively (blue line) correlated with L-dopa and cortisol, while Bacteroidetes showed converse trends to Firmicutes (Figures 5E, F). At the family level, Muribaculaceae and Streptococcus were positively correlated with L-dopa, quercetin 3′-glucuronide, and acrylamide but negatively

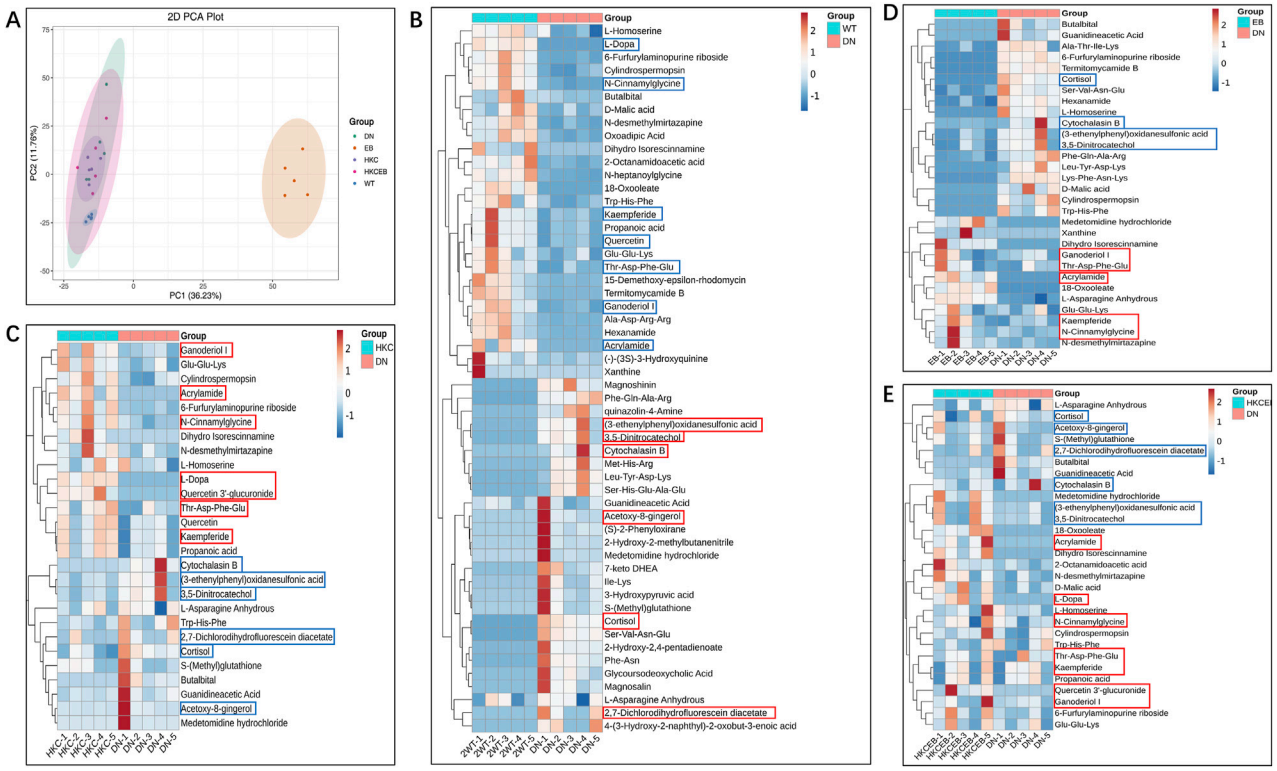


FIGURE 3
Alteration of serum metabolites after treatment with *A. manihot*, irbesartan, and their combination. **(A)** PCA plot of the altered serum metabolites in the WT, DN, HKC, EB, and HKCEB groups. **(B–E)** Cluster heatmaps of the top metabolites in DN compared to the WT group and after treatment with HKC, EB, or HKCEB. The red boxes indicate upregulation, while the blue boxes imply downregulation. PCA, principal component analysis; DN, diabetic nephropathy; WT, non-diabetic control; HKC, Huangkui capsule of *A. manihot*; EB, irbesartan; HKCEB, HKC combined with EB.

correlated with cortisol and acetoxy-8-gingerol (Figures 5G, H). At the genus level, Rikenellaceae and Enterobacteriaceae were positively correlated with cytochalasin B, cortisol, and acetoxy-8-gingerol but negatively correlated with L-dopa, quercetin 3′-glucuronide, and acrylamide (Figures 5I, J). Blautia was positively correlated with L-dopa, quercetin 3′-glucuronide, and acrylamide but negatively correlated with cytochalasin B, cortisol, and acetoxy-8-gingerol (Figure 5K). Desulfovibrio was positively correlated with cytochalasin B and negatively correlated with L-dopa, quercetin 3′-glucuronide, acrylamide, and acetoxy-8-gingerol (Figure 5L).

DEGs in kidneys after treatment with HKC, EB, and their combination

PCoA implicated that the DEGs in the kidneys of the DN mice may be regulated after HKC, EB, and HKCEB treatments (Figure 6A). The DEGs in the kidneys of the DN group compared to the WT, HKC, EB, and HKCEB groups are summarized by Venn diagrams (Supplementary Figures S6A–C). The top-50 genes in the kidneys of the DN, HKC, EB, and HKCEB groups are represented as cluster heatmaps (Figures 6B–E; Supplementary Figures S7A–C). In brief, the *Cstdc5*, *S100a8*, *S100a9*, *Asprv1*, *Il1r2*, *Lcn2*, *Cxcr2*, *Trem1*, *Chil1*, *Slpi*, *Mmp7*, *Sprr2f*, *Ngp*, *1200007C13Rik*, *Mpo*, *Marco*, *Camp*, *Ctsg*, and *Elane* genes were upregulated, while the *Trdn* and *Acsm3* genes were downregulated in the DN group (Figure 6B). In the HKC group, *Trdn* was upregulated, while *Trem1*, *S100a8*, *Mmp7*, *S100a9*, *Asprv1*, *Chil1*, *Il1r2*, *1200007C13Rik*, *Cxcr2*, and *Marco* were downregulated (Figure 6C). In the EB group, all *Fam193b*, *Malat1*, *Rgs11*, *Gm12940*, *Rsrp1*, and *AI480526* genes were downregulated (Figure 6D). In the HKCEB group, *Acsm3* gene expression increased while *Trem1*, *Camp*, *S100a9*, *Asprv1*, *Chil1*, and *Ctsg* gene expressions decreased (Figure 6E).

KEGG pathway enrichment analyses of the DEGs

Volcanic map, gene ontology (GO), and KEGG enrichment analyses of the DEGs in the kidneys of the DN, HKC, EB, and HKCEB groups are shown in Supplementary Figures S8–S10. The genes *Gsta1* and *Ugt1a2* that were upregulated in DN were enriched in the glutathione, pentose, and glucuronate interconversion pathways (Figure 7A). After HKC treatment, *Tlr4* was downregulated and enriched in the inflammatory bowel disease pathway (Figure 7B). After HKCEB treatment, *Selp* and *Itgb2* were enriched in the neutrophil extracellular trap formation pathway (Figure 7C). No specific pathways of the DEGs in the kidneys of the EB group were found.

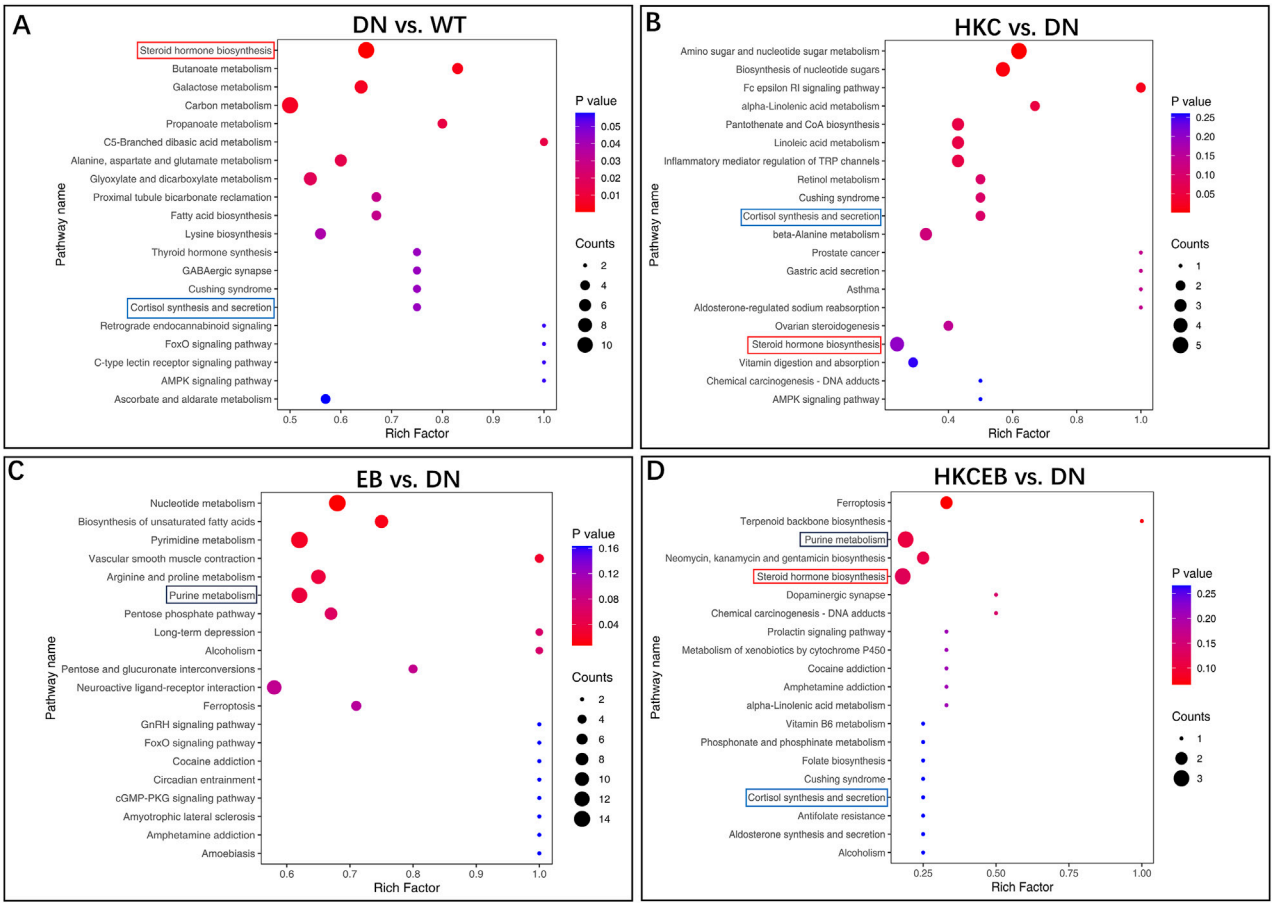


FIGURE 4
KEGG pathway enrichment analyses of the various metabolites. The predicted KEGG pathways of the different serum metabolites **(A)** in DN compared to WT and **(B–D)** after treatment with HKC, EB, or HKCEB are presented. DN, diabetic nephropathy; WT, non-diabetic control; HKC, Huangkui capsule of *A. manihot*; EB, irbesartan; HKCEB, HKC combined with EB.

Correlation between serum metabolites and renal genes

KEGG enrichment bubble maps predicting the correlations between the serum metabolites and genes in the kidneys are shown in Supplementary Figures S11, S12. The 50 DEGs (only 6 in the EB group) and 50 differential metabolites were included in the Spearman correlate analyses, whose heatmaps are shown in Figures 8A–D. The *Trem1*, *S100a9*, *Asprv1*, and *Chil1* genes were upregulated in DN but downregulated in the HKC and HKCEB groups. These genes were positively correlated with metabolites such as cortisol, cytochalasin B, 2,7-dichlorodihydrofluorescein diacetate, medetomidine hydrochloride, and L-cystin but negatively correlated with dihydro isorescinnamine, L-dopa, 18-oxooleate, quercetin 3′-glucuronide, xanthosine, and 18-oxooleate (Figures 8E–H). In addition, the *S100a8*, *Mmp7*, *Il1r2*, and *Cxcr2* genes that were increased in DN but decreased after HKC treatment were positively correlated with cortisol, cytochalasin B, 2,7-dichlorodihydrofluorescein diacetate, medetomidine hydrochloride, and 4-(3-hydroxy-2-naphthyl)-2-oxobut-3-enoic acid but negatively correlated with dihydro isorescinnamine, L-dopa, quercetin 3′-glucuronide, and acrylamide (Figures 8I–L). In the HKCEB group, the *Camp* and *Ctsg* genes were positively correlated with 2,7-dichlorodihydrofluorescein diacetate, 4-(3-hydroxy-2-naphthyl)-2-oxobut-3-enoic acid, L-cystin, butalbital, S-(methyl) glutathione, and thymidine but negatively correlated with dihydro isorescinnamine, L-dopa, quercetin 3′-glucuronide, xanthosine, 18-oxooleate, medetomidine hydrochloride, and acrylamide (Figures 8M, N).

Discussion

In the present study, we investigated the changes in the intestinal flora, serum metabolites, and mRNA expressions in the kidneys of db/db mice with DN. The aim here was to explore the molecular mechanisms of HKC and its combined treatment with EB for T2D-related DN primarily in the gut-kidney axis. After 4 weeks of administration of HKC, EB, and their combination, the UACRs of the db/db mice with DN were found to be significantly reduced, as reported in recent clinical observations (Zhao et al., 2022) and animal experiments (Yu et al., 2023a; Yu et al., 2023b). Furthermore, the molecular mechanisms of HKC and its

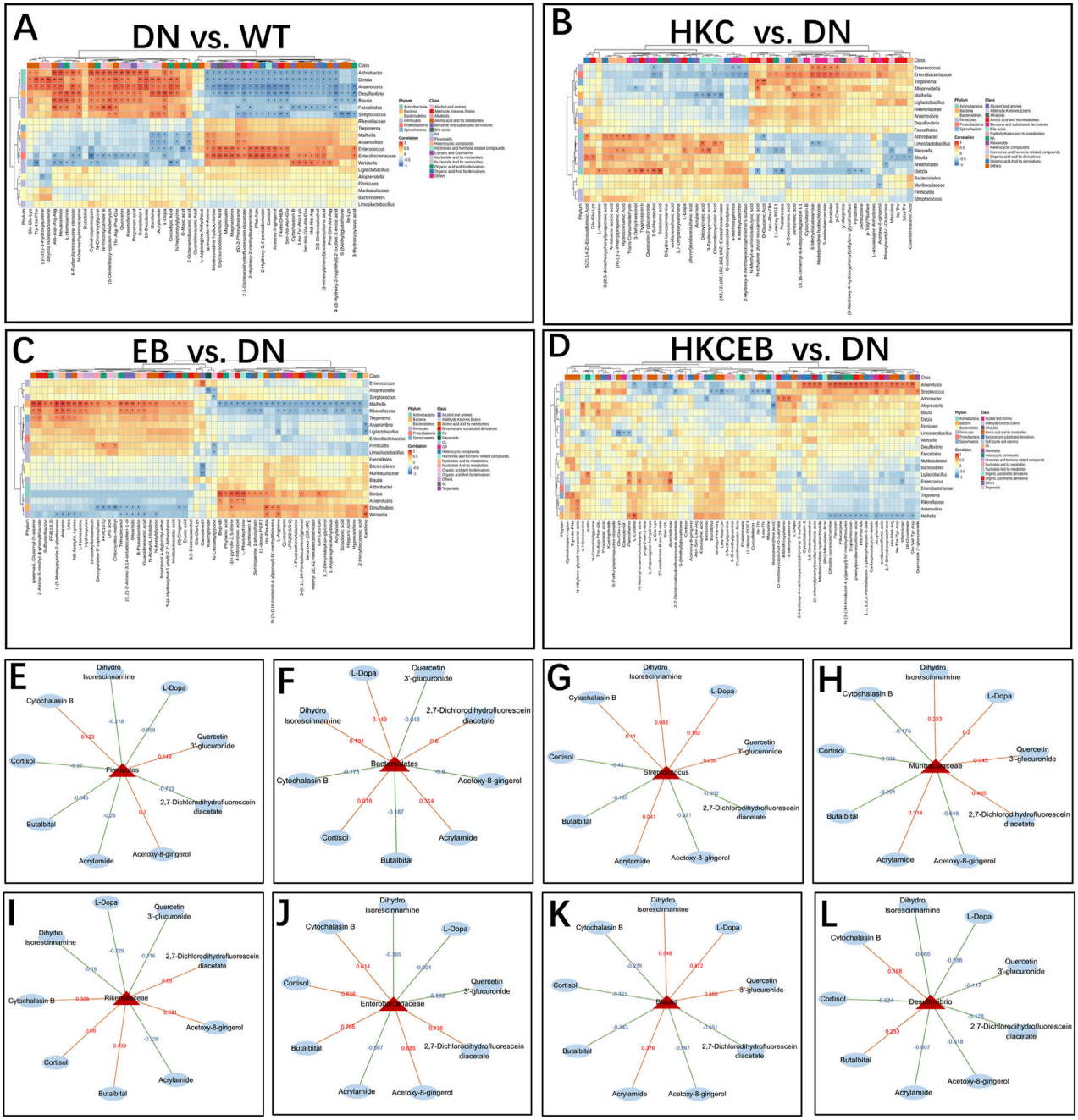


FIGURE 5
Correlation between changes to the gut microbiota and various serum metabolites **(A)** in DN compared to the WT group and **(B–D)** after treatment with HKC, EB, or HKCEB. **(E–L)** The major gut microbiota are shown, and each is correlated with several serum metabolites. The red lines indicate positive correlation while the blue lines indicate negative correlation. DN, diabetic nephropathy; WT, non-diabetic control; HKC, Huangkui capsule of *A. manihot*; EB, irbesartan; HKCEB, HKC combined with EB.

combined treatment with EB for T2D-related DN were predicted from data analyses and summarized in Figure 9.

Based on analyses of the gut microbiota, we found that microorganisms like Alloprevotella, Bacteroides, Desulfovibrio, Enterobacteriaceae, Enterococcus, Firmicutes, Treponema, Rikenellaceae, and Weissella were increased in DN compared to the WT group, while Muribaculaceae, Anaerovibrio Ligilactobacillus, Limosilactobacillus, Faecalitalea were decreased. After treatment with HKC and HKCEB, however, these microbes underwent opposite changes, with the trends in DN transitioning from increasing to decreasing or from decreasing to increasing compared to those in WT. In recent years, increasing evidence has shown that microbiota are associated with diabetes and diabetic complications, including DN (Zhao et al., 2023; Tao et al., 2019). Gut microbiota such as Firmicutes and Bacteroidetes are found to be the dominant species in rats with T2D and DN, while Firmicutes and Bacteroides can be reduced by treatment with the San-Huang-Yi-Shen capsule to improve DN (Su et al., 2022). Weissella in the

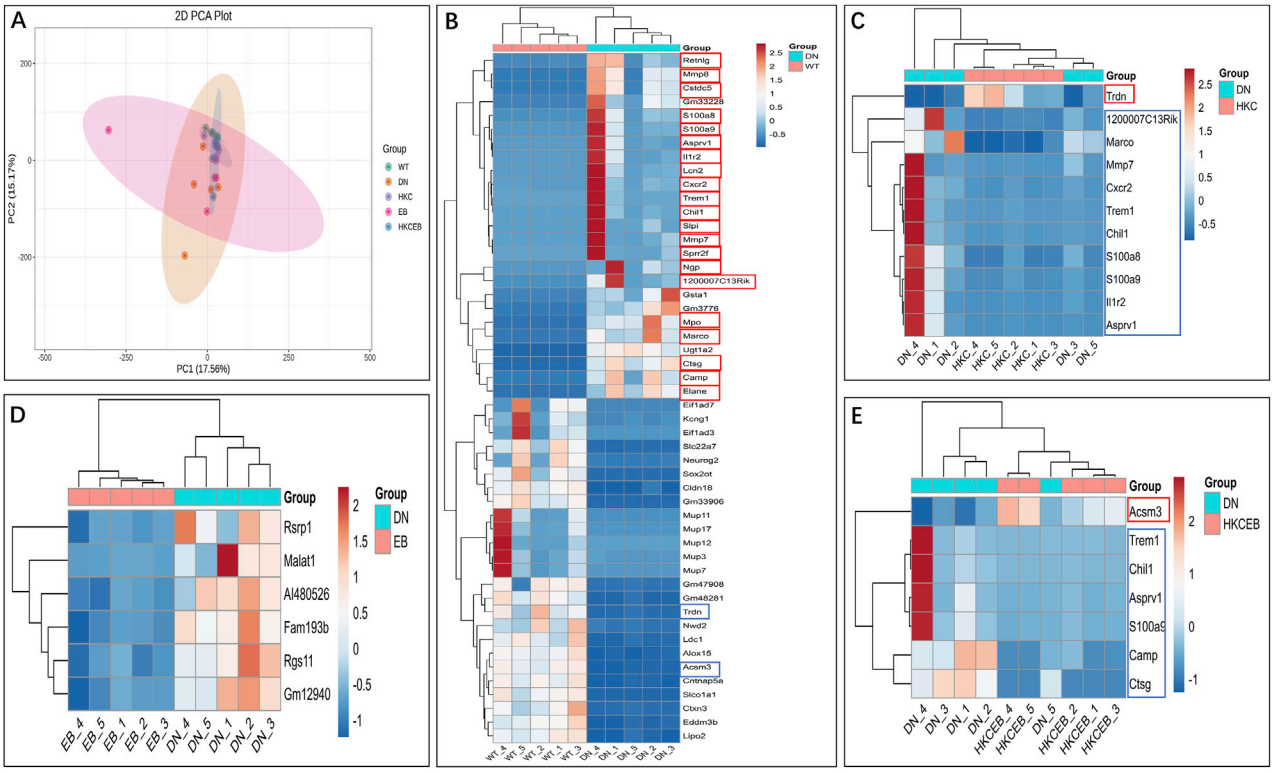


FIGURE 6
Cluster heatmap analyses of the differentially expressed genes in the kidneys. **(A)** PCoA plot and **(B–E)** cluster heatmaps show the differentially expressed genes in the kidneys of DN group compared to the WT group and after treatment with HKC, EB, or HKCEB. The red boxes indicate upregulation, while the blue boxes indicate downregulation. PCoA, principal coordinates analysis; DN, diabetic nephropathy; WT, non-diabetic control; HKC, Huangkui capsule of *A. manihot*; EB, irbesartan; HKCEB, HKC combined with EB.

intestinal flora can be reduced using white common bean extract and subsequently ameliorate T2D and its complications (Feng et al., 2022). Muribaculaceae can be increased using the sodium-glucose cotransporter 2 inhibitors canagliflozin and dapagliflozin to prevent DN progression as well as the onset of end-stage renal disease independent of lowering glucose levels (Wu et al., 2023). Limosilactobacillus could decrease blood glucose levels in db/db mice and alleviate diabetes-mediated liver and kidney damage (Hsieh et al., 2020), so it has been considered as a probiotic in diabetic patients (Lacerda et al., 2022). Furthermore, Enterobacteriaceae, Blautia, and Rikenellaceae are found to be related to proteinuria and albuminuria in DN (Su et al., 2022; He et al., 2021). The Enterobacteriaceae, Enterococcus, and Desulfovibrio were significantly increased in the intestinal flora of patients with diabetic kidney disease, and Enterobacteriaceae was positively correlated with urinary proteins in fecal samples of adult patients with chronic kidney disease and idiopathic nephrotic syndrome (He et al., 2021).

In the analyses of the serum metabolites, several metabolites such as cortisol and cytochalasin B were found to be elevated in the DN group. After treatment with HKC and HKCEB, these two metabolites were regulated in opposite directions. Several clinical studies have shown that cortisol is higher in the plasma of patients with T2D-related DN (Devi et al., 2019). Serum cortisol levels in T2D patients and prediabetic subjects are elevated and associated with high levels of microalbuminuria (Zhang et al., 2020). Cytochalasin B is as a cytopermeable mycotoxin that can inhibit the loss of nephrin in the podocytes to reduce proteinuria (Doublier et al., 2003; Doublier et al., 2005). Furthermore, quercetin and L-dopa were found to be downregulated in the serum of db/db mice with DN. After treatment with HKC, quercetin 3′-glucuronide (the main metabolite of quercetin) was upregulated; however, this was not true for HKCEB. A previous study indicated that quercetin 3′-glucuronide can improve podocyte injury in DN rats by inhibiting oxidative stress and the TGF-β1/Smad pathway (Guo et al., 2013). Interestingly, L-dopa or 3,4-dihydroxyphenylalanine is a chiral amino acid generated via biosynthesis of L-tyrosine (Giuri et al., 2021); experimental studies have suggested that this small peptide can be used to induce hyperhomocysteinemia and therapeutically prevent the progression of Parkinson's disease (Barthelmebs et al., 1991) while suppressing streptozotocin-induced diabetic glomerular hyperfiltration, subsequently preventing the progress of DN (Barthelmebs et al., 1990).

Furthermore, transcriptomics analyses demonstrated that genes such as *Trem1*, *S100a8*, *S100a9*, and *Mmp7* are higher in the kidneys of patients with DN compared to those in the WT group. The triggering receptor expressed on monocytes 1 (*Trem1*) gene amplifies neutrophil- and monocyte-mediated inflammatory responses triggered by bacterial and fungal infections by stimulating the release of proinflammatory chemokines and cytokines. As an amplifier of inflammation, *Trem1* has been shown to have a role in cIgA1-induced kidney injury (Zhao et al., 2018) and in maintaining tubular homeostasis through

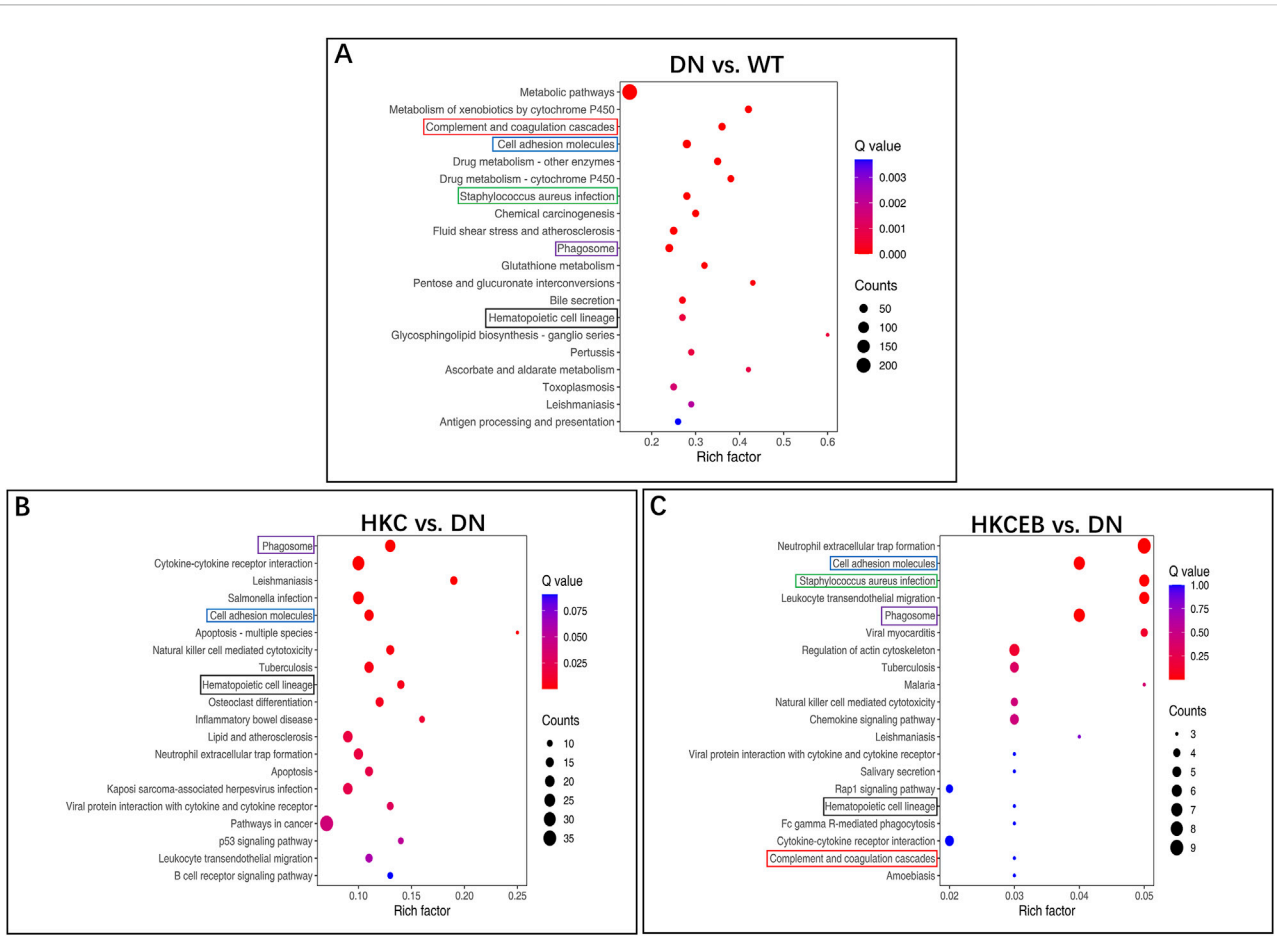


FIGURE 7
KEGG pathway enrichment analysis of the differentially expressed genes in the kidneys **(A)** for DN compared to the WT group and **(B, C)** after treatment with HKC or HKCEB. DN, diabetic nephropathy; WT, non-diabetic control; HKC, Huangkui capsule of *A. manihot*; HKCEB, HKC combined with EB.

regulation of mitochondrial metabolic flexibility (Tammaro et al., 2019). The *S100a8* and *S100a9* genes encode myeloid-related protein 8 (MRP8) and migration inhibitory factor related protein 14 (MRP14), respectively; these two proteins are associated with immune and inflammation responses (Sreejit et al., 2020). Du et al. (2023) recently reported that the activities of S100A8 and S100A9 are increased in the tubular epithelial cells under DN. Kuwabara et al. previously reported that the *S100a8* gene expression in the glomeruli of the kidneys is associated with the progression of proteinuria and albuminuria in patients with obesity and T2D; they suggested that *S100a8* may induce inflammatory changes in macrophages via TLR4 signaling (Kuwabara et al., 2014). The matrix metalloproteinase 7 (*Mmp7*) gene expression is strongly correlated with fibrosis and with eGFR (Yu et al., 2023a; Yu et al., 2023b). Hirohama et al. (2023) recently conducted a proteomics analysis of kidney samples from patients with DN and identified *Mmp7* as a diagnostic marker of kidney fibrosis. Recent studies have shown that *A. manihot* polysaccharide (AMP) fortifies the intestinal mucus barrier by increasing mucus production, which plays a crucial role in AMP-mediated amelioration of colitis. The effects of AMP on mucus production are dependent on IL-10. These findings suggest that plant polysaccharides fortify the intestinal mucus barrier by maintaining homeostasis of the gut microbiome (Wang et al., 2024). MMP-7 is implicated in regulating kidney homeostasis and diseases and barely expressed in normal adult kidney but upregulated in acute kidney injury (AKI) and chronic kidney disease (CKD) (Liu et al., 2020). Decoction of white aconite (DWA) suppressed mRNA expression of fibrosis markers include Collagen I, CTGF, TGF-β, inhibited protein levels of MMP-9, α-SMA, and Galectin-3, while elevating TIMP1 expression (Xing et al., 2024b). Furthermore, Urine MMP7 as a kidney injury biomarker (Avello et al., 2023). In the present study, we found that these three genes were downregulated by HKC; furthermore, the *Trdn* gene expression in the DN group was lower than that in the WT group, which is a unique gene that is downregulated after treatment with HKC but not EB.

Based on the findings regarding the gut microbiota, serum metabolites, and gene activities in the kidneys, we conducted further correlation analysis and found that Enterobacteriaceae, Rikenellaceae, and Blautia were positively correlated with cortisol and cytochalasin B but negatively correlated with quercetin 3′-glucuronide and L-dopa. Furthermore, *Trem1*, *S100a8*, *S100a9*, and *Mmp7* were positively correlated with cortisol and cytochalasin B but negatively correlated with quercetin 3′-glucuronide, L-dopa, and dihydro isorescinnamine. All these

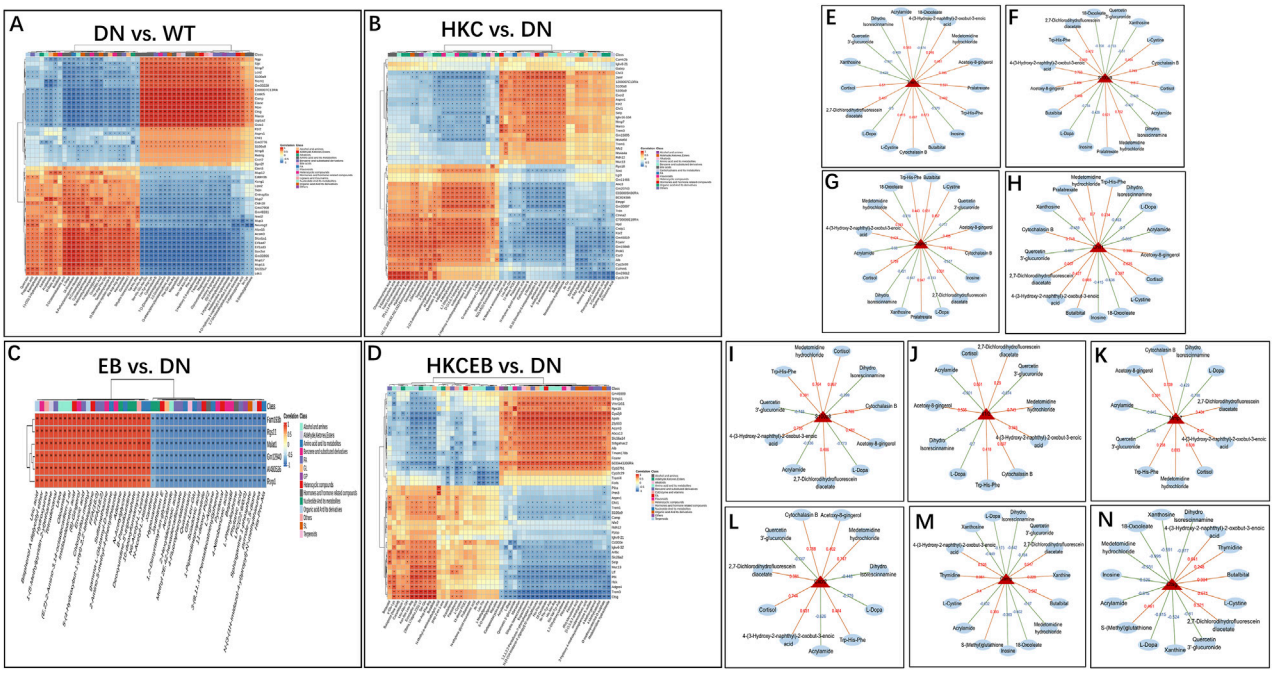


FIGURE 8
Correlation between serum metabolites and differentially expressed genes in the kidneys. **(A–D)** Heatmaps predicted by KEGG enrichment analysis show the correlations between the serum metabolites and renal genes in DN compared to WT and after treatment with HKC, EB, or HKCEB. **(E–N)** The genes expressed in the kidneys are functionally related to metabolites positively (red) or negatively (blue). DN, diabetic nephropathy; WT, non-diabetic control; HKC, Huangkui capsule of *A. manihot*; EB, irbesartan; HKCEB, HKC combined with EB.

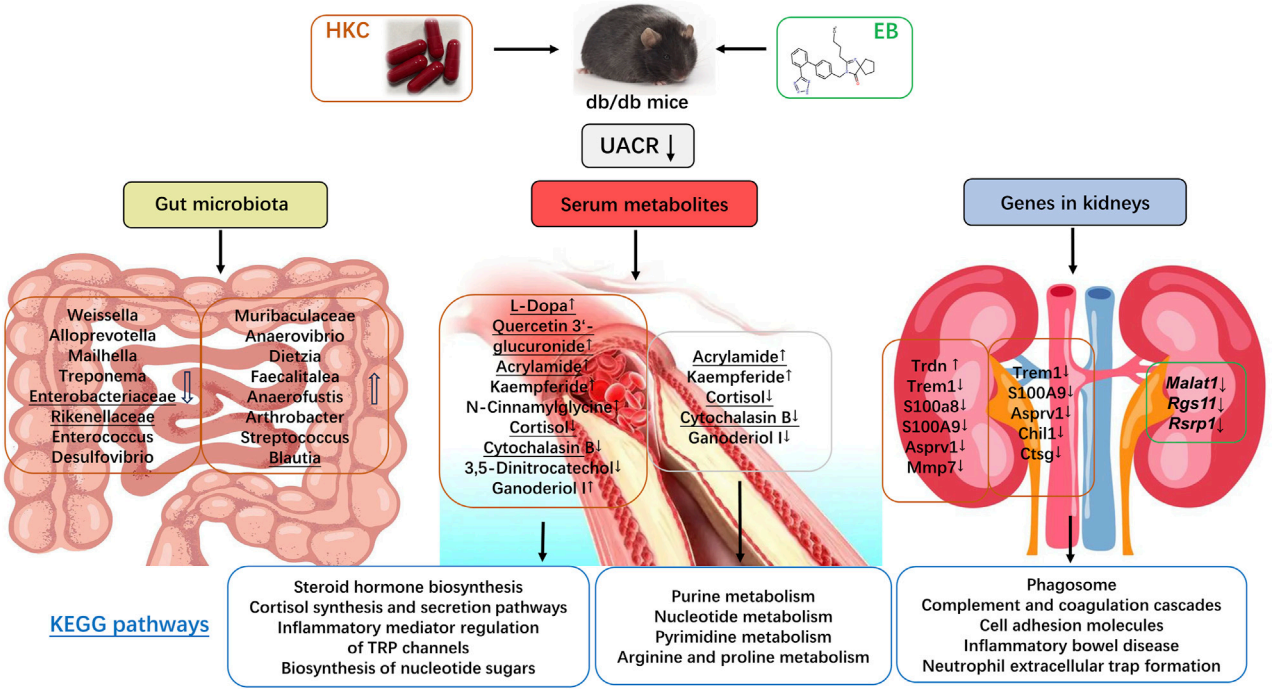


FIGURE 9
Abelmoschus manihot (L.) and its combination with irbesartan regulated the gut–kidney axis.

related changes in the gut microbiota, circulating metabolites, and renal genes have been directly or indirectly proven to be associated with the reduction of urinary protein/albumin [24–45]. Therefore, the findings of the present study suggest that HKC combined with EB has multiple effects on the gut-kidney axis and that they could be involved in reducing inflammation, improving the functions of renal reabsorption and regulation, and delaying the progress of DN.

EB is clinically used in the treatment of essential hypertension and DN (Diao et al., 2024; Doublier et al., 2005). However, no significant effects were observed for EB on regulating blood pressure in db/db mice in this study. Although several serum metabolites were found to be associated with EB, no specific pathways were found for the DEGs in the kidneys. Su et al. (2022) recently reported the effects of the San-Huang-Yi-Shen capsule on rats with DN using EB as the control. Similarly, EB was not found to cause any significant improvements in the blood glucose level and renal function (Li et al., 2017). Given these findings, there are a few limitations to the present study. First, the main active chemical constituents of HKC were found to be rutin, hyperoside, isoquercitrin, gossypetin-8-O-β-D-glucuronide, myricetin, quercetin-3-O-β-D-glucuronide, and quercetin; among these, the flavonoid that has the primary effect on DN is unknown. Second, the plasma lipids and their metabolism are not included in the analyses. Third, female db/db mice were not included in the study because of changes in their estrogen and progesterone levels. Therefore, further investigation of the chemical constituents of *A. manihot* (L.) as well as the effects of HKC on plasma lipid metabolism and females are needed.

Conclusion

The present study provides experimental evidence that *A. manihot* (L.) in the form of HKC has multiple effects on regulation of the gut-kidney axis. The data concerning changes to the gut microbiota, serum metabolites, and DEGs in the kidneys may be useful for improved understanding the mechanisms of HKC in the treatment of DN for reducing albuminuria and proteinuria.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: NCBI (accession: PRJNA1069301).

Ethics statement

The animal study was approved by the ethics committee of China Pharmaceutical University. The study was conducted in accordance with all local legislations and institutional requirements.

Author contributions

HY: Writing–original draft, Methodology, Investigation, Formal Analysis, Data curation. HT: Writing–review and editing, Validation, Resources. RS: Investigation, Writing–review and editing. YS: Writing–review and editing, Investigation. XC: Writing–review and editing, Investigation. JX: Writing–review and editing, Investigation. NL: Validation, Funding acquisition, Writing–review and editing. SC: Writing–review and editing, Validation. HG: Writing–review and editing, Validation. WT: Supervision, Writing–review and editing, Validation. HFG: Visualization, Resources, Project administration, Funding acquisition, Conceptualization, Writing–review and editing, Validation, Supervision.

Funding

The authors declare that financial support was received for the research, authorship, and/or publication of this article. This study was supported by the Cooperation Research Project (CPU20200228) and the National Natural Science Foundation of China (NSFC-82104751).

Conflict of interest

HT and HG were employed by Suzhong Pharmaceutical Group 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.

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.2024.1424968/full#supplementary-material

References

Avello, A., Guerrero-Mauvecin, J., and Sanz, A. B. (2023). Urine MMP7 as a kidney injury biomarker. *Clin. Kidney J.* 17, sfad233. doi:10.1093/ckj/sfad233

Barthelmebs, M., Vailly, B., Grima, M., Velly, J., Stephan, D., Froehly, S., et al. (1991). Effects of dopamine prodrugs and fenoldopam on glomerular hyperfiltration in streptozotocin-induced diabetes in rats. *J. Cardiovasc Pharmacol.* 18, 243–253. doi:10.1097/00005344-199108000-00011

Barthelmebs, M., Vailly, B., Velly, J., Grima, M., and Imbs, J. L. (1990). L-dopa and streptozotocin-induced diabetic nephropathy in rats. *Am. J. Hypertens.* 3, 72S-74S–74S. doi:10.1093/ajh/3.6.72s

Chen, Y., Cai, G., Sun, X., and Chen, X. (2016). Treatment of chronic kidney disease using a traditional Chinese medicine, Flos Abelmoschus manihot (Linnaeus) Medicus (Malvaceae). *Clin. Exp. Pharmacol. Physiol.* 43, 145–148. doi:10.1111/1440-1681.12528

Chong, J., and Xia, J. (2020). Using MetaboAnalyst 4.0 for metabolomics data analysis, interpretation, and integration with other omics data. *Methods Mol. Biol.* 2104, 337–360. doi:10.1007/978-1-0716-0239-3_17

Devi, S., Nongkhlaw, B., Limesh, M., Pasanna, R. M., Thomas, T., Kuriyan, R., et al. (2019). Acyl ethanolamides in Diabetes and Diabetic Nephropathy: novel targets from untargeted plasma metabolomic profiles of South Asian Indian men. *Sci. Rep.* 9, 18117. doi:10.1038/s41598-019-54584-2

Diao, Z., Yu, H., Wu, Y., Sun, Y., Tang, H., Wang, M., et al. (2024). Identification of the main flavonoids of Abelmoschus manihot (L.) medik and their metabolites in the treatment of diabetic nephropathy. *Front. Pharmacol.* 14, 1290868. doi:10.3389/fphar.2023.1290868

Doublier, S., Musante, L., Lupia, E., Candiano, G., Spatola, T., Caridi, G., et al. (2005). Direct effect of plasma permeability factors from patients with idiopatic FSGS on nephrin and podocin expression in human podocytes. *Int. J. Mol. Med.* 16, 49–58. doi:10.3892/ijmm.16.1.49

Doublier, S., Salvidio, G., Lupia, E., Ruotsalainen, V., Verzola, D., Deferrari, G., et al. (2003). Nephrin expression is reduced in human diabetic nephropathy: evidence for a distinct role for glycated albumin and angiotensin II. *Diabetes* 52, 1023–1030. doi:10.2337/diabetes.52.4.1023

Du, L., Chen, Y., Shi, J., Yu, X., Zhou, J., Wang, X., et al. (2023). Inhibition of S100A8/A9 ameliorates renal interstitial fibrosis in diabetic nephropathy. *Metabolism* 144, 155376. doi:10.1016/j.metabol.2022.155376

Feng, Y., Zhu, J., Wang, Q., Cao, H., He, F., Guan, Y., et al. (2022). White common bean extract remodels the gut microbiota and ameliorates type 2 diabetes and its complications: a randomized double-blinded placebo-controlled trial. *Front. Endocrinol. (Lausanne)* 13, 999715. doi:10.3389/fendo.2022.999715

Giuri, D., Ravarino, P., and Tomasini, C. (2021). L-Dopa in small peptides: an amazing functionality to form supramolecular materials. *Org. Biomol. Chem.* 19, 4622–4636. doi:10.1039/d1ob00378j

Guo, J. M., Lu, Y. W., Shang, E. X., Li, T., Liu, Y., Duan, J. A., et al. (2015). Metabolite identification strategy of non-targeted metabolomics and its application for the identification of components in Chinese multicomponent medicine Abelmoschus manihot L. *Phytomedicine* 22, 579–587. doi:10.1016/j.phymed.2015.02.002

Guo, X. D., Zhang, D. Y., Gao, X. J., Parry, J., Liu, K., Liu, B. L., et al. (2013). Quercetin and quercetin-3-O-glucuronide are equally effective in ameliorating endothelial insulin resistance through inhibition of reactive oxygen species-associated inflammation. *Mol. Nutr. Food Res.* 57, 1037–1045. doi:10.1002/mnfr.201200569

He, H., Lin, M., You, L., Chen, T., Liang, Z., Li, D., et al. (2021). Gut microbiota profile in adult patients with idiopathic nephrotic syndrome. *Biomed. Res. Int.* 2021, 8854969. doi:10.1155/2021/8854969

Hirohama, D., Abedini, A., Moon, S., Surapaneni, A., Dillon, S. T., Vassalotti, A., et al. (2023). Unbiased human kidney tissue proteomics identifies matrix metalloproteinase 7 as a kidney disease biomarker. *J. Am. Soc. Nephrol.* 34, 1279–1291. doi:10.1681/ASN.0000000000000141

Hsieh, P. S., Ho, H. H., Hsieh, S. H., Kuo, Y. W., Tseng, H. Y., Kao, H. F., et al. (2020). Lactobacillus salivarius AP-32 and Lactobacillus reuteri GL-104 decrease glycemic levels and attenuate diabetes-mediated liver and kidney injury in db/db mice. *BMJ Open Diabetes Res. Care* 8, e001028. doi:10.1136/bmjdrc-2019-001028

Krautkramer, K. A., Fan, J., and Bäckhed, F. (2021). Gut microbial metabolites as multi-kingdom intermediates. *Nat. Rev. Microbiol.* 19, 77–94. doi:10.1038/s41579-020-0438-4

Kuwabara, T., Mori, K., Mukoyama, M., Kasahara, M., Yokoi, H., and Nakao, K. (2014). Macrophage-mediated glucolipotoxicity via myeloid-related protein 8/toll-like receptor 4 signaling in diabetic nephropathy. *Clin. Exp. Nephrol.* 18, 584–592. doi:10.1007/s10157-013-0922-5

Lacerda, D. C., Trindade da Costa, P. C., Pontes, P. B., Carneiro Dos Santos, L. A., Cruz Neto, J. P. R., Silva Luis, C. C., et al. (2022). Potential role of Limosilactobacillus fermentum as a probiotic with anti-diabetic properties: a review. *World J. Diabetes* 13, 717–728. doi:10.4239/wjd.v13.i9.717

Lai, X., Liang, H., Zhao, Y., and Wang, B. (2009). Simultaneous determination of seven active flavonols in the flowers of Abelmoschus manihot by HPLC. *J. Chromatogr. Sci.* 47, 206–210. doi:10.1093/chromsci/47.3.206

Lewis, E. J., Hunsicker, L. G., Clarke, W. R., Berl, T., Pohl, M. A., Lewis, J. B., et al. (2001). Renoprotective effect of the angiotensin-receptor antagonist irbesartan in patients with nephropathy due to type 2 diabetes. *N. Engl. J. Med.* 345, 851–860. doi:10.1056/NEJMoa011303

Li, N., Tang, H., Wu, L., Ge, H., Wang, Y., Yu, H., et al. (2021). Chemical constituents, clinical efficacy and molecular mechanisms of the ethanol extract of Abelmoschus manihot flowers in treatment of kidney diseases. *Phytother. Res.* 35, 198–206. doi:10.1002/ptr.6818

Li, P., Chen, Y. Z., Lin, H. L., Ni, Z. H., Zhan, Y. L., Wang, R., et al. (2017). Abelmoschus manihot - a traditional Chinese medicine versus losartan potassium for treating IgA nephropathy: study protocol for a randomized controlled trial. *Trials* 18, 170. doi:10.1186/s13063-016-1774-6

Li, P., Lin, H., Ni, Z., Zhan, Y., He, Y., Yang, H., et al. (2020). Efficacy and safety of Abelmoschus manihot for IgA nephropathy: a multicenter randomized clinical trial. *Phytomedicine* 76, 153231. doi:10.1016/j.phymed.2020.153231

Liu, Z., Tan, R. J., and Liu, Y. (2020). The many faces of matrix metalloproteinase-7 in kidney diseases. *Biomolecules* 10, 960. doi:10.3390/biom10060960

Meijers, B., Evenepoel, P., and Anders, H. J. (2019). Intestinal microbiome and fitness in kidney disease. *Nat. Rev. Nephrol.* 15, 531–545. doi:10.1038/s41581-019-0172-1

Meijers, B. K., and Evenepoel, P. (2011). The gut-kidney axis: indoxyl sulfate, p-cresyl sulfate and CKD progression. *Nephrol. Dial. Transpl.* 26, 759–761. doi:10.1093/ndt/gfq818

Pang, Z., Zhou, G., Ewald, J., Chang, L., Hacariz, O., Basu, N., et al. (2022). Using MetaboAnalyst 5.0 for LC-HRMS spectra processing, multi-omics integration and covariate adjustment of global metabolomics data. *Nat. Protoc.* 17, 1735–1761. doi:10.1038/s41596-022-00710-w

Parving, H. H., Lehnert, H., Bröchner-Mortensen, J., Gomis, R., Andersen, S., Arner, P., and Irbesartan in Patients with Type 2 Diabetes and Microalbuminuria Study Group (2001). The effect of irbesartan on the development of diabetic nephropathy in patients with type 2 diabetes. *N. Engl. J. Med.* 345, 870–878. doi:10.1056/NEJMoa011489

Sharma, K., McCue, P., and Dunn, S. R. (2003). Diabetic kidney disease in the db/db mouse. *Am. J. Physiol. Ren. Physiol.* 284, F1138–F1144. doi:10.1152/ajprenal.00315.2002

Shi, R., Tao, Y., Tang, H., Wu, C., Fei, J., Ge, H., et al. (2023). Abelmoschus Manihot ameliorates the levels of circulating metabolites in diabetic nephropathy by modulating gut microbiota in non-obese diabetes mice. *Microb. Biotechnol.* 16, 813–826. doi:10.1111/1751-7915.14200

Sreejit, G., Abdel-Latif, A., Athmanathan, B., Annabathula, R., Dhyani, A., Noothi, S. K., et al. (2020). Neutrophil-derived S100a8/A9 amplify granulopoiesis after myocardial infarction. *Circulation* 141, 1080–1094. doi:10.1161/CIRCULATIONAHA.119.043833

Su, X., Yu, W., Liu, A., Wang, C., Li, X., Gao, J., et al. (2022). San-huang-yi-shen capsule ameliorates diabetic nephropathy in rats through modulating the gut microbiota and overall metabolism. *Front. Pharmacol.* 12, 808867. doi:10.3389/fphar.2021.808867

Tammaro, A., Scantlebery, A. M. L., Rampanelli, E., Borrelli, C., Claessen, N., Butter, L. M., et al. (2019). TREM1/3 deficiency impairs tissue repair after acute kidney injury and mitochondrial metabolic flexibility in tubular epithelial cells. *Front. Immunol.* 10, 1469. doi:10.3389/fimmu.2019.01469

Tao, S., Li, L., Li, L., Liu, Y., Ren, Q., Shi, M., et al. (2019). Understanding the gut-kidney axis among biopsy-proven diabetic nephropathy, type 2 diabetes mellitus and healthy controls: an analysis of the gut microbiota composition. *Acta Diabetol.* 56, 581–592. doi:10.1007/s00592-019-01316-7

Tu, Y. (2016). Artemisinin-A gift from traditional Chinese medicine to the world (nobel lecture). *Angewandte Chemie Int. ed. Engl.* 55, 10210–10226. doi:10.1002/anie.201601967

Wang, B., Chandrasekera, P. C., and Pippin, J. J. (2014). Leptin- and leptin receptor-deficient rodent models: relevance for human type 2 diabetes. *Curr. Diabetes Rev.* 10, 131–145. doi:10.2174/1573399810666140508121012

Wang, Y., Li, C., Li, J., Zhang, S., Zhang, Q., Duan, J., et al. (2024). Abelmoschus manihot polysaccharide fortifies intestinal mucus barrier to alleviate intestinal inflammation by modulating Akkermansia muciniphila abundance. *Acta Pharm. Sin. B* 14, 3901–3915. doi:10.1016/j.apsb.2024.06.002

Wu, J., Chen, Y., Yang, H., Gu, L., Ni, Z., Mou, S., et al. (2023). Sodium glucose co-transporter 2 (SGLT2) inhibition via dapagliflozin improves diabetic kidney disease (DKD) over time associatied with increasing effect on the gut microbiota in db/db mice. *Front. Endocrinol. (Lausanne)* 14, 1026040. doi:10.3389/fendo.2023.1026040

Xing, Z., Chen, Y., Chen, J., Peng, C., Peng, F., and Li, D. (2024a). Metabolomics integrated with mass spectrometry imaging reveals novel action of tetramethylpyrazine in migraine. *Food Chem.* 460, 140614. doi:10.1016/j.foodchem.2024.140614

Xing, Z., Yang, C., Feng, Y., He, J., Peng, C., and Li, D. (2024b). Understanding aconite's anti-fibrotic effects in cardiac fibrosis. *Phytomedicine* 122, 155112. doi:10.1016/j.phymed.2023.155112

Yu, H., Tang, H., Wang, M., Xu, Q., Yu, J., Ge, H., et al. (2023b). Effects of total flavones of Abelmoschus manihot (L.) on the treatment of diabetic nephropathy via the activation of solute carriers in renal tubular epithelial cells. *Biomed. Pharmacother.* 169, 115899. doi:10.1016/j.biopha.2023.115899

Yu, H., Wang, M., Yu, J., Tang, H., Xu, Q., Cheng, N., et al. (2023a). Evaluation of the efficacy of Abelmoschus manihot (L.) on diabetic nephropathy by analyzing biomarkers in the glomeruli and proximal and distal convoluted tubules of the kidneys. *Front. Pharmacol.* 14, 1215996. doi:10.3389/fphar.2023.1215996

Zhang, L., Li, P., Xing, C. Y., Zhao, J. Y., He, Y. N., Wang, J. Q., et al. (2014). Efficacy and safety of Abelmoschus manihot for primary glomerular disease: a prospective, multicenter randomized controlled clinical trial. *Am. J. Kidney Dis.* 64, 57–65. doi:10.1053/j.ajkd.2014.01.431

Zhang, X., Deng, X., Zhou, J., Qiu, K., Deng, M., Lin, Z., et al. (2020). The association of serum cortisol level with microalbuminuria in patients with type 2 diabetes and prediabetes. *Int. J. Med. Sci.* 17, 2998–3004. doi:10.7150/ijms.48742

Zhao, H., Yang, C. E., Liu, T., Zhang, M. X., Niu, Y., Wang, M., et al. (2023). The roles of gut microbiota and its metabolites in diabetic nephropathy. *Front. Microbiol.* 14, 1207132. doi:10.3389/fmicb.2023.1207132

Zhao, J., Tostivint, I., Xu, L., Huang, J., Gambotti, L., Boffa, J. J., et al. (2022). Efficacy of combined Abelmoschus manihot and irbesartan for reduction of albuminuria in patients with type 2 diabetes and diabetic kidney disease: a multicenter randomized double-blind parallel controlled clinical trial. *Diabetes care* 45, e113–e115. doi:10.2337/dc22-0607

Zhao, Y. F., Zhu, L., Liu, L. J., Shi, S. F., Lv, J. C., and Zhang, H. (2018). TREM-1 contributes to inflammation in IgA nephropathy. *Kidney Dis. (Basel)* 4, 29–36. doi:10.1159/000485622

frontiers | Frontiers in Pharmacology

TYPE Original Research
PUBLISHED 15 November 2024
DOI 10.3389/fphar.2024.1480629

Check for updates

OPEN ACCESS

EDITED BY
Swayam Prakash Srivastava,
University of Michigan, United States

REVIEWED BY
Kingsley Omage,
Oregon Health and Science University, United States
Jijia Hu,
Wuhan University, China

*CORRESPONDENCE
Ping Wang,
wp21cn@163.com
Dan Peng,
pengdan202403@163.com

†These authors have contributed equally to this work

RECEIVED 14 August 2024
ACCEPTED 05 November 2024
PUBLISHED 15 November 2024

CITATION
Ke J, Zang H, Liu Y, Teng Q, Hua J, Peng D and Wang P (2024) β-cryptoxanthin suppresses oxidative stress via activation of the Nrf2/HO-1 signaling pathway in diabetic kidney disease. *Front. Pharmacol.* 15:1480629. doi: 10.3389/fphar.2024.1480629

COPYRIGHT
© 2024 Ke, Zang, Liu, Teng, Hua, Peng 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.

β-cryptoxanthin suppresses oxidative stress via activation of the Nrf2/HO-1 signaling pathway in diabetic kidney disease

Jingjing Ke[1†], Hualong Zang[1,2†], Yang Liu[1,2], Qiuping Teng[1,2], Jiao Hua[1,2], Dan Peng[3*] and Ping Wang[1,2*]

[1]Department of Nephrology, Jingmen Central Hospital, Hubei Minzu University, Jingmen, Hubei, China, [2]Department of Nephrology, Jingmen Central Hospital Affiliated to Jingchu University of Technology, Jingmen, Hubei, China, [3]Department of Neonatology, Jingmen Central Hospital Affiliated to Jingchu University of Technology, Jingmen, Hubei, China

Objectives: This study aims to explore the role and investigate mechanisms of β-Cryptoxanthin (BCX) in high glucose (HG)-induced podocyte injury and renal dysfunction.

Methods: In this study, db/db mice were orally treated with BCX. Blood glucose, body weight, urinary albumin creatinine ratio (ACR) were recorded to evaluate the mice renal function. The H&E, PAS staining, and transmission electron microscopy (TEM) were utilized to examine the effect of BCX on the morphological changes of glomeruli in db/db mice. In addition, reactive oxygen species (ROS) content, mitochondrial membrane potential (MMP) level, ATP level, and SA-β-gal staining were used to assess the podocyte oxidative damage, mitochondrial dysfunction and senescence. Furthermore, the effects of BCX on Nrf2/HO-1 signaling pathway were evaluated *in vivo* and *in vitro* through Western blotting, immunohistochemistry and immunofluorescence analysis.

Results: *In vivo*, BCX reversed glomerular mesangial matrix expansion and reduced proteinuria in db/db mice, as well as decreased glomerular oxidative stress and kidney aging. Similarly, *in vitro* study showed that BCX effectively alleviated the oxidative stress, mitochondrial dysfunction, and senescence induced by HG in podocytes. Furthermore, we identified that the antioxidative effects of BCX are associated with the activation of Nrf2/HO-1 signaling pathway, and that Nrf2 knockdown partially abrogated the protective effects of BCX *in vitro*.

Conclusion: Our study demonstrated for the first time that BCX alleviates podocyte injury in DKD by promoting Nrf2/HO-1 signaling pathways. BCX may be a potential candidate compound for preventing Diabetic kidney disease (DKD).

KEYWORDS

β-cryptoxanthin, diabetic kidney disease, podocyte, oxidative stress, Nrf2

1 Introduction

Diabetic kidney disease (DKD) is the leading cause of end-stage renal disease (ESRD) (Rayego-Mateos et al., 2023). Current interventions, including hypoglycemic agents, sodium-glucose cotransporter protein 2 (SGLT2) inhibitors, and renin-angiotensin-aldosterone system (RAAS) blockers are not able to completely prevent DKD progress to ESRD (Dai et al., 2023). Therefore, it is imperative to explore the underlying pathogenesis of DKD and to identify more effective intervention targets. Podocytes constitute the last layer of the glomerular filtration barrier through their foot processes (Li et al., 2023). Numerous studies have confirmed that podocyte injury is closely related to the development of DKD (Lizotte et al., 2023; Salemkour et al., 2023). Thus, elucidating the mechanisms underlying podocyte injury is crucial for developing effective treatments for DKD.

In recent years, oxidative stress has been closely linked to podocyte injury and the development of DKD (Chae et al., 2023; Mohandes et al., 2023). Under physiological conditions, superoxide dismutase (SOD), the glutathione system, and catalase play vital roles in the intracellular defense against reactive oxygen species (ROS), helping to maintain redox balance within the cell (Jomova et al., 2023). However, oxidative stress arises when the production of cellular ROS exceeds the clearance capacity of the antioxidant defense systems (Jomova et al., 2023). Moreover, excessive ROS production can lead to mitochondrial dysfunction by damage in proteins, DNA, and lipids (Su et al., 2023). Oxidative stress is regarded as a major factor in DKD, and its excessive production under diabetic conditions also contributes to podocyte injury (Su et al., 2023). Notably, numerous studies have established a close relationship between oxidative stress and cellular senescence (Li et al., 2024). Senescent cells are known to be present at increased levels in many renal diseases, and it appears that senescence plays a role in maladaptive kidney repair contributing to glomerular sclerosis and proteinuria (Lucas et al., 2023). This suggests that targeting oxidative stress and cellular senescence may be a critical strategy for inhibiting the development of DKD.

Previous studies have reported that nuclear factor erythroid 2 (NFE2)-related factor 2 (Nrf2) is a transcription factor that serves as a major regulator of cellular redox homeostasis, and has been shown to prevent kidney injury and aging (Liu et al., 2023). Nrf2 deficiency is associated with increased ROS production and apoptosis in DKD (Liu et al., 2022a). Thus, pharmacologic activation of Nrf2 has emerges as a promising therapeutic strategy for addressing podocyte injury in diabetic conditions.

Mitochondria-targeted antioxidants such as mitoquinone (MitoQ) and SS-31 (d-Arg-dimethylTyr-Lys-Phe-NH_2) have demonstrated efficacy in treating kidney diseases (Kirkman et al., 2023; Shan et al., 2023). However, the high cost of synthetic antioxidants limits their widespread use. In contrast, β-cryptoxanthin (BCX) is recognized as an antioxidant and is abundantly found in fruits and vegetables like oranges, red peppers, and pumpkins (Clugston, 2023). BCX has several important biological functions that benefit human health (Granot-Hershkovitz et al., 2023). For example, BCX can delay the progression of non-alcoholic fatty liver disease (NAFLD) by alleviating insulin resistance and oxidative stress (Clugston, 2023). Additionally, a recent study found that BCX promoted Nrf2 nuclear expression, maintained mitochondrial function to inhibit H_2O_2-induced oxidative stress and cellular senescence in human renal tubular epithelial (HK-2) cells, suggesting that BCX exerts a protective role by regulating the Nrf2-mediated signaling pathway (Zhang et al., 2023). Moreover, the Mikkabi Cohort Study revealed a negative correlation between BCX and the incidence of dyslipidemia and type 2 diabetes mellitus (Sugiura et al., 2015a). Therefore, we hypothesize that supplementing with BCX may be an effective strategy to protect against podocyte injury in DKD.

In this study, we investigated the protective effect of BCX on oxidative stress, mitochondrial damage and senescence in podocytes both *in vivo* and vitro, and evaluated the role of the Nrf2 pathway in this process.

2 Materials and methods

2.1 Animal studies

Eight-week-old db/db mice and matched db/m mice were purchased from CAVENS Laboratory Animals (Jiangshu, China). In animal experiments, considering that female mice are resistant to type 2 diabetes, only male mice were used (Fu et al., 2020). After 2 weeks of adaptive feeding, the mice were randomly separated into four groups: (1) db/m group (n = 6); (2) db/m + BCX (n = 6); (3) db/db group (n = 6); (4) db/db + BCX (n = 6). Mice were given BCX (10 mg/kg/d) (HY-108059, MCE, China) in a normal diet for 6 weeks (Liu et al., 2022b). Blood glucose, body weight, 24-h proteinuria and the urinary albumin creatinine ratio (ACR) were tested every 2 weeks. At the age of 16 weeks, the kidneys and blood samples were harvested for histological and biochemical analyses. All animal experimental procedures were approved by the Ethics Committee for the Experimental Use of Animals of Hubei Minzu University (No. 2021028).

2.2 Cell culture and treatments

Conditionally immortalized podocytes were kindly provided by Dr. Moin A. Saleem (Bristol University, Bristol, United Kingdom). Briefly, podocytes were cultured at 33°C in RPMI 1640 medium (HyClone, USA) supplemented with 10% fetal bovine serum (Gibco, USA) and 1×insulin-transferrin-selenium (ITS, Gibco) for proliferation. To differentiate, the cells were cultured at 37°C in an ITS-free medium for 7–10 days. The differentiated cells were stimulated for 24 h with high glucose (HG, 30 mM). Prior to HG exposure, the cells were pretreated with 10 μM BCX for 2 h (Zhang et al., 2023). For knockdown of Nrf2, the Nrf2 siRNA (Sangon Biotech, China) was conducted using HiPerFect (Qiagen, Germany) according to the manufacturer's instructions.

2.3 SA-β-galactosidase staining

The β-galactosidase (β-gal) assay was performed using the SA-β-gal staining kit (Beyotime, C0602) according to the manufacturer's instructions. Briefly, podocytes were fixed for 10 min, and were

TABLE 1 Antibody used for Western blotting.

Source	Antibody	Dilutions	Company	Item No.
Rabbit	Nrf2	1:1,000	Proteintech	#16396-1-AP
Rabbit	HO-1	1:1,000	Proteintech	#10701-1-AP
Rabbit	Caspase3	1:1,000	CST	#9662
Mouse	Cleaved-caspase3	1:1,000	Immunoway	#YM3431
Mouse	β-actin	1:1,000	Proteintech	#66009-1-Ig
Rabbit	P16	1:1,000	Proteintech	#10883-1-AP
Rabbit	P21	1:1,000	Proteintech	#10355-1-AP
Mouse	P53	1:1,000	Proteintech	#60283-2-Ig

stained with the SA-β-gal–staining solution at pH 6.0 for 12 h. A fluorescence microscope (Leica, Germany) was used for capturing the images.

2.4 Transmission electron microscopy

For mice kidney tissues, transmission electron microscopy (TEM) was used for analysis. After harvesting the mice kidney tissues, they were fixed using glutaraldehyde and prepared as electron microscopic specimens. The ultrastructure of glomerular podocytes was assessed using transmission electron microscopy with randomly selected fields of view according to standard procedures.

2.5 Detection of mitochondrial membrane potential (MMP, △Ψm), intracellular reactive oxygen species (ROS) and ATP

The MMP of podocytes was measured by using JC-1 fluorescence probe Staining Kit (C2005, Beyotime, China); the ATP production in podocytes and kidney tissues were assayed by ATP Assay Kit (S0026, Beyotime) and relative light unit (RLU) was recorded by microplate reader. ROS production in podocytes and renal tissues was analyzed using 2′-7′-dichlorofluorescein diacetate (DCFH-DA, S0033M, Beyotime) and dihydroethidium (DHE), respectively. All procedures performed were in accordance with the manufacturer's protocol. Fluorescence microscope (Leica, Germany) was used for capturing the images.

2.6 Malonaldehyde (MDA) and glutathione (GSH) detection

The reagent in the MDA detection kit (Beyotime, S0131S) was added to the cell lysis supernatant and tissue homogenates and the mixture was incubated in a water-bath at 100°C for 15 min. After cooling to room temperature, the mixture was centrifuged for 10 min 200 μL of the supernatant was transferred into a 96-well plate and the absorbance value at 532 nm was recorded using a microplate reader (EnSight; PerkinElmer, USA).

The total GSH content in podocytes and mouse kidney tissues were measured using a GSH kit (Beyotime, S0052). Briefly, the cell lysis supernatants were collected after two cycles of freeze and thaw. Then the GSH reagent was added into the supernatants or tissue homogenates. Finally, the levels of total glutathione were measured by detecting the absorbance at 412 nm.

2.7 Western blot analysis

The total proteins were separated by 8%–12% SDS-PAGE gels and transferred onto PVDF membranes (Millipore, USA), which were blocked with 5% bovine serum albumin (BSA) solution for 2 h at room temperature. Then, the membranes were incubated with the specific primary antibodies at 4°C overnight (Table 1). After washing with TBST (Tris buffered saline with tween 20) three times, the membranes were incubated with HRP-conjugated secondary anti-rabbit or anti-mouse antibody for 1 h. Finally, the protein bands were visualized using the ImageLab software (BioRad, USA). β-actin density was used as internal control to normalize the protein expression.

2.8 Immunofluorescence staining

Five-micrometer-thick mice kidney sections (5 μm) were deparaffinized, blocked with 5% BSA buffer, and incubated overnight with corresponding primary antibodies (WT-1, 1:100, Novus, #NBP2-44607; Nrf2, 1:50, Proteintech, #16396-1-AP; Synaptopodin, 1:100, Progen, #65194) at 4°C. After washing with TBST, the sections were then incubated in horseradish peroxidase (HRP)-conjugated or Cy3-labeled secondary antibodies for 2 h and counterstained with DAPI (1 μg/mL, Invitrogen, USA) for 10 min to visualize the nuclei.

Podocytes seeded on slides were fixed with 4% paraformaldehyde for 25 min, permeabilized with 0.1% triton X-100 (Sigma, USA) for 15 min, and blocked with 1% BSA buffer for 40 min, then the podocytes were incubated with primary antibodies against Nrf2 (Proteintech, 1:100, #16396-1-AP), or (γ-H2AX Ser139, 1:100, #ab81299) at 4 °C overnight. After washing with TBST buffer, the slides were incubated with Alex Fluro 594-conjugated secondary antibody (AntGene, 1:150, China) for 35 min. The confocal microscope (Leica, Germany) was used to visualize images.

2.9 Flow cytometry

Podocytes apoptosis was determined using an Annexin V-PE/7-ADD kit (BD, USA) according to the manufacturer's instructions. Briefly, cells were seeded in six-well plates and exposed to HG and pretreated with BCX for 2 h. After digestion and centrifugation, the cells were resuspended with 100 μL binding buffer, which then supplemented with 2.5 μL Annexin V-PE and 2.5 μL 7-AAD and incubated for 20 min in the dark. The apoptotic cells were measured by CytoFLEX (Beckman, USA) and analyzed by FlowJo Software.

10.3389/fphar.2024.1480629

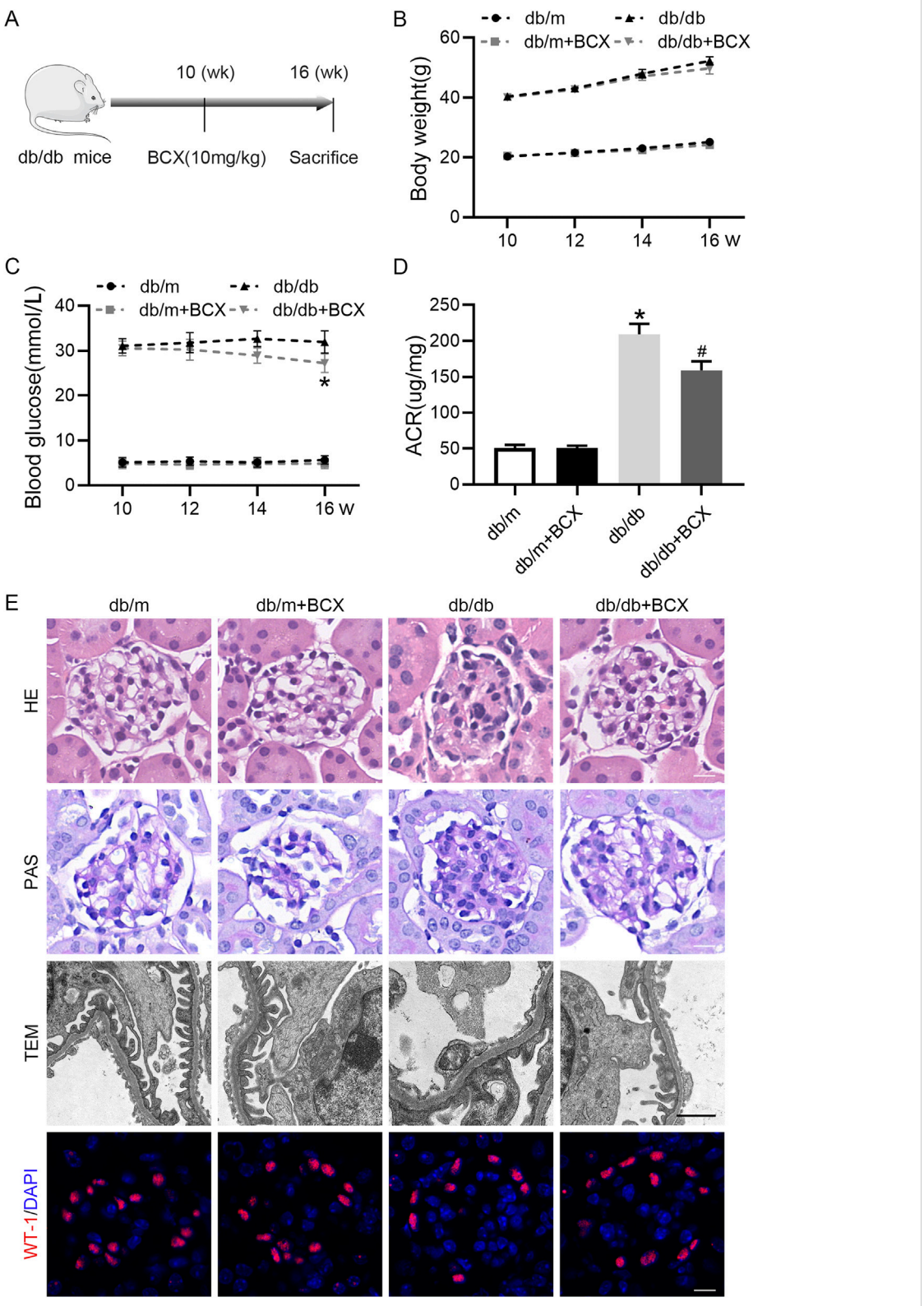


FIGURE 1
Effects of BCX on functional and morphological characteristics in kidney of db/db mice. **(A)** A schematic diagram showing the intervention-study design. **(B)** Body weight changes in each group. **(C)** Blood glucose levels in each group. **(D)** Urine ACR levels in each group. **(E)** Representative HE stanning images (scale bar = 50 μm), PAS stanning images (scale bar = 50 μm) transmission electron microscopy (TEM) images (scale bar = 1 μm), and Wilms' Tumor 1 (WT1, red) IF images (scale bar = 50 μm) in glomeruli from each group. *$p < 0.05$ vs. control group; #$p < 0.05$ vs. db/db group, n = 6.

10.3389/fphar.2024.1480629

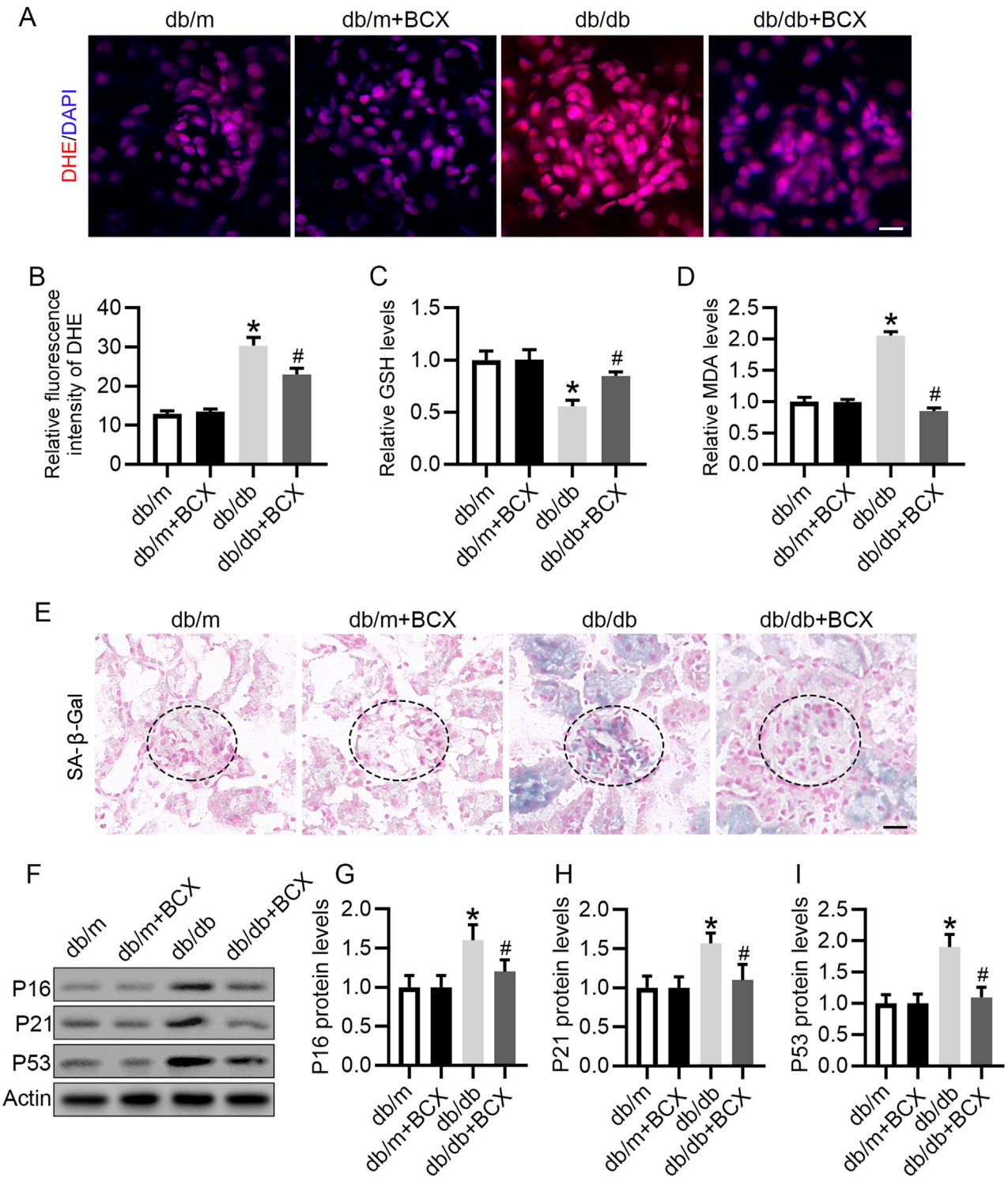


FIGURE 2
BCX attenuates oxidative stress and aging in glomeruli of db/db mice. **(A)** Oxidative stress level of glomeruli was evaluated by DHE staining from different groups, Scale bar = 10 μm. **(B)** Quantification of DHE fluorescence intensity from panel A. **(C)** GSH concentrations in kidney cortexes from each group. **(D)** MDA concentrations in kidney cortexes from each group. **(E)** Representative SA-β-gal activity staining in kidney from each group, Scale bar = 25 μm. **(F)** Representative images of P16, P21, and P53 protein expression in glomeruli from different groups, n = 6. **(G–I)** Quantitative analysis of P16, P21, and P53 protein levels from panel F $^{*}p < 0.05$ vs. control group; $^{\#}p < 0.05$ vs. db/db group, n = 6.

2.10 Statistical analysis

The data were expressed as the mean ± SD and were analyzed using GraphPad Prism 9.0 Software (GraphPad, USA). One-way analysis of variance (ANOVA) with Tukey's *post hoc* test was used to compare the difference among three or more groups. $p < 0.05$ was considered to be statistically significant.

3 Results

3.1 Effects of BCX on functional and morphological characteristics in kidneys of db/db mice

First, to evaluate the renal protective effect of BCX, we measured body weight, blood glucose, and urine albumin creatinine ratio (ACR) in mice (Figures 1A–D). The results showed that compared to db/m mice, db/db mice exhibited higher body weight and blood glucose levels. Treatment with BCX alleviated hyperglycemia at 16 weeks but had no effect on body weight (Figures 1B, C). In addition, the ACR level was significantly elevated in db/db mice, and treatment with BCX significantly reduced ACR levels (Figure 1D). Meanwhile, the HE, PAS staining, and TEM were utilized to observe the effect of BCX on the morphological changes of glomeruli in db/db mice (Figure 1E). The results revealed that db/db mice exhibited significant pathological changes, including glomerulosclerosis, mesangial matrix expansion, glomerular basement membrane (GBM) thickening, and diffuse foot process fusion, while the administration of BCX alleviated these pathological changes (Figure 1E). Moreover, WT-1 staining demonstrated that the administration of BCX also reduced diabetes-induced podocyte loss (Figure 1E).

3.2 BCX attenuates oxidative stress and aging in glomeruli of db/db mice

Considering the important role oxidative stress plays in glomerular injury in DKD (Su et al., 2023), and since BCX has been shown to exert antioxidant effects (Zhang et al., 2023), we investigated whether BCX could mitigate oxidative stress in glomeruli. DHE staining revealed a significantly increased ROS content in the glomeruli of db/db mice compared with the control group, while treatment with BCX significantly alleviated ROS accumulation in the glomeruli of db/db mice (Figures 2A, B). Additionally, the data showed that GSH level was significantly reduced and MDA level was significantly increased in the renal cortex of db/db mice, while BCX significantly inhibited the reduction of GSH level and the increase of MDA content (Figures 2C, D). Since the accumulation of oxidative stress is closely related to aging, we further examined whether BCX can mitigate kidney aging. The SA-β-gal staining results showed a markedly increased SA-β-gal activity in the glomeruli of db/db mice, which was significantly reduced by BCX treatment (Figure 2E). A defining trait of cellular senescence is cell cycle arrest, which is mainly mediated by p53/p21 and p16 pathways occurring in upregulation of p16, p21 or p53 (Lucas et al., 2023). Interestingly, BCX also inhibited the expression of senescence-related proteins such as P16, P21, and P53 in glomeruli of db/db mice (Figures 2F–I). Collectively, these results indicate that BCX can inhibit oxidative stress damage and aging in the glomeruli of diabetic mice.

3.3 BCX ameliorates HG-induced oxidative stress, mitochondrial dysfunction in podocytes

BCX has been shown to attenuate mitochondrial oxidative damage in hepatic cells (Nishino et al., 2021), but its protective effect on podocytes remains unknown. Our study found that HG exposure significantly increased ROS production in podocytes, whereas BCX pre-treatment significantly decreased ROS production (Figures 3A, C). To further investigate the effects of BCX on mitochondrial function, MMP and cellular ATP production were assessed in podocytes. HG exposure significantly decreased MMP and cellular ATP content, while pre-treatment with BCX significantly prevented these changes (Figures 3B–E). Taken together, these results suggest that BCX can mitigate oxidative stress and mitochondrial dysfunction induced by HG in podocytes.

3.4 BCX inhibits HG-induced senescence in podocytes

In vivo studies have confirmed that BCX can reduce kidney aging in db/db mice. We further explored whether BCX can reduce HG-induced podocyte senescence *in vitro*. SA-β-gal staining showed that HG significantly increase the proportion of SA-β-gal-positive cells, and pre-treatment with BCX significantly reduced SA-β-gal-positive cells (Figures 4A, B). In addition, γ-H2AX (a cellular senescence marker) staining showed that BCX markedly decreased the expression of γ-H2AX in podocytes induced by HG (Figure 4C). Moreover, BCX also inhibited HG-induced increases in senescence-related proteins such as P16, P21, and P53 (Figures 4D–G). Together, these results suggest that BCX alleviates HG-induced podocyte senescence *in vitro*.

3.5 Effects of BCX on Nrf2/HO-1 pathway in podocytes both *in vivo* and *in vitro*

Based on the previous results, we speculated that the protective effect of BCX against oxidative stress, mitochondrial dysfunction and senescence might be attributed to the activation of antioxidant genes. Since Nrf2/heme oxygenase-1 (HO-1) signaling pathway plays a key role in antioxidative stress (Lin et al., 2023), we investigated whether Nrf2 activation was involved in the protective effects of BCX. Western blot analysis revealed a significant decrease in Nrf2 and HO-1 protein levels in the glomeruli of db/db mice compared to db/m mice (Figures 5A–C). Moreover, the reduced Nrf2 protein expression in glomeruli and podocytes from db/db mice were also confirmed

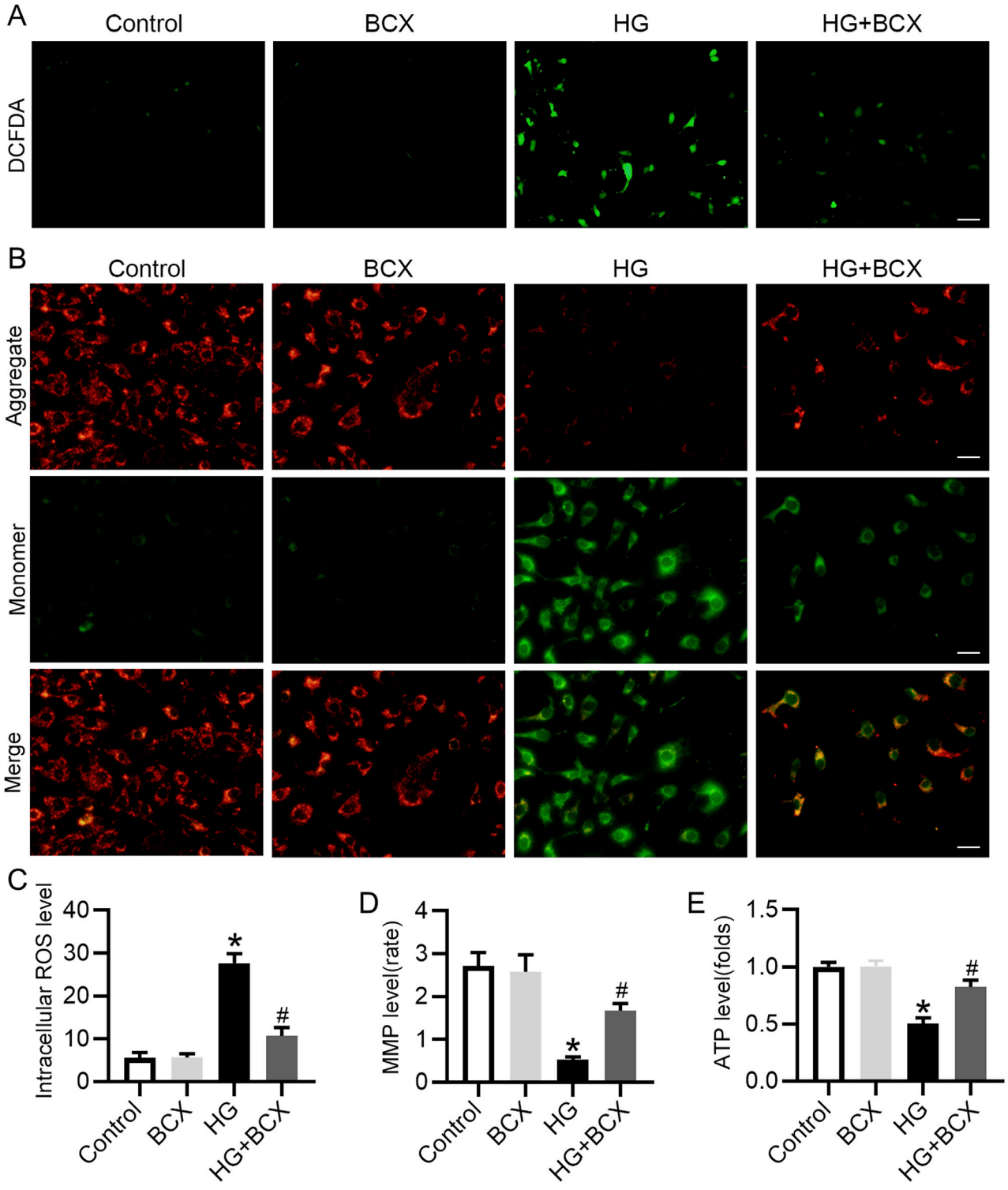


FIGURE 3
BCX ameliorates HG-induced oxidative stress, mitochondrial dysfunction in podocytes. **(A)** A representative image of DCFDA staining in podocytes from each group, Scale bar = 100 μm, n = 3. **(B)** A representative image of JC-1 staining in podocytes from each group, Scale bar = 100 μm, n = 3. **(C)** Quantitative of ROS content from panel A. **(D)**: Quantitative of mitochondrial membrane potential from panel B. **(E)**: Relative ATP content in podocytes from each group, n = 3. *$p < 0.05$ vs. control group; $^{\#}p < 0.05$ vs. HG group.

by immunohistochemical and immunofluorescence double staining (Figures 5D, E). Interestingly, BCX treatment reversed the reduction of Nrf2 and HO-1 protein levels in glomeruli and podocytes from db/db mice (Figures 5D, E). Similar to the results observed *in vivo*, the Nrf2 and HO-1 expression were decreased in HG-stimulated podocytes, which was also reversed by BCX pre-treatment (Figures

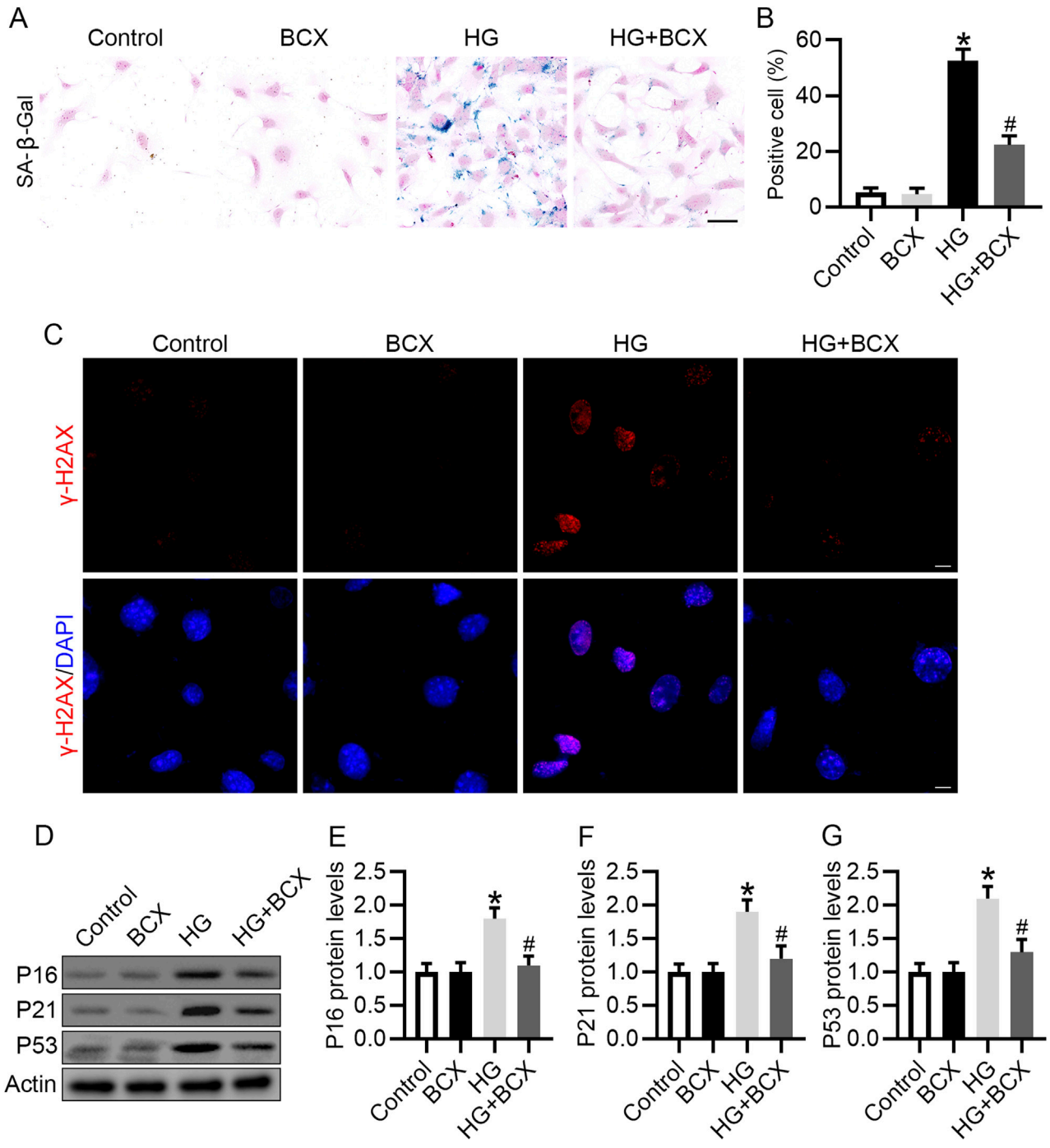


FIGURE 4
BCX inhibits HG-induced senescence in podocytes. **(A)** Representative SA-β-gal activity staining in podocytes from each group, Scale bar = 50 μm, n = 3. **(B)** Quantification of positive cells from panel A. **(C)** Representative γ-H2AX staining (red) in podocytes from each group, Scale bar = 10 μm, n = 3. **(D)** Representative images of P16, P21, and P53 protein expression in podocytes from different groups, n = 3. **(E–G)** Quantitative analysis of P16, P21, and P53 protein levels from panel D. *$p < 0.05$ vs. control group; $^{\#}p < 0.05$ vs. HG group.

5F–H). Furthermore, immunofluorescence was used to monitor the nuclear translocation of Nrf2. The results revealed a significant decrease in nuclear Nrf2 expression in HG-stimulated podocytes, which was restored by BCX pre-treatment (Figures 5I, J). Taken together, these data suggest that Nrf2 signaling may be involved in the protective effects of BCX against HG-induced oxidative stress, mitochondrial dysfunction, and senescence in podocytes.

3.6 Knockdown of Nrf2 blocked anti-oxidative stress effect of BCX in podocytes

To further validate the role of Nrf2 in the mitigation of oxidative stress and protection of mitochondrial function by BCX, Nrf2 siRNA was used to knockdown the expression of Nrf2 (Figures 6A–C). DCFH-DA staining showed that the ROS

10.3389/fphar.2024.1480629

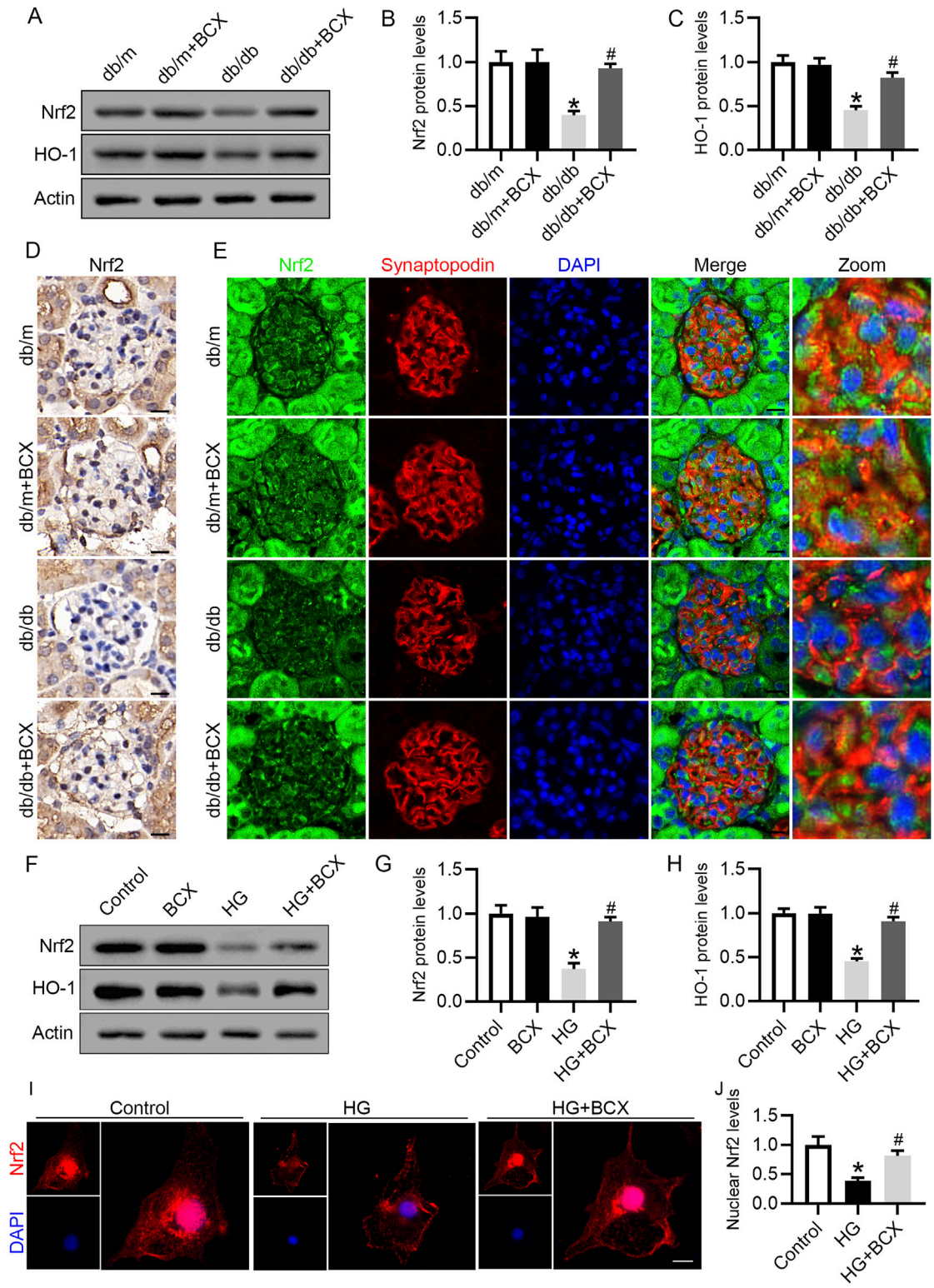


FIGURE 5
Effects of BCX on Nrf2/HO-1 pathway in podocytes *in vivo* and *in vitro* **(A)** Representative images of Nrf2 and HO-1 protein expression in glomeruli in the different groups, n = 6. **(B, C)** Quantitative analysis of Nrf2 and HO-1 protein levels from panel A. **(D)** Immunohistochemical analysis of Nrf2 in glomeruli from different groups, Scale bar = 10 μm, n = 6. **(E)** Representative fluorescence microscope images of Nrf2 (green), Synaptopodin (red), DAPI (blue) in glomeruli from different groups, Scale bar = 10 μm, n = 6. **(F)** Representative images of Nrf2 and HO-1 protein expression in podocytes, n = 3. **(G, H)** Quantitative analysis of Nrf2 and HO-1 protein levels from panel F. **(I)** Representative fluorescence microscope images of Nrf2 in podocytes, Scale bar = 20 μm, n = 3. **(J)** Quantitative analysis of nuclear expression levels of Nrf2. *$p < 0.05$ vs. db/m or control group; #$p < 0.05$ vs. db/db or HG group.

10.3389/fphar.2024.1480629

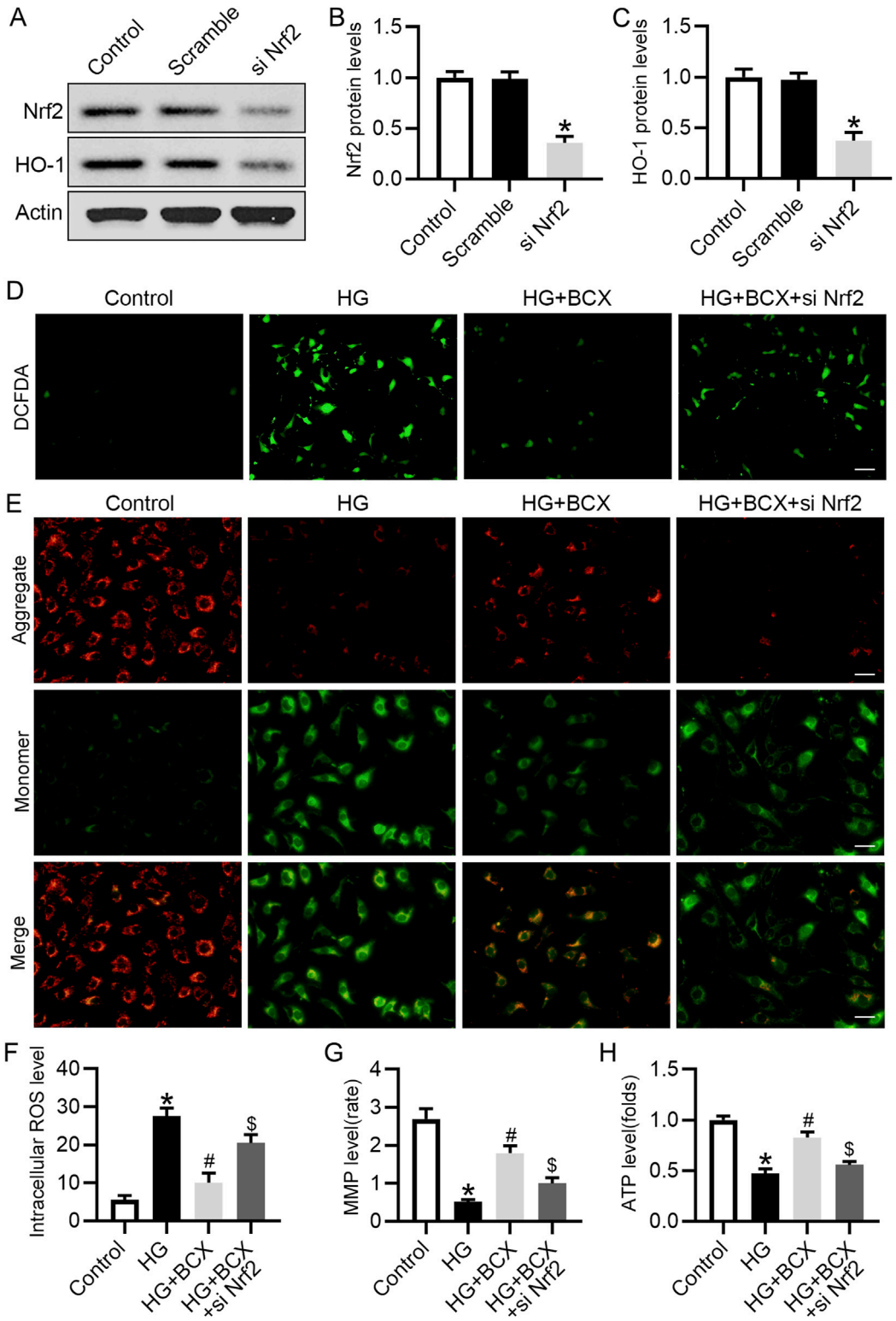


FIGURE 6
Knockdown of Nrf2 blocked anti-oxidative stress effect of BCX in podocytes. **(A)** Representative images of Nrf2 and HO-1 protein expression in podocytes, n = 3. **(B, C)** Quantitative analysis of Nrf2 and HO-1 protein levels from panel A. **(D)** A representative image of DCFDA staining in podocytes from each group, Scale bar = 100 μm, n = 3. **(E)** A representative image of JC-1 staining in podocytes from each group, Scale bar = 100 μm, n = 3. **(F)** Quantitative of ROS content from panel D. **(G)**: Quantitative of mitochondrial membrane potential from panel E. **(H)**: Relative ATP content in podocytes from each group, n = 3. *$p < 0.05$ vs. control group; $^{\#}p < 0.05$ vs. HG group, $^{\$}p < 0.05$ vs. HG + BCX group.

10.3389/fphar.2024.1480629

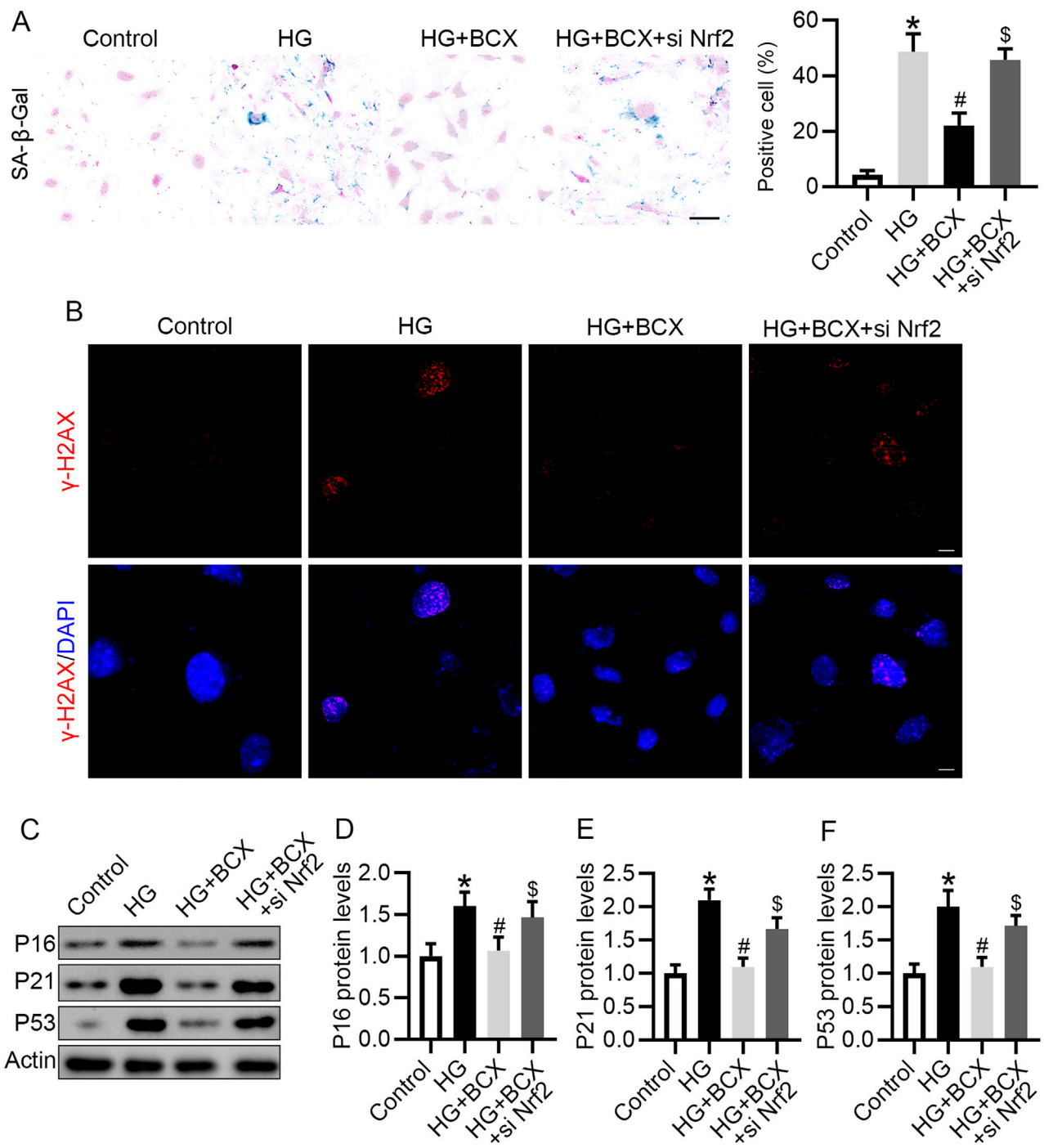


FIGURE 7
Knockdown of Nrf2 blocked the anti-senescence effect of BCX in podocytes. **(A)** Representative SA-β-gal activity staining in podocytes and the quantification of positive cells from each group, Scale bar = 50 μm, n = 3. **(B)** Representative γ-H2AX staining (red) in podocytes from each group, Scale bar = 10 μm, n = 3. **(C)** Representative images of P16, P21, and P53 protein expression in podocytes from different groups, n = 3. **(D–F)** Quantitative analysis of P16, P21, and P53 protein levels from **(D)**. $^{*}p < 0.05$ vs. control group; $^{\#}p < 0.05$ vs. HG group, $^{\$}p < 0.05$ vs. HG + BCX group.

content was significantly increased in podocytes under HG conditions, but was decreased by BCX pre-treatment, and this effect was partially abolished by Nrf2 siRNA transfection (Figures 6D, F). In addition, BCX inhibited the HG-induced decrease in MMP and ATP levels, and these effects were abolished by Nrf2 siRNA transfection (Figures 6E–H). These results suggest that BCX mitigates oxidative stress and mitochondrial damage partially through the Nrf2/HO-1 pathway.

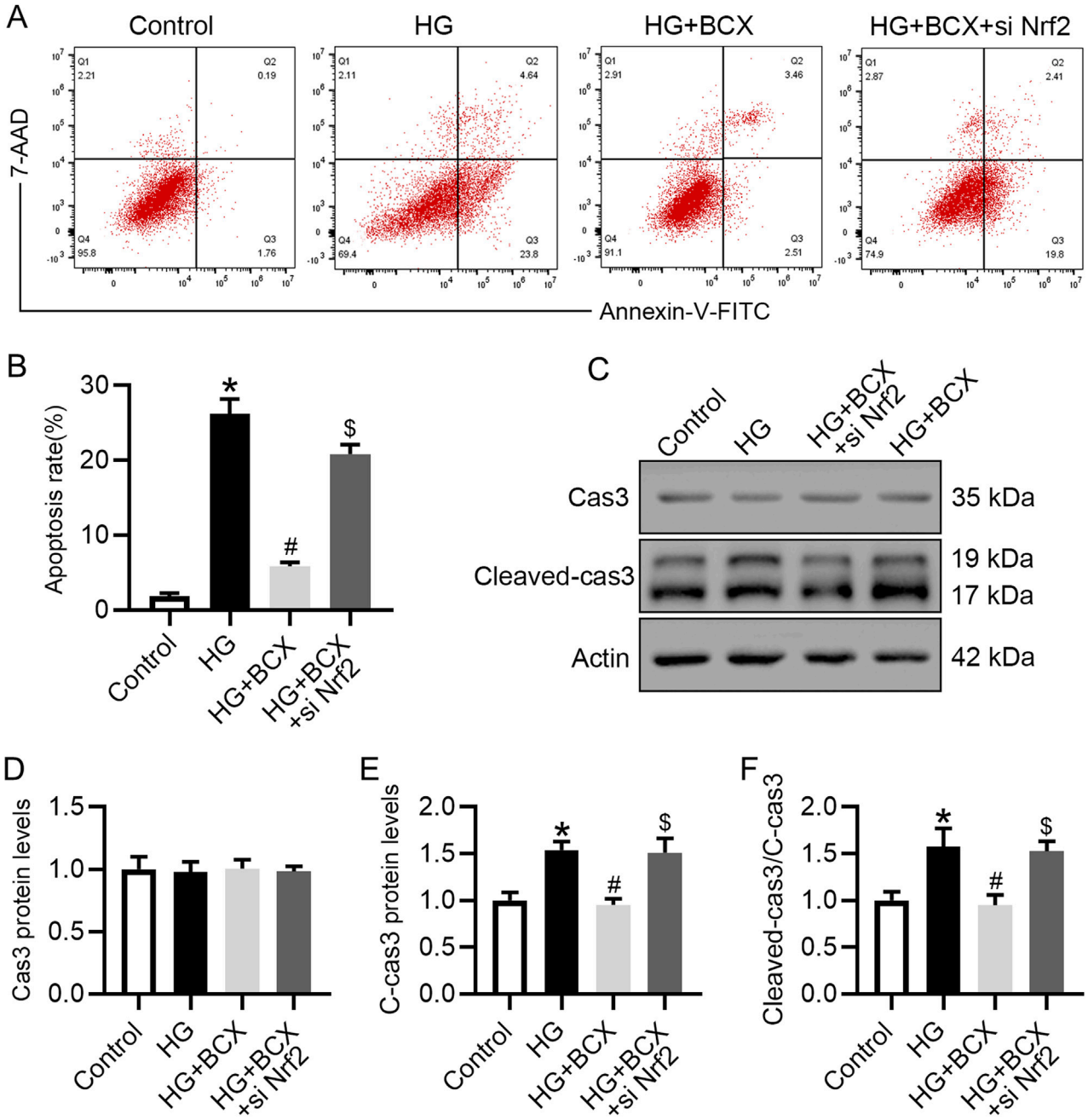


FIGURE 8
Silencing Nrf2 reversed the protective effect of BCX against the HG-induced podocyte apoptosis. **(A)** Flow cytometry analysis of podocyte apoptosis in different groups, n = 3. **(B)** The apoptosis rates from panel A. **(C)** Representative images of Cas3 and cleaved-cas3 protein expression in podocytes, n = 3. **(D–F)** Quantitative analysis of Cas3, cleaved-cas3 protein levels and cleaved-cas3/Cas3 ration from panel C. $^{*}p < 0.05$ vs. control group; $^{\#}p < 0.05$ vs. HG group, $^{\$}p < 0.05$ vs. HG + BCX group.

3.7 Knockdown of Nrf2 blocked the anti-senescence effect of BCX in podocytes

Next, we evaluated whether BCX plays an anti-senescence role by activating Nrf2. BCX significantly reduced the number of senescent cells under HG conditions, however, knockdown of Nrf2 partially abolished the effect of BCX on the clearance of senescent cells (Figure 7A). Consistent with previous results, pre-treatment with BCX significantly decreased the expression of senescence-related proteins, including γ-H2AX, P16, P21, and P53, while knockdown of Nrf2 blocked the downregulation of senescence-related proteins induced by BCX treatment (Figures 7B–F).

3.8 Silencing Nrf2 reversed anti-apoptotic effects of BCX in podocytes

Previous studies have shown that oxidative stress, mitochondrial damage and senescence can initiate cellular apoptosis (Su et al.,

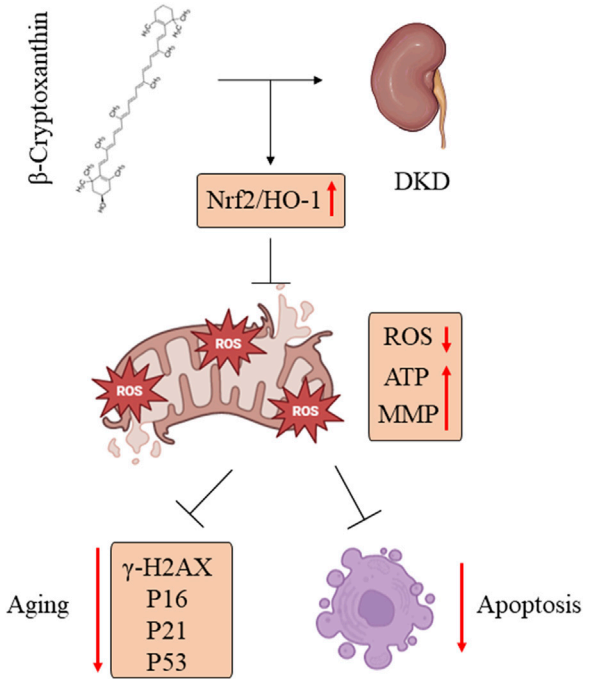


FIGURE 9
BCX alleviated podocyte oxidative stress, mitochondrial dysfunction, and senescence in DKD by promoting Nrf2/HO-1 signaling pathways.

2023). Therefore, we also explored whether BCX could ameliorate podocyte apoptosis under HG conditions and evaluated the role of Nrf2 in this process. Podocyte apoptosis was measured using flow cytometry, and the data indicated that treatment with BCX significantly ameliorated HG-induced podocyte apoptosis. However, this effect was partially diminished by Nrf2 siRNA transfection (Figures 8A, B). In addition, the Western blot showed that HG increased the expression of apoptosis marker cleaved-caspase3 (cleaved-cas3) rather than total Cas3 in podocytes, and BCX treatment decreased upregulated cleaved-cas3, and this effect was eliminated by Nrf2 siRNA transfection (Figures 8C–F).

4 Discussion

In the present study, we demonstrated that BCX was able to inhibit HG-induced oxidative stress, mitochondrial damage, and senescence in podocytes. Additionally, we explored the potential mechanisms underlying these effects. Our findings indicated that the protective effect of BCX on podocytes is partly mediated by activating the Nrf2/HO-1 signaling pathway (Figure 9). Overall, our findings provide experimental support for further investigation of the protective role of BCX in DKD.

Numerous studies have established that oxidative stress is a major factor in the development and progression of DKD (Ma et al., 2023; Wu et al., 2023a). The kidney is one of the most energy-demanding organs and is second only to the heart in the expression of proteins related to mitochondrial function and oxygen consumption (Hoogstraten et al., 2024). This enormous energy consumption can sometimes lead to increased oxidative stress (Teixeira et al., 2023). In the oxidative phosphorylation (OXPHOS) pathway, the electron transport chain (ETC) generates most of the mitochondrial ROS (mtDNA), and excessive ROS production progressively damages mitochondria, leading to a decrease in the efficiency of the ETS, a further increase in the level of ROS and a decrease in the MMP and ATP production, which ultimately trigger apoptosis and senescence (Raut and Khullar, 2023). Furthermore, oxidative stress is a crucial factor driving senescence of podocytes (Chen et al., 2024). ROS can cause DNA damage and double-strand breaks, activating the p53-mediated DNA damage response, which upregulates p21 and p16, leading to the cell cycle arrest and checkpoint enforcement, ultimately resulting in cellular senescence (Achanta and Huang, 2004). Recent studies have also shown that oxidative damage and senescence of podocytes are involved in the progression of DKD (Chen et al., 2024). Our study confirmed that HG can induce oxidative stress, mitochondrial damage, and apoptosis in podocytes. Besides, our data showed that HG stimulation increases the number of SA-β-gal positive cells, as well as the expression of P16, P21 and P53, and promotes the production of the cellular senescence marker γ-H2AX in podocyte. Therefore, inhibiting oxidative stress damage and senescence is an important measure to protect podocyte injury in the diabetic state.

Currently, many researchers are focusing on the development of potent antioxidant drugs (Sakashita et al., 2021; Wu et al., 2023b), such as MitoQ and SS-31 (Kirkman et al., 2023; Shan et al., 2023). These antioxidants have shown efficacy in treating kidney diseases (Hou et al., 2016; Xiao et al., 2017). For example, MitoQ has been demonstrated to attenuate renal tubular oxidative stress injury by reducing mitochondrial fission and promoting mitochondrial autophagy (Xiao et al., 2017). SS-31 is a mitochondria-targeted tetrapeptide that can scavenge mtROS and inhibit the opening of the mitochondrial permeability transition pore (mPTP) (Szeto et al., 2011). SS-31 significantly reduces oxidative stress and inflammation after ischemia-reperfusion (IR) injury and promote the proliferation of surviving renal tubular cells (Szeto et al., 2011). The clinical potential of such synthetic antioxidants has been described but has not yet advanced to the clinical trial stage (Gutierrez-Mariscal et al., 2020). In addition, the cost of synthetic antioxidants is relatively high, making them unsuitable for widespread use. Therefore, the search for natural antioxidants with minimal side effects and lower costs, suitable for use in foods or as medicine, remains an active area of research.

Humans primarily obtain dietary carotenoids from foods such as green and yellow vegetables and algae (Wang et al., 2024). Carotenoids have been reported to be effective in reducing the risk of lifestyle-related diseases (Terao, 2023). BCX is an antioxidant carotenoid that has recently gained attention for its role in reducing the risk of NAFLD (Clugston, 2023). BCX ameliorates NAFLD progression through a multifaceted approach, primarily due to its antioxidative stress effects (Clugston, 2023). A recent study has also found that BCX attenuates the development of NAFLD in mice by suppressing inflammation (Haidari et al., 2020). In addition, BCX has been shown to prevent cognitive dysfunction and oxidative damage in aging mice (Ma et al., 2023). This evidence suggests that BCX is an antioxidant with therapeutic potential; however, its

efficacy in delaying the progression of DKD remains unclear. We have demonstrated for the first time that BCX lowers blood glucose and attenuates glomerulosclerosis and proteinuria levels in diabetic mice. Furthermore, our findings indicate that BCX inhibited HG-induced oxidative stress, mitochondrial damage, senescence and apoptosis in podocytes *in vitro*.

The present study also explored the underlying mechanism by which BCX regulates oxidative stress, mitochondrial damage in podocytes. It is well-known that Nrf2 is a key transcription factor for maintaining intracellular redox homeostasis, and delaying cellular senescence (Lin et al., 2023). Under physiological conditions, Nrf2 is retained in the cytoplasm by Kelch-like ECH-associated protein 1 (Keap1) and is degraded through ubiquitination (Dinkova-Kostova and Copple, 2023). However, upon oxidative stimulation or in the presence of Nrf2-stimulating factors, Nrf2 dissociates from Keap1 and then translocate to the nucleus, where it activates a series of cell-protective and antioxidant genes, including HO-1 and NAD(P)H quinone dehydrogenase 1(NQO-1) (Dinkova-Kostova and Copple, 2023). Numerous studies have confirmed that Nrf2/HO-1 pathway has a protective effect on renal oxidative stress injury in diabetic mice (Liu et al., 2022b; Xiao et al., 2017). Activation of Nrf2 can delay the progression of DKD by inhibiting mitochondrial fission, ferroptosis, and inflammatory responses as well as promoting mitochondrial autophagy (Lu et al., 2023; Xiao et al., 2017). However, it remains uncertain whether BCX can attenuate HG-induced podocyte injury by activating Nrf2. A recent study found that BCX maintained mitochondrial function by promoting Nrf2 nuclear translocation to inhibit oxidative stress in HK-2 cells (Zhang et al., 2023). To determine whether BCX also activates Nrf2 in podocytes, we assessed the expression of Nrf2 and HO-1 by different methods. Our results showed that BCX reversed HG-induced downregulation of Nrf2 and HO-1 in podocytes both *in vivo* and *in vitro*. Furthermore, BCX promoted Nrf2 nuclear translocation to enhance its anti-oxidative stress capacity. Importantly, knockdown of Nrf2 in podocytes partially abolished the anti-oxidative stress and mitochondrial protective effects of BCX, suggesting that BCX confers its protective effects by activating Nrf2/HO-1 signaling pathway.

Currently, the research on BCX primarily focuses on fundamental mechanism studies and lacks convincing clinical research, particularly large-scale prospective randomized controlled trials (RCTs). The most notable study is the 'Mikkabi Cohort Study' conducted in Japan, which initially investigated the association between serum concentrations of six dietary carotenoids (including BCX) and the risk of developing lifestyle-related diseases among residents in Mikkabi town, Shizuoka Prefecture. This study revealed a negative correlation between BCX and the incidence of dyslipidemia and type 2 diabetes mellitus; however, it did not address potential adverse reactions associated with BCX (Sugiura et al., 2015a; Sugiura et al., 2015b). Therefore, there is a need for further investigation into potential adverse reactions that may arise from extensive clinical application of BCX as a pharmaceutical agent - an issue that should be addressed by the scientific community. Nevertheless, based on these findings and other research conclusions, we believe that BCX exhibits promising potential for the treatment of DKD and warrants further exploration through RCT studies.

This study also has some limitations. First, our research did not reveal how BCX activates the Nrf2 pathway. Glycogen synthase kinase 3β (GSK) may be the upstream molecule regulating Nrf2, which recent studies have confirmed that GSK3β is hyperactive in glomerular podocytes, associated with impaired Nrf2 response and premature senescence (Chen et al., 2024). Second, although this study confirmed that BCX can activate Nrf2, it did not verify *in vivo* experiments whether inhibiting Nrf2 could block the protective effect of BCX on podocyte injury in db/db mice.

In conclusion, this study demonstrates for the first time that BCX, as an antioxidant, prevents hyperglycemic-induced podocyte injury by inhibiting oxidative stress and mitochondrial dysfunction by activating the Nrf2/HO-1 signaling pathway. These findings strongly support a therapeutic potential of BCX in the treatment of podocyte injury under diabetic conditions.

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 Ethics Committee for the Experimental Use of Animals of Hubei Minzu University. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

JK: Writing–original draft, Visualization, Formal Analysis, Data curation. HZ: Writing–original draft, Methodology, Formal Analysis, Data curation. YL: Writing–original draft, Methodology. QT: Writing–original draft, Methodology. JH: Writing–original draft, Methodology. DP: Writing–review and editing, Writing–original draft, Supervision, Project administration, Funding acquisition, Conceptualization. PW: Writing–review and editing, Writing–original draft, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal Analysis, Conceptualization.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. This study was supported by the National Natural Science Foundation of China (No. 82260150), the Natural Science Foundation of Hubei Province (No. 2021CFB445), and the Science and Technology Key Project of Jingmen City (No. 2021YFZD026).

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.

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

Achanta, G., and Huang, P. (2004). Role of p53 in sensing oxidative DNA damage in response to reactive oxygen species-generating agents. *Cancer Res.* 64, 6233–6239. doi:10.1158/0008-5472.CAN-04-0494

Chae, S. Y., Kim, Y., and Park, C. W. (2023). Oxidative stress induced by lipotoxicity and renal hypoxia in diabetic kidney disease and possible therapeutic interventions: targeting the lipid metabolism and hypoxia. *Antioxidants-Basel* 12, 2083. doi:10.3390/antiox12122083

Chen, M., Fang, Y., Ge, Y., Qiu, S., Dworkin, L., and Gong, R. (2024). The redox-sensitive GSK3β is a key regulator of glomerular podocyte injury in type 2 diabetic kidney disease. *Redox Biol.* 72, 103127. doi:10.1016/j.redox.2024.103127

Clugston, R. D. (2023). β-cryptoxanthin and fatty liver disease: new insights. *Hepatobil Surg. Nutr.* 12, 450–452. doi:10.21037/hbsn-23-201

Dai, Z. C., Chen, J. X., Zou, R., Liang, X. B., Tang, J. X., and Yao, C. W. (2023). Role and mechanisms of SGLT-2 inhibitors in the treatment of diabetic kidney disease. *Front. Immunol.* 14, 1213473. doi:10.3389/fimmu.2023.1213473

Dinkova-Kostova, A. T., and Copple, I. M. (2023). Advances and challenges in therapeutic targeting of NRF2. *Trends Pharmacol. Sci.* 44, 137–149. doi:10.1016/j.tips.2022.12.003

Fu, Y., Sun, Y., Wang, M., Hou, Y., Huang, W., Zhou, D., et al. (2020). Elevation of JAML promotes diabetic kidney disease by modulating podocyte lipid metabolism. *Cell Metab.* 32, 1052–1062. doi:10.1016/j.cmet.2020.10.019

Granot-Hershkovitz, E., He, S., Bressler, J., Yu, B., Tarraf, W., Rebholz, C. M., et al. (2023). Plasma metabolites associated with cognitive function across race/ethnicities affirming the importance of healthy nutrition. *Alzheimers Dement.* 19, 1331–1342. doi:10.1002/alz.12786

Gutierrez-Mariscal, F. M., Arenas-de, L. A., Limia-Perez, L., Romero-Cabrera, J. L., Yubero-Serrano, E. M., and López-Miranda, J. (2020). Coenzyme Q (10) supplementation for the reduction of oxidative stress: clinical implications in the treatment of chronic diseases. *Int. J. Mol. Sci.* 21, 7870. doi:10.3390/ijms21217870

Haidari, F., Hojhabrimanesh, A., Helli, B., Seyedian, S. S., and Ahmadi-Angali, K. (2020). An energy-restricted high-protein diet supplemented with β-cryptoxanthin alleviated oxidative stress and inflammation in nonalcoholic fatty liver disease: a randomized controlled trial. *Nutr. Res.* 73, 15–26. doi:10.1016/j.nutres.2019.08.009

Hoogstraten, C. A., Hoenderop, J. G., and de Baaij, J. (2024). Mitochondrial dysfunction in kidney tubulopathies. *Annu. Rev. Physiol.* 86, 379–403. doi:10.1146/annurev-physiol-042222-025000

Hou, Y., Li, S., Wu, M., Wei, J., Ren, Y., Du, C., et al. (2016). Mitochondria-targeted peptide SS-31 attenuates renal injury via an antioxidant effect in diabetic nephropathy. *Am. J. Physiol-Renal* 310, F547–F559. doi:10.1152/ajprenal.00574.2014

Jomova, K., Raptova, R., Alomar, S. Y., Alwasel, S. H., Nepovimova, E., Kuca, K., et al. (2023). Reactive oxygen species, toxicity, oxidative stress, and antioxidants: chronic diseases and aging. *Arch. Toxicol.* 97, 2499–2574. doi:10.1007/s00204-023-03562-9

Kirkman, D. L., Stock, J. M., Shenouda, N., Bohmke, N. J., Kim, Y., Kidd, J., et al. (2023). Effects of a mitochondrial-targeted ubiquinol on vascular function and exercise capacity in chronic kidney disease: a randomized controlled pilot study. *Am. J. Physiol-Renal* 325, F448–F456. doi:10.1152/ajprenal.00067.2023

Li, D., Yu, Q., Wu, R., Tuo, Z., Wang, J., Ye, L., et al. (2024). Interactions between oxidative stress and senescence in cancer: mechanisms, therapeutic implications, and future perspectives. *Redox Biol.* 73, 103208. doi:10.1016/j.redox.2024.103208

Li, X., Zhang, Y., Xing, X., Li, M., Liu, Y., Xu, A., et al. (2023). Podocyte injury of diabetic nephropathy: novel mechanism discovery and therapeutic prospects. *Biomed. Pharmacother.* 168, 115670. doi:10.1016/j.biopha.2023.115670

Lin, D. W., Hsu, Y. C., Chang, C. C., Hsieh, C. C., and Lin, C. L. (2023). Insights into the molecular mechanisms of NRF2 in kidney injury and diseases. *Int. J. Mol. Sci.* 24, 6053. doi:10.3390/ijms24076053

Liu, C., Rafacho, B., and Wang, X. D. (2022a). Xanthophyll β-cryptoxanthin treatment inhibits hepatic steatosis without altering vitamin A status in β-carotene 9',10'-oxygenase knockout mice. *Hepatobil Surg. Nutr.* 11, 188–198. doi:10.21037/hbsn-20-404

Liu, Y., Uruno, A., Saito, R., Matsukawa, N., Hishinuma, E., Saigusa, D., et al. (2022b). Nrf2 deficiency deteriorates diabetic kidney disease in Akita model mice. *Redox Biol.* 58, 102525. doi:10.1016/j.redox.2022.102525

Liu, Y., Wang, S., Jin, G., Gao, K., Wang, S., Zhang, X., et al. (2023). Network pharmacology-based study on the mechanism of ShenKang injection in diabetic kidney disease through Keap1/Nrf2/Ho-1 signaling pathway. *Phytomedicine* 118, 154915. doi:10.1016/j.phymed.2023.154915

Lizotte, F., Rousseau, M., Denhez, B., Lévesque, D., Guay, A., Liu, H., et al. (2023). Deletion of protein tyrosine phosphatase SHP-1 restores SUMOylation of podocin and reverses the progression of diabetic kidney disease. *Kidney Int.* 104, 787–802. doi:10.1016/j.kint.2023.06.038

Lu, Q., Yang, L., Xiao, J. J., Liu, Q., Ni, L., Hu, J. W., et al. (2023). Empagliflozin attenuates the renal tubular ferroptosis in diabetic kidney disease through AMPK/NRF2 pathway. *Free Radic. Bio Med.* 195, 89–102. doi:10.1016/j.freeradbiomed.2022.12.088

Lucas, V., Cavadas, C., and Aveleira, C. A. (2023). Cellular senescence: from mechanisms to current biomarkers and senotherapies. *Pharmacol. Rev.* 75, 675–713. doi:10.1124/pharmrev.122.000622

Ma, X., Ma, J., Leng, T., Yuan, Z., Hu, T., Liu, Q., et al. (2023). Advances in oxidative stress in pathogenesis of diabetic kidney disease and efficacy of TCM intervention. *Ren. Fail.* 45, 2146512. doi:10.1080/0886022X.2022.2146512

Mohandes, S., Doke, T., Hu, H., Mukhi, D., Dhillon, P., and Susztak, K. (2023). Molecular pathways that drive diabetic kidney disease. *J. Clin. Invest.* 133, e165654. doi:10.1172/JCI165654

Nishino, A., Maoka, T., and Yasui, H. (2021). Preventive effects of β-cryptoxanthin, a potent antioxidant and provitamin A carotenoid, on lifestyle-related diseases-A central focus on its effects on non-alcoholic fatty liver disease (NAFLD). *Antioxidants-Basel* 11, 43. doi:10.3390/antiox11010043

Raut, S. K., and Khullar, M. (2023). Oxidative stress in metabolic diseases: current scenario and therapeutic relevance. *Mol. CELL Biochem.* 478, 185–196. doi:10.1007/s11010-022-04496-z

Rayego-Mateos, S., Rodrigues-Diez, R. R., Fernandez-Fernandez, B., Mora-Fernández, C., Marchant, V., Donate-Correa, J., et al. (2023). Targeting inflammation to treat diabetic kidney disease: the road to 2030. *Kidney Int.* 103, 282–296. doi:10.1016/j.kint.2022.10.030

Sakashita, M., Tanaka, T., and Inagi, R. (2021). Metabolic changes and oxidative stress in diabetic kidney disease. *Antioxidants-Basel* 10, 1143. doi:10.3390/antiox10071143

Salemkour, Y., Yildiz, D., Dionet, L., T, H. D., Verheijden, K., Saito, R., et al. (2023). Podocyte injury in diabetic kidney disease in mouse models involves TRPC6-mediated calpain activation impairing autophagy. *J. Am. Soc. Nephrol.* 34, 1823–1842. doi:10.1681/ASN.0000000000000212

Shan, Z., Wang, Y., Qiu, T., Zhou, Y., Zhang, Y., Hu, L., et al. (2023). SS-31 alleviated nociceptive responses and restored mitochondrial function in a headache mouse model via Sirt3/Pgc-1α positive feedback loop. *J. Headache Pain* 24, 65. doi:10.1186/s10194-023-01600-6

Su, S., Ma, Z., Wu, H., Xu, Z., and Yi, H. (2023). Oxidative stress as a culprit in diabetic kidney disease. *Life Sci.* 322, 121661. doi:10.1016/j.lfs.2023.121661

Sugiura, M., Nakamura, M., Ogawa, K., Ikoma, Y., and Yano, M. (2015a). High serum carotenoids associated with lower risk for the metabolic syndrome and its components among Japanese subjects: Mikkabi cohort study. *Brit J. Nutr.* 114, 1674–1682. doi:10.1017/S0007114514503268

Sugiura, M., Nakamura, M., Ogawa, K., Ikoma, Y., and Yano, M. (2015b). High-serum carotenoids associated with lower risk for developing type 2 diabetes among Japanese subjects: Mikkabi cohort study. *Bmj Open Diab Res. CA* 3, e000147. doi:10.1136/bmjdrc-2015-000147

Szeto, H. H., Liu, S., Soong, Y., Wu, D., Darrah, S. F., Cheng, F. Y., et al. (2011). Mitochondria-targeted peptide accelerates ATP recovery and reduces ischemic kidney injury. *J. Am. Soc. Nephrol.* 22, 1041–1052. doi:10.1681/ASN.2010080808

Teixeira, R. B., Pfeiffer, M., Zhang, P., Shafique, E., Rayta, B., Karbasiafshar, C., et al. (2023). Reduction in mitochondrial ROS improves oxidative phosphorylation and provides resilience to coronary endothelium in non-reperfused myocardial infarction. *Basic Res. Cardiol.* 118, 3. doi:10.1007/s00395-022-00976-x

Terao, J. (2023). Revisiting carotenoids as dietary antioxidants for human health and disease prevention. *Food Funct.* 14, 7799–7824. doi:10.1039/d3fo02330c

Wang, Y., Yang, F., Liu, T., Zhao, C., Gu, F., Du, H., et al. (2024). Carotenoid fates in plant foods: chemical changes from farm to table and nutrition. *Crit. Rev. Food Sci.* 64, 1237–1255. doi:10.1080/10408398.2022.2115002

Wu, J., Shang, H., Zhang, A., He, Y., Tong, Y., Huang, Q., et al. (2023a). Antioxidant nanozymes in kidney injury: mechanism and application. *Nanoscale* 15, 13148–13171. doi:10.1039/d3nr01954c

Wu, Q., Guan, Y. B., Zhang, K. J., Li, L., and Zhou, Y. (2023b). Tanshinone IIA mediates protection from diabetes kidney disease by inhibiting oxidative stress induced pyroptosis. *J. Ethnopharmacol.* 316, 116667. doi:10.1016/j.jep.2023.116667

Xiao, L., Xu, X., Zhang, F., Wang, M., Xu, Y., Tang, D., et al. (2017). The mitochondria-targeted antioxidant MitoQ ameliorated tubular injury mediated by mitophagy in diabetic kidney disease via Nrf2/PINK1. *Redox Biol.* 11, 297–311. doi:10.1016/j.redox.2016.12.022

Zhang, Y., Mao, H., Li, Y., Xiong, Y., Liu, X., Wang, L., et al. (2023). β-Cryptoxanthin maintains mitochondrial function by promoting NRF2 nuclear translocation to inhibit oxidative stress-induced senescence in HK-2 cells. *Int. J. Mol. Sci.* 24, 3851. doi:10.3390/ijms24043851

frontiers | Frontiers in Pharmacology

TYPE Original Research
PUBLISHED 03 January 2025
DOI 10.3389/fphar.2024.1500458

Check for updates

OPEN ACCESS

EDITED BY
Swayam Prakash Srivastava,
University of Michigan, United States

REVIEWED BY
Hao Du,
UCONN Health, United States
Nisha Sharma,
National Institute of Pharmaceutical Education and Research, Mohali, India

*CORRESPONDENCE
Yongping Zhang,
zgygpg@126.com
Bin Zhang,
zhangbin7@126.com
Xiaobo Sun,
sun_xiaobo163@163.com

RECEIVED 23 September 2024
ACCEPTED 06 December 2024
PUBLISHED 03 January 2025

CITATION
Zhang Y, Wang F, Zhang C, Yao F, Zhang B, Zhang Y and Sun X (2025) FGF21 ameliorates diabetic nephropathy through CDK1-dependently regulating the cell cycle. *Front. Pharmacol.* 15:1500458. doi: 10.3389/fphar.2024.1500458

COPYRIGHT
© 2025 Zhang, Wang, Zhang, Yao, Zhang, Zhang and Sun. 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.

FGF21 ameliorates diabetic nephropathy through CDK1-dependently regulating the cell cycle

Yudie Zhang[1,2,3,4,5], Fan Wang[2,3,4,5], Chongyang Zhang[2,3,4,5], Fan Yao[2,3,4,5], Bin Zhang[2,3,4,5]*, Yongping Zhang[1]* and Xiaobo Sun[2,3,4,5]*

[1]Guizhou University of Traditional Chinese Medicine, Guiyang, China, [2]Institute of Medicinal Plant Development, Peking Union Medical College and Chinese Academy of Medical Sciences, Beijing, China, [3]Key Laboratory of Bioactive Substances and Resources Utilization of Chinese Herbal Medicine, Ministry of Education, Beijing, China, [4]Beijing Key Laboratory of Innovative Drug Discovery of Traditional Chinese Medicine (Natural Medicine) and Translational Medicine, Beijing, China, [5]Key Laboratory of efficacy evaluation of Chinese Medicine Against Glyeolipid Metabolism Disorder Disease, State Administration of Traditional Chinese Medicine, Beijing, China

Background: Diabetic nephropathy (DN) is a prevalent global renal illness and one of the main causes of end-stage renal disease (ESRD). FGF21 has been shown to ameliorate diabetic nephropathy, and in addition FGF-21-treated mice impeded mitogenicity, whereas it is unclear whether FGF21 can influence DN progression by regulating the cell cycle in diabetic nephropathy.

Methods: In order to create a diabetic model, STZ injections were given to C57BL/6J mice for this investigation. Then, FGF21 was administered, and renal tissue examination and pathological observation were combined with an assessment of glomerular injury, inflammation, oxidative stress, and the fibrinogen system in mice following the administration of the intervention. Furthermore, we used db/db mice and FGF21 direct therapy for 8 weeks to investigate changes in fasting glucose and creatinine expression as well as pathological changes in glomeruli glycogen deposition, fibrosis, and nephrin expression. To investigate the mechanism of action of FGF21 in the treatment of glycolytic kidney, transcriptome sequencing of renal tissues and KEGG pathway enrichment analysis of differential genes were performed.

Results: The study's findings demonstrated that FGF21 intervention increased clotting time, decreased oxidative stress and inflammation, and avoided thrombosis in addition to considerably improving glomerular filtration damage. After 8 weeks of FGF21 treatment, glomerular glycogen deposition, fibrosis, and renin expression decreased in db/db mice. Moreover, there was a notable reduction of creatinine and fasting blood glucose levels. Additionally, the CDK1 gene, a key player in controlling the cell cycle, was discovered through examination of the transcriptome sequencing data. It was also shown that FGF21 dramatically reduces the expression of CDK1, which may help diabetic nephropathy by averting mitotic catastrophe and changing the renal cell cycle.

Conclusion: In short, FGF21 improved the development of diabetic nephropathy in diabetic nephropathy-affected animals by reducing glomerular filtration damage, inflammation, and oxidative stress, inhibiting the formation of thrombus, and controlling the cell cycle through CDK1.

KEYWORDS

FGF21, diabetic nephropathy, CDK1, cell cycle, glomerular filtration barrier

1 Introduction

Diabetes is becoming more common as people's lifestyles and material circumstances change. Diabetes affects over 415 million people globally (Falkevall et al., 2017). Diabetes-related microvascular complications such as diabetic nephropathy (DN) are widespread and represent a major global health issue. It is also a major cause of end-stage renal disease (ESRD) with a high incidence rate (Wu et al., 2018). Many investigations have shown that lipid metabolism abnormalities are the primary cause of DN and that they worsen over time. DN is usually defined by changes in metabolism and hemodynamics brought on by hyperglycemia (Jiang et al., 2005; Goldberg et al., 2012; Liu et al., 2009). Metabolic disorders also cause oxidative stress, inflammation, and renal fibrosis (Grabias and Konstantopoulos, 2014; Alicic et al., 2017; Tuttle et al., 1991). A further sign of diabetic nephropathy (DN) is deterioration of the glomerular filtration barrier, which is characterized by damage to glomerular podocytes that develop epithelial mesenchymal transition (EMT) in response to particular stimuli and abnormalities in markers specific to podocytes. At EMT, podocytes separate from the glomerular basement membrane, flush into the urine, and eventually exit the body (Simonson, 2007). The podocyte serves as the last line of defense to stop protein loss from the blood as it passes through the glomerulus. Thus, podocytes play a crucial role in preserving the glomerular filtration barrier (Haley et al., 2018).

Renal fibrosis occurs in the later stages of diabetic nephropathy progression. During this period, endothelial-mesenchymal transition (EndMT) is a common cellular phenotypic transition in which endothelial cells lose specific morphology and acquire myofibroblast-like features including α-smooth muscle actin (αSMA), poikilodulin, and fibronectin. Podocyte epithelial-mesenchymal transition (EMT) is a maladaptive response to injury that results in impaired glomerular filtration barrier integrity and proteinuria (Dong et al., 2023; Manetti et al., 2017). Wnt/β-catenin signaling plays a key role in promoting podocyte injury and dysfunction (Dai et al., 2009). Wnts induces dephosphorylation of β-catenin and stimulates its translocation to the nucleus, whereas β-catenin activation inhibits the expression of renin expression, specifically disrupting the slit septum of the glomerular filtration membrane (Chen et al., 2022; MacDonald et al., 2009). In addition to the above signaling, Notch signaling and Hedgehog signaling play important roles in the development of diabetic nephropathy (Tuttle et al., 2022; Zhao et al., 2023). Notably, it has been shown that endothelial glucocorticoid receptor (GR) deficiency leads to upregulation of the classical Wnt signaling pathway, impaired fatty acid oxidation and thus accelerated fibrosis (Przybyciński et al., 2021; Srivastava et al., 2021a). Interestingly, GR deficiency in podocytes leads to similar results (Srivastava et al., 2021b). Similarly, FGFR1 and SIRT3-mediated mechanisms have been shown to ameliorate diabetic renal fibrosis (Li et al., 2020a; Srivastava et al., 2018). Depending on the pathogenesis of DN, some potential drugs corresponding to DN have also been shown to have therapeutic effects on DN in recent years, such as empagliflozin, where Linagliptin -mediated inhibition of dipeptidyl peptidase 4 (DPP4) hinders EMT to ameliorate renal fibrosis in DN (Kanasaki et al., 2014; Li et al., 2020b). In addition, sodium-glucose cotransporter protein-2 inhibitors, glycolysis inhibitors, mineralocorticoid antagonists, renin-angiotensin system inhibitors, and the peptide AcSDKP ameliorate DN by improving glycolysis, metabolic reprogramming, and inhibiting EMT (Li et al., 2017; Srivastava et al., 2020a; Srivastava et al., 2020b; Shi and Bhalla, 2023;Lin et al., 2024).

FGF21 is a member of the fibroblast growth factor (FGF) superfamily, which is a collection of signaling proteins with 150–300 amino acids with a conserved core of about 120 amino acids. It was initially discovered in 2000. The human chromosome 19 gene FGF21 is 75% homologous to the mouse FGF21 gene (Nishimura et al., 2000; Itoh and Ornitz, 2011). Early-stage diabetic nephropathy is intimately linked to FGF21 (Esteghamati et al., 2017; Xu et al., 2017). It has been demonstrated that little daily doses of FGF21 markedly improved morphological glomerular defects, insulin resistance, and lipid levels in diabetic db/db mice (Kim et al., 2013). Type 1 diabetic nephropathy is lessened by FGF21-mediated activation of the AMPK and antioxidant pathways (Weng et al., 2020; Cheng et al., 2016; Cheng et al., 2020). A deficit in FGF21 exacerbates inflammation, oxidative stress, and fibrosis in free fatty acid-induced nephropathy (Zhang et al., 2013).

Numerous investigations have demonstrated that podocyte loss over a particular threshold results in glomerulosclerosis, suggesting that podocytes are the cause of most glomerular disorders (Kriz and LeHir, 2005; Kim et al., 2001; Kretzler, 2005). A lower number of podocytes is a predictor of diabetic nephropathy because the degree of podocyte reduction influences the degree of proteinuria (Barisoni et al., 2007; Wiggins, 2007). Furthermore, the loss of podocytes is linked to the mitotic catastrophe it is going through (Lasagni et al., 2010; Kriz et al., 1995). Furthermore it was found that premature activation of CDK1 can lead to mitotic catastrophe (Castedo et al., 2002). Importantly, FGF-21-treated mice impeded mitogenicity and inhibited hepatocyte value-addition (Kharitonenkov et al., 2005). While there is a lack of research on whether FGF21 can affect the progression of DN by regulating the cell cycle in diabetic nephropathy, this article is based on this to explore the novel mechanism of FGF21 in the treatment of diabetic nephropathy.

2 Material and methods

2.1 Reagents

Recombinant FGF21 protein (KYS202002, later referred to as FGF21) was provided by Jiangsu Kangyuan Pharmaceuticals Co., Ltd. (Jiangsu, China). Engeletin was purchased from Master of Bioactive Molecules (USA). Electronic balance was purchased from Mettler Toledo Instruments (Shanghai) Co., Ltd. (Shanghai, China). AU680 automatic biochemical analyzer was purchased from Beckman Coulter (United States).

2.2 Animal

Male 5-week-old db/db and dbm mice were purchased from Jiangsu Xishan Animal Technology (Jiangsu, China), and male 7-8-week-old C57BL/6J mice were purchased from Beijing Vitae Laboratory Animal Science and Technology (Beijing, China), and were housed in ventilated cages at temperature (20°C–25°C), relative humidity (60%), light/dark cycle (12 h), and free access to food and water, etc. were maintained in ventilated cages. All animal care and experimental procedures were ethically approved by the Animal Care and Use Committee of the Chinese Academy of Medical Sciences and Peking Union Medical College Affiliated Institutions. Measures were taken to reduce the number of animals used and to ensure that suffering was minimized.

2.2.1 STZ-induced diabetic nephropathy model

Eighty 7–8 weeks old male C57BL/6J mice were taken from the streptozotocin (STZ) diabetic nephropathy model and randomly divided into 6 groups of 12 mice each. One group was a blank control group, which was injected with saline after 4 weeks of normal diet. The remaining group of mice, after 4 weeks of high-fat diet (HFD), were injected once with STZ (140 mg/kg) to induce the diabetic nephropathy model. Three of the groups were FGF21 administration groups, which were injected subcutaneously with FGF21 after modeling, namely FGF21-L [HFD + STZ + FGF21 (3 mg/kg)], FGF21-M [HFD + STZ + FGF21 (6 mg/kg)], and FGF21-H [HFD + STZ + FGF21 (12 mg/kg)]. The last group was the positive drug control group, where mice modeled for gavage administration of engeletin. After these mice were administered for 4 weeks, fasting blood glucose was tested, urine was collected, and blood and kidneys were taken after anesthesia for later experiments.

2.2.2 Spontaneous type II diabetes model in dbdb mice

After a week of acclimation feeding, five-week-old db/db mice were split into three groups of ten mice each: the Model group, the FGF21 treatment group, and the other group. A low dose group [FGF21-L, FGF21 (3 mg/kg)] and a high dose group [FGF21-H FGF21 (6 mg/kg)] were created from the administered groups. Ten additional dbm mice served as the blank control group. After 8 weeks of administering FGF21 subcutaneously in the administered group, in addition to the blank group and model group by saline gavage, fasting blood glucose was assessed, blood was collected for biochemical testing, and kidneys were removed to observe pathological alterations.

2.3 Ethics approval

All animal experiments were approved by the Experimental Animal Ethics Committee of the Institute of Medicinal Plant Development, Peking Union Medical College, in accordance with the National Guidelines for the Care and Use of Animals (SCXK-2012-0001, SYXK-2023-0008).

2.4 Biochemical assays

2.4.1 Urine tests

Urine was collected from each group after 4 weeks of drug administration, and urine albumin (uALB), albumin (ALB), uric acid (UA) levels, β2-microglobulin (β2-MG) with kidney injury factor-1 (KIM-1) levels and N-acetyl-b-D-glucosaminoglycans (NAG) levels were measured by using automatic biochemistry instrument.

2.4.2 Serum indicator tests

At the end of the experiment, mice were fasted overnight and anesthetized with isoflurane, and blood was obtained from the eye sockets. These blood samples were centrifuged at 3,500 rpm for 15 min to collect serum samples, which were then immediately frozen at −80°C for biochemical assays. Lipid-related markers [triglycerides (TG), total cholesterol (TC), low-density lipoprotein (LDL-c), lactate dehydrogenase (LDH)] in serum were measured using an automated biochemistry instrument along with liver-function-related markers including alanine aminotransferase (ALT), alanoglutamic aminotransferase (AST), and alkaline phosphatase (ALP). Serum creatinine (CREA), serum urea (UREA) were also tested. Oxidative stress related indicators including glutathione peroxidase (GSH-PX), heme oxygenase 1 (HO-1) and oxidized malondialdehyde (MDA) levels were tested. Plasma indicator tests.

2.5 Enzyme-linked immunosorbent assay (ELISA)

ELISA kits (purchased from Huaying Institute of Biotechnology, Beijing, China) were used to detect the levels of inflammatory vesicles (NLRP3), IL-6 and C-reactive protein (CRP) in mouse serum.

2.6 Histopathological evaluation

Kidney tissue fixed with 4% paraformaldehyde was eluted with a gradient of water and ethanol, de-ethanolized with xylene, and embedded in paraffin. Paraffin blocks were sectioned, transparent with xylene, and then eluted with ethanol and water in a reverse gradient. Hematoxylin and eosin (H&E) staining, periodate-schiff

(PAS) staining and Masson staining were used to assess the histopathological changes and degree of renal fibrosis.

2.7 Immunohistochemical

Changes in Nephrin expression observed by immunohistochemistry. Samples of tissue were embedded in paraffin, fixed in paraformaldehyde, and then sectioned into 4 μm thick pieces. After being dried at 60°C, the parts were cleaned with xylene to get rid of the wax. Following rehydration in graded alcohol, sections were incubated in 30% hydrogen peroxide. For the purpose of antigen repair, sections were incubated at 95°C in 10 mM citrate buffer (pH 6.0). The Nephrin (PRS2265, Sigma-Aldrich) antibody was then incubated on slides for a whole night at 4°C. The sections were also restained with hematoxylin after being stained with diaminobenzidine chromogen (ZSGB-BIO, China) and treated with secondary antibody for one hour at room temperature. A light microscope was used to view the stained slices.

2.8 Fasting blood glucose measurement

After 4 weeks of treatment, blood glucose levels were measured by tail vein using a glucometer (ACCU-CHEK, Germany), and the mice were fasted for 6 h before the measurement.

2.9 Western blot analysis

Total kidney protein was extracted using protein extraction reagent (ComWin Biotech, China). Equal amounts of proteins were demyelinated, separated by SDS-PAGE, and transferred to nitrocellulose membranes. After sealing, the membrane was incubated overnight at 4°C with primary antibodies, including CDK1 (abcam, United Kingdom), PAK1 (abcam, UK), Chk1 (abcam, United Kingdom), and β-actin (Proteintech). Subsequently, the membranes were washed again at room temperature and incubated with goat anti-rabbit antibody or rabbit anti-mouse antibody coupled with horseradish peroxidase for 1 h. The blots were then washed again and incu-blated using the Enhanced Chemiluminescent Protein Blotting Detection Kit (ComWin Biotech, China) followed by visualization of specific protein bands using eBlot.

2.10 Quantitative polymerase chain reaction assay

TRIzol reagent was used to isolate total RNA from cerebral ischemic infarction or colon tissue. RNA concentration was determined using a NanoDrop spectrophotometer (Thermo Fisher Scientific, United States). RNA was reverse transcribed to cDNA by using a reverse transcription kit (RR036A; TaKaRa Bio, Shiga, Japan). A fluorescence quantification kit was used (RR820A; TaKaRa Bio, Shiga, Japan). Relative quantification of mRNA expression was calculated using the $2^{-\Delta\Delta CT}$ algorithm. Primers were as follows:

2.11 RNA-seq analysis

Standard extraction procedures were used to extract RNA from kidney tissues. Agilent 2100 bioanalyzer was primarily used for the rigorous quality control of the RNA samples, which allowed for the reliable detection of RNA integrity. To create the library, the 250–300 bp cDNA was screened using AMPure XP beads, followed by PCR amplification and further purification of the PCR products using AMPure XP beads. To find genes with significant variations in expression levels across states, gene expression was quantified and the expression data statistically analyzed. Additionally, GO function enrichment analysis and KEGG pathway enrichment analysis were carried out based on the functional roles of the genes.

2.12 Statistical analysis

GraphPad Prism 9.4 and SPSS 25.0 were used for statistical analysis. Data were presented as the mean ± SD. Multiple comparisons were performed using one-way ANOVA, followed

Gene	Primer sequence (5′–3′)	
	Forward	Reverse
Cdk1	AGAAGGTACTTACGGTGTGT	GAGAGATTTCCCGAATTGCAGT
CHK1	ACTTACTGCAATGCTCGCTG	TTGAGGGGTTTGTTGTACCATC
p53	CCCCTGTCATCTTTTGTCCCT	AGCTGGCAGAATAGCTTATTGAG
PAK1	CACCAGCACTATGATTGGAC	ATTCCCGTAAACTCCCCTGTG
FGFR1	TAATACCACCGACAAGGAAATGG	TGATGGGAGAGTCCGATAGAGT
SIRT3	CTACATGCACGGTCTGTCGAA	GCCAAAGCGAAGTCAGCCATA
GR	AGCTCCCCCTGGTAGAGAC	GGTGAAGACGCAGAAACCTTG
ANGPTL4	CATCCTGGGACGAGATGAACT	TGACAAGCGTTACCACAGGC

by Tukey's multiple comparisons test. The Kruskal-Wallis test was used for mNSS and immunofluorescence analysis. Statistical significance was set at $p < 0.05$.

3 Results

3.1 FGF21 improves renal function and glomerular filtration in mice with diabetic nephropathy

Diabetic nephropathy was induced in C57BL/6J mice by injection of STZ after high-fat 4, followed by subcutaneous injection of FGF21 for 4 weeks of treatment (Figure 1A). We examined the level of FGFR1, a high-affinity key receptor of FGF21, which significantly increased the mRNA level of FGFR1 (Figure 1B). Urine levels of urinary albumin (uALB), albumin (ALB), and uric acid (UA) were dramatically upregulated following STZ modeling, but subcutaneous injection of FGF21 significantly decreased the levels of these indexes (Figure 1C). Impaired glomerular filtration barrier after STZ modeling. Furthermore, STZ led to markedly increased levels of kidney injury factor-1 (KIM-1) and β2-microglobulin (β2-MG), as well as N-acetyl-b-D-glucosaminoglycans (NAG); remarkably, FGF21 was able to repair this injury (Figure 1B). Nevertheless, following modeling, serum urea (UREA) and creatinine (CREA) considerably rose, and FGF21 intervention decreased the levels (Figure 1D). Histopathology was used to investigate the morphological changes in the kidney. The results of H&E staining revealed that, following modeling, glomerular enlargement, thickening of the glomerular basement membrane, and dilatation of the thylakoid region were seen (Figure 1E). Crucially, the glomerular structural abnormalities considerably improved following the treatment of FGF21. Nephrin is a structural protein of the podocyte cleavage membrane that is only expressed in glomerular podocytes. Nephrin plays a vital function in maintaining the glomerular filtration barrier. Using immunohistochemistry, we were able to observe the expression of nephrin in the glomerular podocytes of mice in the model group, which was found to be significantly reduced (Figure 1F). In contrast, nephrin was significantly more expressed in the podocytes of mice in the administered group following the intervention of FGF21, which significantly improved the mice's impaired glomerular filtration barrier caused by STZ.

3.2 FGF21 improves glycolipid metabolism in mice with diabetic nephropathy

Since disruption of glycolipid metabolism is the primary cause of diabetic nephropathy, we investigated the impact of FGF21 on this process. We found that STZ significantly increased the levels of lactate (LD), glycated serum protein (GSP), and fasting blood glucose (FBG) in mice (Figure 2A), but that FGF21 significantly attenuated the effect and reduced the disruption of glycolipid metabolism. On the other hand, FGF21 attenuated the influence of serum levels of triglycerides (TG) with total cholesterol (TC), low-density lipoprotein (LDL-c) with lactate dehydrogenase (LDH) in the mouse model group (Figure 2B). Since liver metabolic function has an important role in the development of diabetic nephropathy, we examined the changes of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP), and found that their levels were significantly increased after modeling, and this effect was suppressed by the intervention of FGF21 (Figure 2C). In addition we examined the levels of Sirtuin3 (SIRT3), a key protein for fatty acid oxidation, and glucocorticoid receptor (GR) and Angiopoietin Like 4 (ANGPTL4), key molecules for glycolipid metabolism. FGF21 was found to increase the mRNA levels of SIRT3 and GR, in addition to decreasing the mRNA levels of ANGPTL4 compared to the model group (Figure 2D). Meanwhile, we observed the effect of FGF21 on glycogen deposition in the kidneys by PAS staining, and we could see that after modeling, a large amount of glycogen deposition appeared in the glomerular thylakoid region, the glomerular basement membrane and around the tubules of mice, which was improved in FGF21-treated mice (Figure 2E).

3.3 FGF21 ameliorates oxidative stress and inflammation in mice with diabetic nephropathy

Hyperglycemia increases the *in vivo* glycosylation of antioxidant enzymes and reduces their activity, resulting in the build-up of free radicals that worsen kidney injury by increasing cytotoxicity, lipid peroxidation, and other related processes. As a result, we looked at the markers for oxidative stress and lipid peroxidation. After modeling, mice showed markedly elevated serum levels of inflammatory components and increased oxidative stress. When compared to the model group, FGF21 reduced the amount of oxidized malondialdehyde (MDA) and enhanced the production of antioxidant enzymes such as glutathione peroxidase (GSH-PX) and heme oxygenase 1 (HO-1) (Figure 3A). Furthermore, inflammatory vesicles (NLRP3), IL-6, and C-reactive protein (CRP) were all markedly decreased by FGF21 (Figure 3B). According to the information above, FGF21 was able to reduce inflammation and oxidative stress that accompanied the metabolism of glycolipids in diabetic nephropathy mice.

3.4 FGF21 can ameliorate the fibrinogen activation system in diabetic nephropathy mice, slow down fibrin deposition and improve renal fibrosis

Patients with diabetes were shown to have reduced fibrinolytic activity, as demonstrated by aberrant tissue plasminogen activator (tPA) and plasminogen activator inhibitor-1 (PAI-1) activity. We therefore looked at the indexes related to plasma fibrinolytic activity and discovered that, following modeling, tPA was significantly reduced in mouse plasma, but levels of PAI-1, vascular hemophilic factor (VWF%), and plasma fibrinogen (FIB) were significantly elevated, which was reversed by FGF21 treatment (Figure 4A).

Among diabetic patients, vascular disease and injury are the leading causes of death. It was shown that following modeling, mice

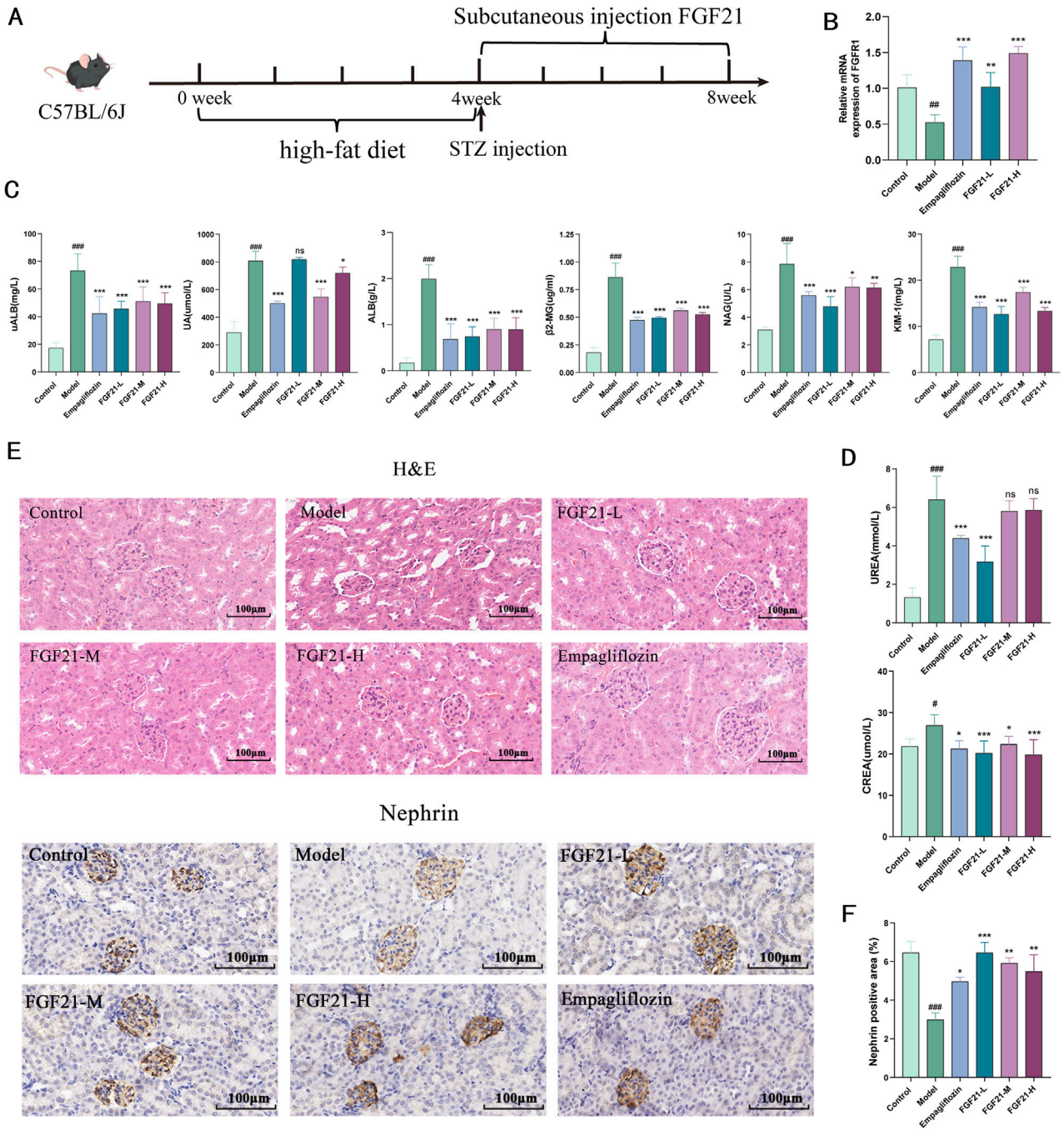


FIGURE 1
FGF21 protects glomerular filtration function and slows down renal injury in mice with diabetic nephropathy. **(A)** Diabetic nephropathy was induced in C57BL/6J mice by injection of STZ after high-fat 4, followed by subcutaneous injection of FGF21 for 4 weeks of treatment (n = 12 per group). **(B)** FGFR1 is a high-affinity receptor for FGF21 (n = 5 per group). **(C)** Urine was collected from mice after FGF21 treatment, and biochemistry instrumentation detected significant downregulation of urinary albumin (uALB), albumin (ALB) and uric acid (UA) levels, and decreased the levels of β2-microglobulin (β2-MG), and kidney injury factor-1 (KIM-1), and decreased the levels of N-acetyl-b-D-glucosylglucomutase (NAG) (n = 12 per group). **(D)** Biochemistry testing of serum, FGF21 decreased serum creatinine (CREA) decreased serum urea (UREA) levels (n = 12 per group). **(E)** H&E staining showed that FGF21 slowed down the modeling-induced glomerular swelling, glomerular basement membrane thickening with dilatation of the tethered area. Immunohistochemistry shows that FGF21 increases nephrin expression in glomeruli (n = 12 per group). **(F)** Quantitative results of Nephrin immunohistochemistry (n = 3 per group). Scale bars:100 μm. Data are presented as mean ± standard deviation (SD). $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ versus the Model group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, and $^{\#\#\#}p < 0.001$ vs. the control group.

had lower plasma levels of anticoagulation-related substances, and that FGF21 intervention extended the duration of clotting (prolongation of prothrombin time (TT), activated partial thromboplastin time (APTT), and prothrombin time (PT). FGF21 also markedly raised antithrombin III (ATIII) and lowered thromboxane B2 (TXB2) and D-dimer (D-DIMER)

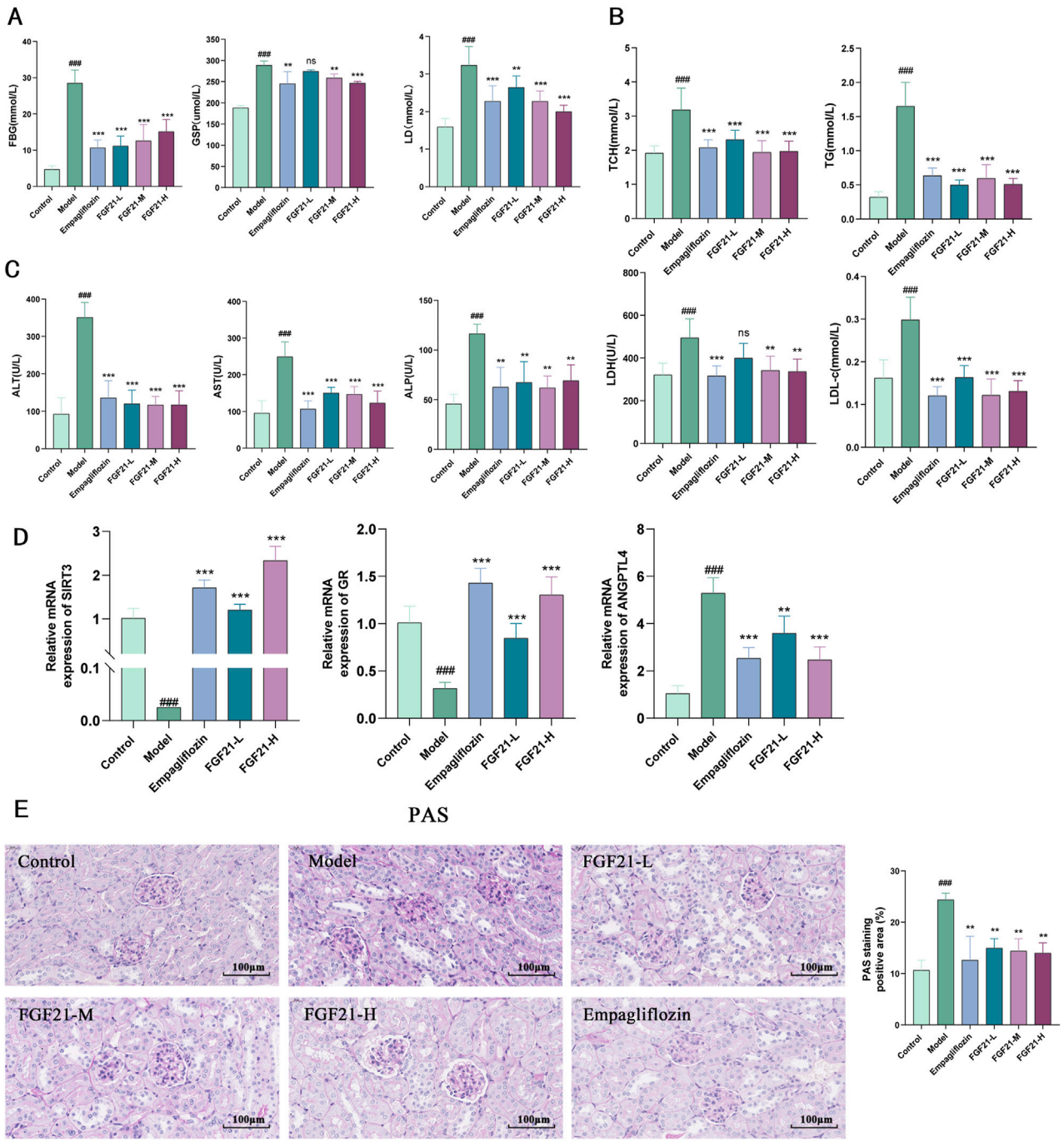


FIGURE 2
FGF21 slows diabetic nephropathy by improving disorders of glucolipid metabolism. **(A)** In mice, FGF21 lowers the levels of lactate (LD), glycated serum protein (GSP), and fasting blood glucose (FBG) (n = 12 per group). **(B)** Serum levels of lactate dehydrogenase (LDH), low-density lipoprotein (LDL-c), total cholesterol (TC), and triglycerides (TG) were all decreased by FGF21 (n = 12 per group). **(C)** Alkaline phosphatase (ALP), azelotransferase (AST), and alanine aminotransferase (ALT) were all found to be lowered in serum levels by FGF21 (n = 12 per group). **(D)** FGF21 elevated mRNA levels of Sirtuin3 (SIRT3) and glucocorticoid receptor (GR) and decreased mRNA levels of Angiopoietin Like 4 (ANGPTL4) compared to the model group (n = 5 per group). **(E)** PAS staining revealed that the amount of glomerular glycogen was decreased by FGF21 and quantification of PAS-stained positive areas is shown on the right side (n = 3 per group). Scale bars: 100 μm. Data are presented as mean ± standard deviation (SD). $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ versus the Model group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, and $^{\#\#\#}p < 0.001$ vs. the control group.

levels (Figure 4B). Furthermore, we used Masson staining to observe the kidney's fibrosis and noticed that STZ caused large collagenous fibrillar lesions in the glomeruli, which were ameliorated by FGF21 (Figure 4C). This indicates that FGF21 can alleviate renal fibrosis, slow down fibrin deposition, and activate the fibrinogen activation pathway in diabetic nephropathy mice.

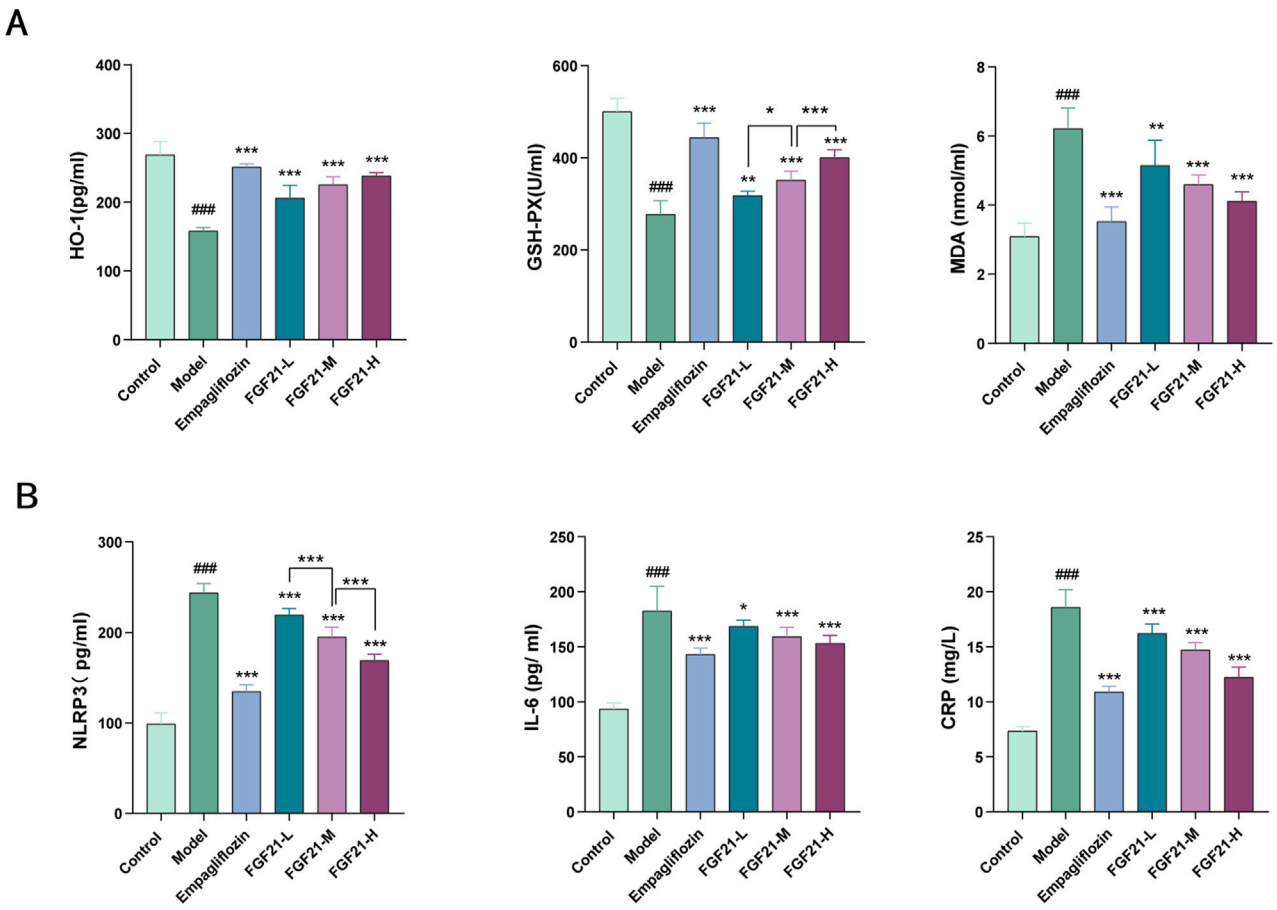


FIGURE 3
FGF21 attenuates oxidative stress, lipid peroxidation and inflammation ameliorates diabetic nephropathy. **(A)** FGF21 increased the expression of antioxidant enzymes [glutathione peroxidase (GSH-PX), heme oxygenase 1 (HO-1)] while decreasing the levels of oxidized malondialdehyde (MDA) (n = 12 per group). **(B)** FGF21 also significantly reduced the levels of inflammatory vesicles (NLRP3), IL-6 and C-reactive protein (CRP) (n = 12 per group). Data are presented as mean ± standard deviation (SD). $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ versus the Model group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, and $^{\#\#\#}p < 0.001$ vs. the control group.

3.5 FGF21 ameliorates diabetic nephropathy in db/db mice by reducing glomerular glycogen deposition and fibrosis

In order to corroborate the validity of the previously mentioned findings, we performed the pharmacodynamic analysis below using db/db mice, a type II diabetes model mouse caused by a mutation in the leptin receptor gene. Five-week-old db/db mice, acclimatized and fed for 1 week, were tested after 8 weeks of FGF21 treatment by subcutaneous injection (Figure 5A). We discovered that in db/db mice, FGF21 intervention was able to considerably lessen blood glucose levels (Figure 5B). Furthermore, we discovered that FGF21 could lower blood levels of urea (UREA) and creatinine (CERA) through biochemical testing on serum (Figure 5C). In addition we performed histopathological observations. H&E results showed that FGF21 improved glomerular swelling and thickening of glomerular basement membrane (Figures 5D, E). Results from masson staining indicated that FGF21 intervention improved fibrosis. Furthermore, as demonstrated by PAS staining results, FGF21 also decreased the amount of glomerular glycogen accumulation (Figures 5D, E). Additionally, we noticed that following FGF21 injection, there was an increase in the expression of the glomerular marker nephrin (Figures 5D, E). To sum up, FGF21 was effective in improving the defective glomerular filtration barrier and mitigating glycogen accumulation and fibrosis in the glomeruli of db/db mice.

3.6 FGF21 ameliorates diabetic nephropathy by mediating CDK1 regulation of the cell cycle

Transcriptome sequencing of kidney tissue from STZ-modeled C57BL/6J mice, we were able to determine the mechanism of action of FGF21. We discovered that 2203 genes were elevated in the model group when compared to the control group, and 182 genes were downregulated in the FGF21 intervention group when compared to the model group. KEGG analysis of these genes that were significantly downregulated in the FGF21 administration group relative to the model group showed that d large number of genes were enriched in the cell cycle and the differences were most significant (Figure 6A). In addition we obtained 47 genes by

10.3389/fphar.2024.1500458

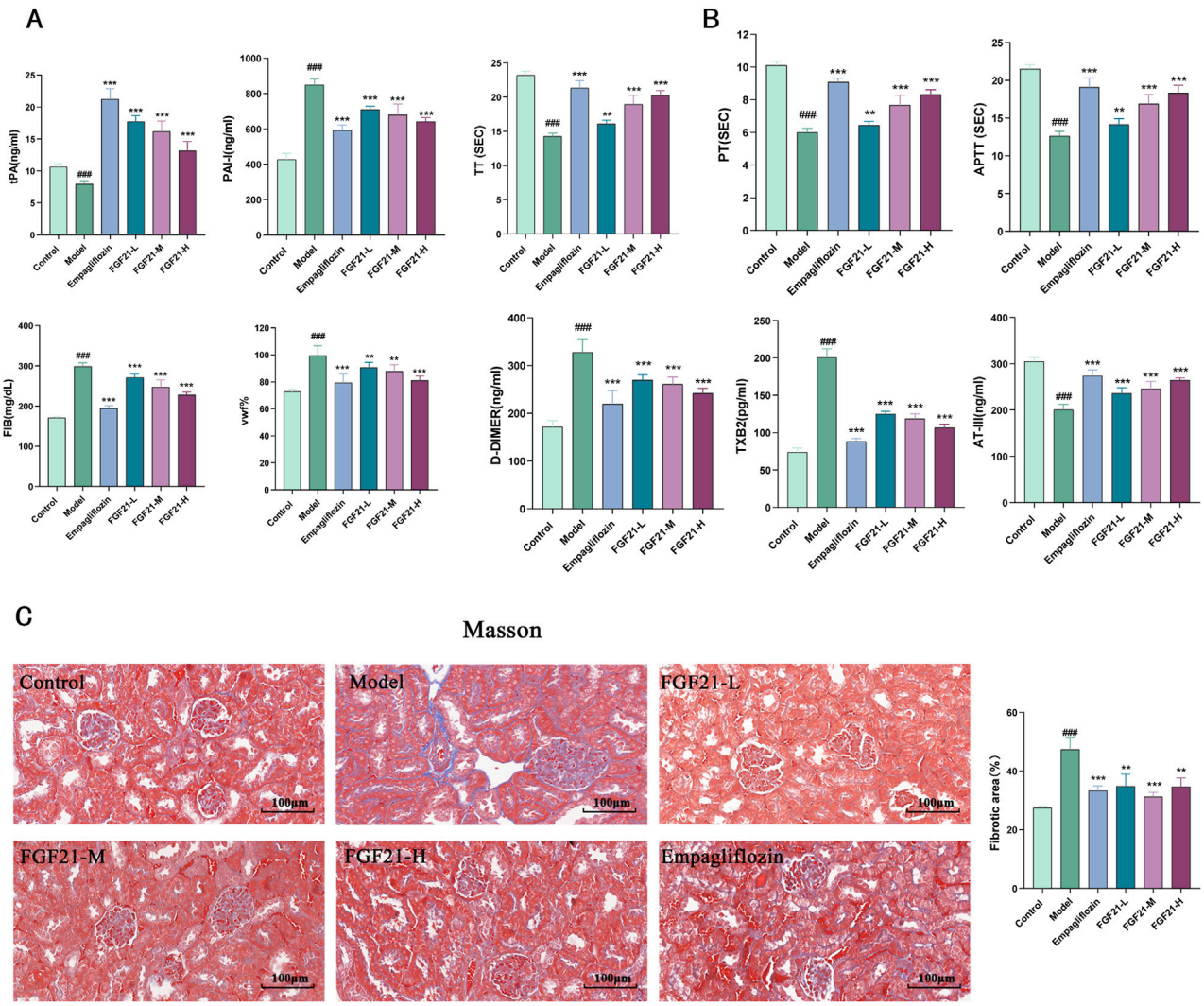


FIGURE 4
FGF21 protects renal function in mice with diabetic nephropathy by modulating renal hemodynamics. **(A)** FGF21 elevated plasma tissue plasminogen activator (tPA) levels and decreased fibrinogen activator inhibitor-1 (PAI-1), vascular hemophilic factor (VWF%), and plasma fibrinogen (FIB) levels (n = 12 per group). **(B)** FGF21 intervention prolonged prothrombin time (TT), activated partial thromboplastin time (APTT), and prothrombin time (PT), decreased thromboxane B2 (TXB2) and D-dimer (D-DIMER) levels, and increased antithrombin III (ATIII) levels (n = 12 per group). **(C)** Masson staining showed that FGF21 reduced collagenous fibrillar lesions appearing in glomeruli and Quantification of Masson stained fibrotic areas is shown on the right side (n = 3 per group). Scale bars: 100 μm. Data are presented as mean ± standard deviation (SD). *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ versus the Model group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, and $^{\#\#\#}p < 0.001$ vs. the control group.

taking the intersection of genes downregulated in the FGF21 group vs. the model group and genes upregulated in the model group vs. the control group (Figure 6B). Among these genes, we discovered that cell cycle protein-dependent kinase (CDK1), to be a central molecule in the overall cell cycle regulatory mechanism. The results showed that FGF21 significantly reduced the expression of the CDK1 gene as well as upregulated the expression of its upstream factor Checkpoint kinase 1 (Chk1) (Figures 6C, D). In addition, it decreased the expression of P21(RAC1)-activated kinase 1 (PAK1) and increased the level of P53, a negatively regulated gene located upstream of CDK1 (Figures 6C, D). The above was supported by the Western blot analysis results. This indicates that via regulating CDK1 to regulate the cell cycle in renal tissues, FGF21 is likely to slow down the mitotic catastrophe and, consequently, the progression of diabetic nephropathy.

4 Discussion

In this paper, using two models of diabetes, we demonstrated that FGF21 ameliorates proteinuria, oxidative stress and inflammation, as well as fibrosis and glomerular podocyte loss or swelling, and impaired glomerular filtration barrier in mice with diabetic nephropathy. In addition, transcriptomic analysis and experimental validation were performed on the kidneys of C57BL/6J mice. FGF21 was found to reduce the expression of the core cell cycle molecule CDK1 by decreasing the elevated levels of P53 and Chk1. This may impede the loss of podocytes, which in turn improves glycolipid metabolism (AST, LDH), inflammation (IL-6) and oxidative stress (HO-1), as well as the fibrinogen system (tPA, PAI-1) that accompanies diabetic nephropathy (Figure 7).

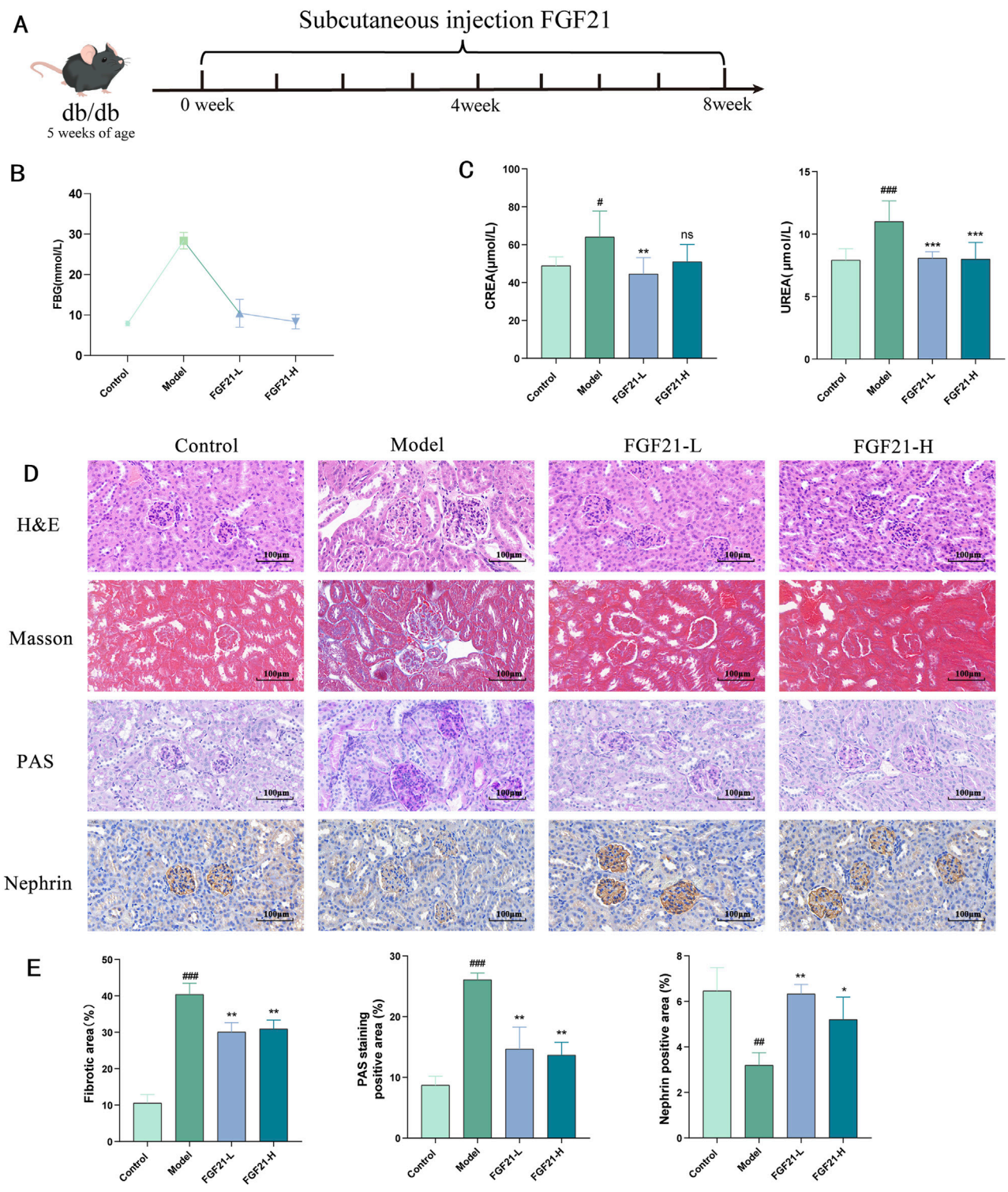


FIGURE 5
FGF21 ameliorates diabetic nephropathy in db/db mice by reducing glomerular glycogen deposition and fibrosis. **(A)** Pattern of FGF21 subcutaneous injection in 5-week-old mice for 8 weeks of treatment (n = 10 per group). **(B)** FGF21 significantly reduces fasting blood glucose in mice (n = 10 per group). **(C)** FGF21 could lower blood levels of urea (UREA) and creatinine (CERA) through biochemical testing on serum (n = 10 per group). **(D)** H&E results showed that FGF21 improved glomerular swelling and thickening of glomerular basement membrane. Masson staining showed that FGF21 intervention improved fibrosis. In addition, FGF21 reduced the amount of glomerular glycogen accumulation as shown by PAS staining. Furthermore, the results of immunohistochemistry demonstrated that FGF21 also raised the expression of nephrin. **(E)** Quantification of Masson stained fibrotic areas, PAS stained positive areas and nephrin immunohistochemistry positive areas (n = 3 per group). Scale bars: 100 μm. Data are presented as mean ± standard deviation (SD). $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ versus the Model group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, and $^{\#\#\#}p < 0.001$ vs. the control group.

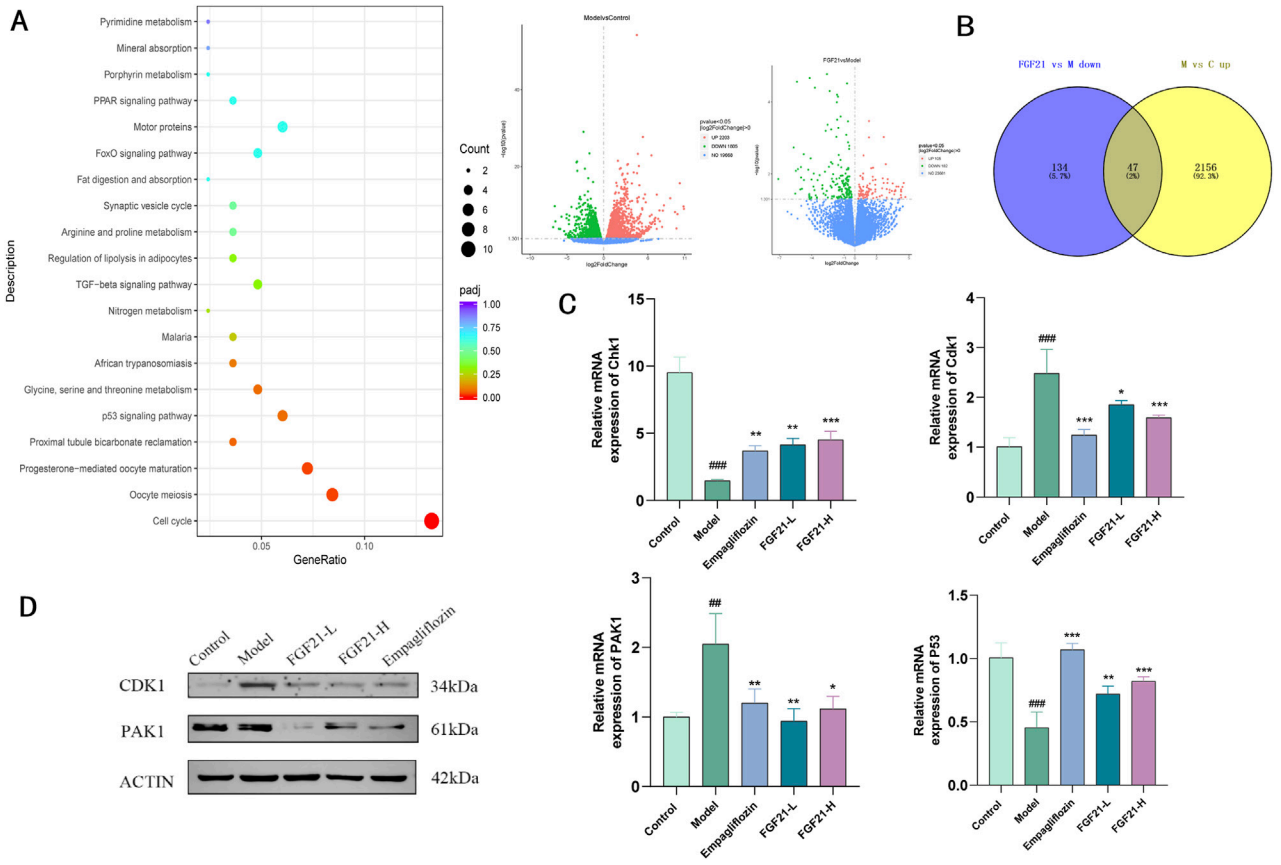


FIGURE 6
FGF21 regulation of cell growth cycle ameliorates diabetic nephropathy by modulating CDK1 expression. **(A)** KEGG analysis bubble plot results of genes significantly downregulated in the FGF21 administration group vs. the model group (left), the model group vs. the control group differential gene and the FGF21 administration group vs. the model group differential gene (right) (n = 4 per group). **(B)** Wayne plots of significantly upregulated genes in the model group vs. control group vs. significantly downregulated genes in the FGF21 administration group vs. model group (n = 4 per group). **(C)** FGF21 elevated cell cycle regulator-cell cycle protein-dependent kinase (CDK1) with P21 (RAC1) activated kinase 1 (PAK1) gene levels, while decreasing Checkpoint kinase 1 (Chk1) with P53 gene levels (n = 4 per group). **(D)** FGF21 reduced CDK1 and PAK1 protein levels (n = 4 per group). Data are presented as mean ± standard deviation (SD). $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ versus the Model group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, and $^{\#\#\#}p < 0.001$ vs. the control group.

One of the main pathogenic processes of diabetic nephropathy is metabolic abnormalities; hyperglycemia changes the metabolism of endothelium and podocyte cells, overtaxing proximal tubular cells, a major source of cellular stress in the kidney (Mohandes et al., 2023; Audzeyenka et al., 2022). Hemodynamic changes are a result of diabetic nephropathy; further research has demonstrated that lipid metabolism abnormalities are a major factor in the onset and course of diabetic nephropathy. The buildup of free fatty acids in the diabetic kidney's tubular epithelial cells causes lipotoxicity, which can result in inflammation, oxidative stress, and even cell death (Kim et al., 2018). FGF21 was found to improve glucose-lipid metabolism in this study by significantly lowering blood glucose levels, triglyceride and total cholesterol levels, and inhibiting blood glucose elevation (Figure 2B). We also observed decreased expression of inflammatory factors and attenuated oxidative stress (Figure 3). This shows that in the early stages of diabetic nephropathy, FGF21 may have a preventative and ameliorative effect.

The imbalance of the ADAMTS13-VWF axis due to chronic hyperglycemia may lead to thrombotic vascular disease in diabetic patients, while intrarenal thrombosis aggravates diabetic nephropathy (Rurali et al., 2013; Taniguchi et al., 2010; Dhanesha et al., 2017). Plasma tPA selectively activates fibrinogen to produce fibrinolytic enzymes, which can lead to the destruction of fibrin and thrombi to maintain blood flow to prevent ischemic injury and the accompanying inflammatory response (Thibord et al., 2021). Increased levels of PAI-1 in plasma diminish local fibrinolysis, decreasing the function of removing fibrin from blood vessels, and placing the organism in a hypercoagulable state, which results in an increase in blood viscosity increase, vascular stenosis and occlusion, insufficient perfusion, and small ischemic areas in the vessel wall, leading to secondary thrombosis (Kolseth et al., 2017; Le et al., 2008). In short, dysregulation of the dynamic balance between tPA and PAI-1 increases the risk of vascular and glomerulosclerosis and causes the blood to become hypercoagulable (Le et al., 2008). The current study found that FGF21 was able to significantly downregulate the level of PAI-1 in plasma while also raising the levels of the four coagulation components (PT, TT, APTT, and D-DIMER) (Figures 4A, B). This suggests that by promoting PAI-1 expression,

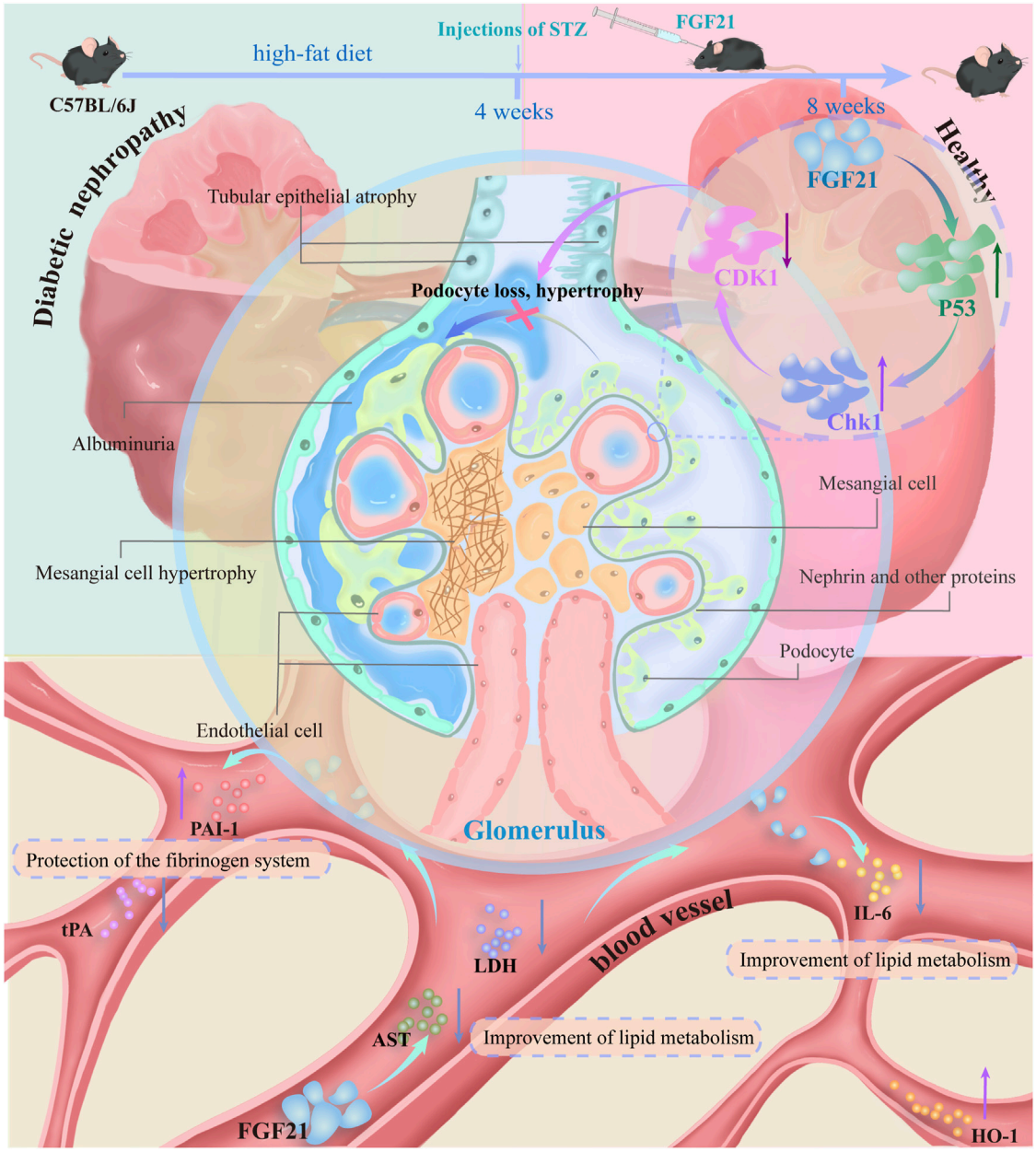


FIGURE 7
C57BL/6J mice on a high-fat diet for 4 weeks were injected with STZ resulting in mice becoming diabetic nephropathic mice. Mice with diabetic nephropathy will develop proteinuria, and the kidneys exhibit significant oxidative stress and inflammation with some fibrosis. In addition there may be concomitant vascular lesions. Glomerular podocytes are lost or swollen and the glomerular filtration barrier is impaired. This was followed by 4 weeks of treatment with subcutaneous injections of FGF21. FGF21 reduces the expression of the core cell cycle molecule CDK1 by decreasing the expression levels of elevated P53 and Chk1. This impedes the loss of podocytes, which in turn improves glycolipid metabolism (AST, LDH), inflammation (IL-6), and oxidative stress (HO-1), as well as the fibrinogen system (tPA, PAI-1) that accompanies diabetic nephropathy.

FGF21 may be able to lower the risk of secondary thrombosis and delay the onset of diabetic nephropathy.

The development of glomerular disease into glomerulosclerosis and chronic kidney disease is largely dependent on the loss of foot cells. Podocytes are also unable to properly complete cytoplasmic divisions due to genetic, mechanical, immunologic, or toxic forms of triggers. This frequently results in the production of aneuploid podocytes, which rapidly separate and die, causing a mitotic catastrophe (Lasagni et al., 2010; Petermann et al., 2002; Neef et al., 2006). The podocyte undergoes a catastrophic mitotic event that damages it and results in podocyte loss, which compromises the glomerular filtration barrier and causes proteinuria (Brenner, 1985). Finally, terminally differentiated podocyte mitosis instead speeds up the loss of podocytes and glomerulosclerosis. Finding ways to enhance podocyte regeneration from other sources remains a challenging goal for

improving chronic kidney disease treatment in the future (Lasagni et al., 2013). In the current investigation, we discovered that the mice experienced severe proteinuria, glomerular fibrosis, and podocyte loss in the model group. On the other hand, FGF21 treatment reduced proteinuria and enhanced the expression of the podocyte marker nephrin. We analyzed the mechanism of action of FGF21 through transcriptome analysis and performed KEGG signaling pathway enrichment analysis on genes that were significantly downregulated compared to the model group and found that cell cycle related genes accounted for the largest proportion and were highly significant. Taking the intersection of the genes downregulated by FGF21 relative to the model group and the genes upregulated by the model group and the control group, it was found that among these genes CDK1 is a core regulatory gene for cell cycle regulation, and further validated that FGF21 did significantly downregulate the expression of CDK1 gene and its protein (Figures 6A–C). CDK1 is a core protein in the regulation of the cell cycle G2/M, and inhibition of CDK1 will prevent cells from entering mitosis (Enomoto et al., 2009). It also inhibited the expression of PAK1 and P53, where PAK1 inhibition terminated mitosis and blocked cells in the G1 phase (Figure 6C) (Yi et al., 2023). It was found that CDK1 overexpression in adrenocortical carcinoma cell (ACC) lines locked ACC cells at the G2/M checkpoint by interacting with UBE2C and AURKA/B, promoting cancer cell proliferation and inducing epithelial mesenchymal transition (EMT) (Ren et al., 2022). In addition cellular G2/M arrest induces fibrosis (Wang et al., 2022). Importantly, it was found that premature activation of CDk1 can lead to mitotic catastrophe, which is an important factor in inducing podocyte loss (Castedo et al., 2002).

This indicates that in the treatment of diabetic nephropathy, CDK1 is anticipated to be a key target for controlling the cell cycle and reducing the loss of glomerular podocytes and associated fibrosis. Furthermore, by preserving the equilibrium of glycolipid metabolism, FGF21 was shown in this study to be able to further reduce the consequent oxidative stress, inflammation, and fibrosis. Moreover, by inhibiting the expression of CDK1, regulating the cell cycle, slowing down mitotic catastrophe, and reducing the loss of podocytes and eventually alleviating proteinuria caused by glomerular filtration barrier damage in diabetic nephropathic mice, FGF21 is expected to become a promising therapeutic drug to reduce podocyte loss and improve the prospect of chronic kidney disease.

Data availability statement

The original contributions presented in the study are publicly available. This data can be found here: https://www.ncbi.nlm.nih.gov/sra/?term=PRJNA1203032.

Ethics statement

The animal study was approved by Experimental Animal Ethics Committee of the Institute of Medicinal Plant Development, Peking Union Medical College. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

YuZ: Conceptualization, Data curation, Formal Analysis, Methodology, Project administration, Validation, Writing–original draft, Writing–review and editing. FW: Conceptualization, Methodology, Supervision, Writing–review and editing. CZ: Methodology, Writing–review and editing. FY: Supervision, Writing–review and editing. BZ: Funding acquisition, Project administration, Resources, Supervision, Writing–review and editing. YoZ: Resources, Supervision, Writing–review and editing. XS: Funding acquisition, Project administration, Resources, Supervision, Writing–review and editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This work was financially supported by the CAMS Innovation Fund for Medical Sciences (CIFMS) (No. 2022-I2M-1-018, 2021-I2M-1-031) and the National Key Research and Development Program of China (2022YFC3501803).

Acknowledgments

I would like to thank all the authors for their help with my experiments and the corresponding authors for their guidance of my article as well as financial support.

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.

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.2024.1500458/full#supplementary-material

References

Alicic, R. Z., Rooney, M. T., and Tuttle, K. R. (2017). Diabetic kidney disease: challenges, progress, and possibilities. *Clin. J. Am. Soc. Nephrol.* 12, 2032–2045. doi:10.2215/CJN.11491116

Audzeyenka, I., Bierżyńska, A., and Lay, A. C. (2022). Podocyte bioenergetics in the development of diabetic nephropathy: the role of mitochondria. *Endocrinology* 163, bqab234. doi:10.1210/endocr/bqab234

Barisoni, L., Schnaper, H. W., and Kopp, J. B. (2007). A proposed taxonomy for the podocytopathies: a reassessment of the primary nephrotic diseases. *Clin. J. Am. Soc. Nephrol.* 2, 529–542. doi:10.2215/CJN.04121206

Brenner, B. M. (1985). Nephron adaptation to renal injury or ablation. *Am. J. Physiol.* 249, F324–F337. doi:10.1152/ajprenal.1985.249.3.F324

Castedo, M., Perfettini, J.-L., Roumier, T., and Kroemer, G. (2002). Cyclin-dependent kinase-1: linking apoptosis to cell cycle and mitotic catastrophe. *Cell Death Differ.* 9, 1287–1293. doi:10.1038/sj.cdd.4401130

Chen, X., Tan, H., Xu, J., Tian, Y., Yuan, Q., Zuo, Y., et al. (2022). Klotho-derived peptide 6 ameliorates diabetic kidney disease by targeting Wnt/β-catenin signaling. *Kidney Int.* 102, 506–520. doi:10.1016/j.kint.2022.04.028

Cheng, Y., Zhang, J., Guo, W., Li, F., Sun, W., Chen, J., et al. (2016). Up-regulation of Nrf2 is involved in FGF21-mediated fenofibrate protection against type 1 diabetic nephropathy. *Free Radic. Biol. Med.* 93, 94–109. doi:10.1016/j.freeradbiomed.2016.02.002

Cheng, Y., Zhang, X., Ma, F., Sun, W., Wang, W., Yu, J., et al. (2020). The role of Akt2 in the protective effect of fenofibrate against diabetic nephropathy. *Int. J. Biol. Sci.* 16, 553–567. doi:10.7150/ijbs.40643

Dai, C., Stolz, D. B., Kiss, L. P., Monga, S. P., Holzman, L. B., and Liu, Y. (2009). Wnt/beta-catenin signaling promotes podocyte dysfunction and albuminuria. *J. Am. Soc. Nephrol.* 20, 1997–2008. doi:10.1681/ASN.2009010019

Dhanesha, N., Doddapattar, P., Chorawala, M. R., Nayak, M. K., Kokame, K., Staber, J. M., et al. (2017). ADAMTS13 retards progression of diabetic nephropathy by inhibiting intrarenal thrombosis in mice. *Arterioscler. Thromb. Vasc. Biol.* 37, 1332–1338. doi:10.1161/ATVBAHA.117.309539

Dong, R., Zhang, X., Liu, Y., Zhao, T., Sun, Z., Liu, P., et al. (2023). Rutin alleviates EndMT by restoring autophagy through inhibiting HDAC1 via PI3K/AKT/mTOR pathway in diabetic kidney disease. *Phytomedicine* 112, 154700. doi:10.1016/j.phymed.2023.154700

Enomoto, M., Goto, H., Tomono, Y., Kasahara, K., Tsujimura, K., Kiyono, T., et al. (2009). Novel positive feedback loop between Cdk1 and Chk1 in the nucleus during G2/M transition. *J. Biol. Chem.* 284, 34223–34230. doi:10.1074/jbc.C109.051540

Esteghamati, A., Khandan, A., Momeni, A., Behdadnia, A., Ghajar, A., Nikdad, M. S., et al. (2017). Circulating levels of fibroblast growth factor 21 in early-stage diabetic kidney disease. *Ir. J. Med. Sci.* 186, 785–794. doi:10.1007/s11845-017-1554-7

Falkevall, A., Mehlem, A., Palombo, I., Heller Sahlgren, B., Ebarasi, L., He, L., et al. (2017). Reducing VEGF-B signaling ameliorates renal lipotoxicity and protects against diabetic kidney disease. *Cell Metab.* 25, 713–726. doi:10.1016/j.cmet.2017.01.004

Goldberg, I. J., Trent, C. M., and Schulze, P. C. (2012). Lipid metabolism and toxicity in the heart. *Cell Metab.* 15, 805–812. doi:10.1016/j.cmet.2012.04.006

Grabias, B. M., and Konstantopoulos, K. (2014). The physical basis of renal fibrosis: effects of altered hydrodynamic forces on kidney homeostasis. *Am. J. Physiol. Ren. Physiol.* 306, F473–F485. doi:10.1152/ajprenal.00503.2013

Haley, K. E., Kronenberg, N. M., Liehm, P., Elshani, M., Bell, C., Harrison, D. J., et al. (2018). Podocyte injury elicits loss and recovery of cellular forces. *Sci. Adv.* 4, eaap8030. doi:10.1126/sciadv.aap8030

Itoh, N., and Ornitz, D. M. (2011). Fibroblast growth factors: from molecular evolution to roles in development, metabolism and disease. *J. Biochem.* 149, 121–130. doi:10.1093/jb/mvq121

Jiang, T., Wang, Z., Proctor, G., Moskowitz, S., Liebman, S. E., Rogers, T., et al. (2005). Diet-induced obesity in C57BL/6J mice causes increased renal lipid accumulation and glomerulosclerosis via a sterol regulatory element-binding protein-1c-dependent pathway. *J. Biol. Chem.* 280, 32317–32325. doi:10.1074/jbc.M500801200

Kanasaki, K., Shi, S., Kanasaki, M., He, J., Nagai, T., Nakamura, Y., et al. (2014). Linagliptin-mediated DPP-4 inhibition ameliorates kidney fibrosis in streptozotocin-induced diabetic mice by inhibiting endothelial-to-mesenchymal transition in a therapeutic regimen. *Diabetes* 63, 2120–2131. doi:10.2337/db13-1029

Kharitonenkov, A., Shiyanova, T. L., Koester, A., Ford, A. M., Micanovic, R., Galbreath, E. J., et al. (2005). FGF-21 as a novel metabolic regulator. *J. Clin. Invest.* 115, 1627–1635. doi:10.1172/JCI23606

Kim, H. W., Lee, J. E., Cha, J. J., Hyun, Y. Y., Kim, J. E., Lee, M. H., et al. (2013). Fibroblast growth factor 21 improves insulin resistance and ameliorates renal injury in db/db mice. *Endocrinology* 154, 3366–3376. doi:10.1210/en.2012-2276

Kim, Y., Lim, J. H., Kim, M. Y., Kim, E. N., Yoon, H. E., Shin, S. J., et al. (2018). The adiponectin receptor agonist AdipoRon ameliorates diabetic nephropathy in a model of type 2 diabetes. *J. Am. Soc. Nephrol.* 29, 1108–1127. doi:10.1681/ASN.2017060627

Kim, Y. H., Goyal, M., Kurnit, D., Wharram, B., Wiggins, J., Holzman, L., et al. (2001). Podocyte depletion and glomerulosclerosis have a direct relationship in the PAN-treated rat. *Kidney Int.* 60, 957–968. doi:10.1046/j.1523-1755.2001.060003957.x

Kolseth, I. B. M., Reine, T. M., Parker, K., Sudworth, A., Witczak, B. J., Jenssen, T. G., et al. (2017). Increased levels of inflammatory mediators and proinflammatory monocytes in patients with type I diabetes mellitus and nephropathy. *J. Diabetes Complicat.* 31, 245–252. doi:10.1016/j.jdiacomp.2016.06.029

Kretzler, M. (2005). Role of podocytes in focal sclerosis: defining the point of no return. *J. Am. Soc. Nephrol.* 16, 2830–2832. doi:10.1681/ASN.2005080841

Kriz, W., Hähnel, B., Rösener, S., and Elger, M. (1995). Long-term treatment of rats with FGF-2 results in focal segmental glomerulosclerosis. *Kidney Int.* 48, 1435–1450. doi:10.1038/ki.1995.433

Kriz, W., and LeHir, M. (2005). Pathways to nephron loss starting from glomerular diseases-insights from animal models. *Kidney Int.* 67, 404–419. doi:10.1111/j.1523-1755.2005.67097.x

Lasagni, L., Ballerini, L., Angelotti, M. L., Parente, E., Sagrinati, C., Mazzinghi, B., et al. (2010). Notch activation differentially regulates renal progenitors proliferation and differentiation toward the podocyte lineage in glomerular disorders. *Stem Cells* 28, 1674–1685. doi:10.1002/stem.492

Lasagni, L., Lazzeri, E., Shankland, S. J., Anders, H.-J., and Romagnani, P. (2013). Podocyte mitosis - a catastrophe. *Curr. Mol. Med.* 13, 13–23. doi:10.2174/1566524011307010013

Le, DSNT, Miles, R., Savage, P. J., Cornell, E., Tracy, R. P., Knowler, W. C., et al. (2008). The association of plasma fibrinogen concentration with diabetic microvascular complications in young adults with early-onset of type 2 diabetes. *Diabetes Res. Clin. Pract.* 82, 317–323. doi:10.1016/j.diabres.2008.08.019

Li, J., Liu, H., Srivastava, S. P., Hu, Q., Gao, R., Li, S., et al. (2020a). Endothelial FGFR1 (fibroblast growth factor receptor 1) deficiency contributes differential fibrogenic effects in kidney and heart of diabetic mice. *Hypertension* 76, 1935–1944. doi:10.1161/HYPERTENSIONAHA.120.15587

Li, J., Liu, H., Takagi, S., Nitta, K., Kitada, M., Srivastava, S. P., et al. (2020b). Renal protective effects of empagliflozin via inhibition of EMT and aberrant glycolysis in proximal tubules. *JCI Insight* 5, e129034. doi:10.1172/jci.insight.129034

Li, J., Shi, S., Srivastava, S. P., Kitada, M., Nagai, T., Nitta, K., et al. (2017). FGFR1 is critical for the anti-endothelial mesenchymal transition effect of N-acetyl-seryl-aspartyl-lysyl-proline via induction of the MAP4K4 pathway. *Cell Death Dis.* 8, e2965. doi:10.1038/cddis.2017.353

Lin, Z., Huo, H., Huang, M., Tao, J., Yang, Y., and Guo, J. (2024). Fufang Zhenzhu Tiaozhi (FTZ) capsule ameliorates diabetic kidney disease in mice via inhibiting the SGLT2/glycolysis pathway. *J. Ethnopharmacol.* 335, 118698. doi:10.1016/j.jep.2024.118698

Liu, L., Shi, X., Bharadwaj, K. G., Ikeda, S., Yamashita, H., Yagyu, H., et al. (2009). DGAT1 expression increases heart triglyceride content but ameliorates lipotoxicity. *J. Biol. Chem.* 284, 36312–36323. doi:10.1074/jbc.M109.049817

MacDonald, B. T., Tamai, K., and He, X. (2009). Wnt/beta-catenin signaling: components, mechanisms, and diseases. *Dev. Cell* 17, 9–26. doi:10.1016/j.devcel.2009.06.016

Manetti, M., Romano, E., Rosa, I., Guiducci, S., Bellando-Randone, S., De Paulis, A., et al. (2017). Endothelial-to-mesenchymal transition contributes to endothelial dysfunction and dermal fibrosis in systemic sclerosis. *Ann. Rheum. Dis.* 76, 924–934. doi:10.1136/annrheumdis-2016-210229

Mohandes, S., Doke, T., Hu, H., Mukhi, D., Dhillon, P., and Susztak, K. (2023). Molecular pathways that drive diabetic kidney disease. *J. Clin. Invest.* 133, e165654. doi:10.1172/JCI165654

Neef, R., Klein, U. R., Kopajtich, R., and Barr, F. A. (2006). Cooperation between mitotic kinesins controls the late stages of cytokinesis. *Curr. Biol.* 16, 301–307. doi:10.1016/j.cub.2005.12.030

Nishimura, T., Nakatake, Y., Konishi, M., and Itoh, N. (2000). Identification of a novel FGF, FGF-21, preferentially expressed in the liver. *Biochim. Biophys. Acta* 1492, 203–206. doi:10.1016/s0167-4781(00)00067-1

Petermann, A. T., Hiromura, K., Blonski, M., Pippin, J., Monkawa, T., Durvasula, R., et al. (2002). Mechanical stress reduces podocyte proliferation *in vitro*. *Kidney Int.* 61, 40–50. doi:10.1046/j.1523-1755.2002.00102.x

Przybyciński, J., Drożdżal, S., Domański, L., Dziedziejko, V., and Pawlik, A. (2021). Role of endothelial glucocorticoid receptor in the pathogenesis of kidney diseases. *Int. J. Mol. Sci.* 22, 13295. doi:10.3390/ijms222413295

Ren, L., Yang, Y., Li, W., Zheng, X., Liu, J., Li, S., et al. (2022). CDK1 serves as a therapeutic target of adrenocortical carcinoma via regulating epithelial-mesenchymal transition, G2/M phase transition, and PANoptosis. *J. Transl. Med.* 20, 444. doi:10.1186/s12967-022-03641-y

Rurali, E., Noris, M., Chianca, A., Donadelli, R., Banterla, F., Galbusera, M., et al. (2013). ADAMTS13 predicts renal and cardiovascular events in type 2 diabetic patients and response to therapy. *Diabetes* 62, 3599–3609. doi:10.2337/db13-0530

Shi, Y., and Bhalla, V. (2023). Kidney single-cell transcriptomes uncover SGLT2i-induced metabolic reprogramming via restoring glycolysis and fatty acid oxidation. *bioRxiv* doi:10.1101/2023.10.31.564836

Simonson, M. S. (2007). Phenotypic transitions and fibrosis in diabetic nephropathy. *Kidney Int.* 71, 846–854. doi:10.1038/sj.ki.5002180

Srivastava, S. P., Goodwin, J. E., Kanasaki, K., and Koya, D. (2020a). Metabolic reprogramming by N-acetyl-seryl-aspartyl-lysyl-proline protects against diabetic kidney disease. *Br. J. Pharmacol.* 177, 3691–3711. doi:10.1111/bph.15087

Srivastava, S. P., Goodwin, J. E., Kanasaki, K., and Koya, D. (2020b). Inhibition of angiotensin-converting enzyme ameliorates renal fibrosis by mitigating DPP-4 level and restoring antifibrotic MicroRNAs. *Genes (Basel)* 11, 211. doi:10.3390/genes11020211

Srivastava, S. P., Li, J., Kitada, M., Fujita, H., Yamada, Y., Goodwin, J. E., et al. (2018). SIRT3 deficiency leads to induction of abnormal glycolysis in diabetic kidney with fibrosis. *Cell Death Dis.* 9, 997. doi:10.1038/s41419-018-1057-0

Srivastava, S. P., Zhou, H., Setia, O., Dardik, A., Fernandez-Hernando, C., and Goodwin, J. (2021b). Podocyte glucocorticoid receptors are essential for glomerular endothelial cell homeostasis in diabetes mellitus. *J. Am. Heart Assoc.* 10, e019437. doi:10.1161/JAHA.120.019437

Srivastava, S. P., Zhou, H., Setia, O., Liu, B., Kanasaki, K., Koya, D., et al. (2021a). Loss of endothelial glucocorticoid receptor accelerates diabetic nephropathy. *Nat. Commun.* 12, 2368. doi:10.1038/s41467-021-22617-y

Taniguchi, S., Hashiguchi, T., Ono, T., Takenouchi, K., Nakayama, K., Kawano, T., et al. (2010). Association between reduced ADAMTS13 and diabetic nephropathy. *Thromb. Res.* 125, e310–e316. doi:10.1016/j.thromres.2010.02.013

Thibord, F., Song, C., Pattee, J., Rodriguez, B. A. T., Chen, M.-H., O'Donnell, C. J., et al. (2021). FGL1 as a modulator of plasma D-dimer levels: exome-wide marker analysis of plasma tPA, PAI-1, and D-dimer. *J. Thromb. Haemost.* 19, 2019–2028. doi:10.1111/jth.15345

Tuttle, K. R., Agarwal, R., Alpers, C. E., Bakris, G. L., Brosius, F. C., Kolkhof, P., et al. (2022). Molecular mechanisms and therapeutic targets for diabetic kidney disease. *Kidney Int.* 102, 248–260. doi:10.1016/j.kint.2022.05.012

Tuttle, K. R., Bruton, J. L., Perusek, M. C., Lancaster, J. L., Kopp, D. T., and DeFronzo, R. A. (1991). Effect of strict glycemic control on renal hemodynamic response to amino acids and renal enlargement in insulin-dependent diabetes mellitus. *N. Engl. J. Med.* 324, 1626–1632. doi:10.1056/NEJM199106063242304

Wang, P., Huang, Z., Peng, Y., Li, H., Lin, T., Zhao, Y., et al. (2022). Circular RNA circBNC2 inhibits epithelial cell G2-M arrest to prevent fibrotic maladaptive repair. *Nat. Commun.* 13, 6502. doi:10.1038/s41467-022-34287-5

Weng, W., Ge, T., Wang, Y., He, L., Liu, T., Wang, W., et al. (2020). Therapeutic effects of fibroblast growth factor-21 on diabetic nephropathy and the possible mechanism in type 1 diabetes mellitus mice. *Diabetes Metab. J.* 44, 566–580. doi:10.4093/dmj.2019.0089

Wiggins, R. C. (2007). The spectrum of podocytopathies: a unifying view of glomerular diseases. *Kidney Int.* 71, 1205–1214. doi:10.1038/sj.ki.5002222

Wu, K., Tian, R., Huang, J., Yang, Y., Dai, J., Jiang, R., et al. (2018). Metformin alleviated endotoxemia-induced acute lung injury via restoring AMPK-dependent suppression of mTOR. *Chem. Biol. Interact.* 291, 1–6. doi:10.1016/j.cbi.2018.05.018

Xu, L., Niu, M., Yu, W., Xia, W., Gong, F., and Wang, O. (2017). Associations between FGF21, osteonectin and bone turnover markers in type 2 diabetic patients with albuminuria. *J. Diabetes Complicat.* 31, 583–588. doi:10.1016/j.jdiacomp.2016.11.012

Yi, Q., Chen, T., Zhou, K., Ma, Q., Sun, Z., and Shi, H. (2023). PAK1 inhibition suppresses the proliferation, migration and invasion of glioma cells. *Curr. Protein Pept. Sci.* 24, 178–189. doi:10.2174/1389203724666221226150329

Zhang, C., Shao, M., Yang, H., Chen, L., Yu, L., Cong, W., et al. (2013). Attenuation of hyperlipidemia- and diabetes-induced early-stage apoptosis and late-stage renal dysfunction via administration of fibroblast growth factor-21 is associated with suppression of renal inflammation. *PLoS One* 8, e82275. doi:10.1371/journal.pone.0082275

Zhao, X.-P., Chang, S.-Y., Pang, Y., Liao, M.-C., Peng, J., Ingelfinger, J. R., et al. (2023). Hedgehog interacting protein activates sodium-glucose cotransporter 2 expression and promotes renal tubular epithelial cell senescence in a mouse model of type 1 diabetes. *Diabetologia* 66, 223–240. doi:10.1007/s00125-022-05810-6

frontiers | Frontiers in Pharmacology

TYPE Original Research
PUBLISHED 21 January 2025
DOI 10.3389/fphar.2024.1453668

Check for updates

OPEN ACCESS

EDITED BY
Swayam Prakash Srivastava,
University of Michigan, United States

REVIEWED BY
Wei-Ting Kuo,
National Taiwan University, Taiwan
Palash Mandal,
Charotar University of Science and Technology, India

*CORRESPONDENCE
Liangbin Zhao,
zhaoliangbin@cdutcm.edu.cn
Dengpiao Xie,
2876522253@qq.com
Naijing Ye,
756992322@qq.com

†These authors have contributed equally to this work

RECEIVED 23 June 2024
ACCEPTED 19 November 2024
PUBLISHED 21 January 2025

CITATION
Ye Y, Huang X, Li X, Gao F, Zhong W, Tang A, Zhao L, Xie D and Ye N (2025) Shenshuaikang enema restores the intestinal barrier and microbiota-gut-kidney axis balance to alleviate chronic kidney disease via NF-κB pathway. *Front. Pharmacol.* 15:1453668. doi: 10.3389/fphar.2024.1453668

COPYRIGHT
© 2025 Ye, Huang, Li, Gao, Zhong, Tang, Zhao, Xie and Ye. 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.

Shenshuaikang enema restores the intestinal barrier and microbiota-gut-kidney axis balance to alleviate chronic kidney disease via NF-κB pathway

Yan Ye[1,2†], Xiaopeng Huang[1,2,3†], Xueying Li[2,4†], Fei Gao[5], Wenzhen Zhong[5], Anqi Tang[1,2], Liangbin Zhao[2*], Dengpiao Xie[2*] and Naijing Ye[1,2*]

[1]TCM Regulating Metabolic Diseases Key Laboratory of Sichuan Province, Hospital of Chengdu University of Traditional Chinese Medicine, Chengdu, China, [2]Hospital of Chengdu University of Traditional Chinese Medicine, Chengdu, China, [3]Department of Ministry of Science, Hospital of Chengdu University of Traditional Chinese Medicine, Chengdu, China, [4]School of Clinical Medicine, Chengdu University of Traditional Chinese Medicine, Chengdu, China, [5]State Key Laboratory of Southwestern Chinese Medicine Resources, Chengdu University of Traditional Chinese Medicine, Chengdu, China

Introduction: Chronic kidney disease (CKD) is a chronic progressive disease characterized by abnormalities in kidney structure or function caused by variousfactors. It has become a significant public health problem, posing a threat to human health worldwide. Shenshuaikang enema (SSKE) has demonstrated notable efficacy and safety in treating CKD, although its mechanism of action remains unclear.

Methods: The CKD rat model was induced using 2.5% adenine, and the effect of SSKE was evaluated by detecting uremic toxins, inflammatory cytokines, and renal function. The structure of the intestine and kidney was observed using electron microscopy. Pathological changes in the intestine and kidney were detected by H&E staining. The expression of Occludin, Claudin-1, and ZO-1 in the intestine was detected by immunohistochemistry. The degree of renal fibrosis was observed using Masson and PAS staining. The expression of NF-κB and MyD88 protein in the intestine, and the expression of F4/80, TLR4, NF-κB and MyD88 in the kidney were detected by immunofluorescence staining. NF-κB-RE-Luc transgenic mice were used to construct a CKD mouse model, and changes in fluorescence intensity in mice and isolated kidney tissues were detected within 1–6 days using a small animal live imager. Finally, 16S rRNA amplicon sequencing was used to monitor changes in intestinal flora in CKD patients before and after SSKE treatment.

Results: We found that SSKE improves renal function, attenuates renal fibrosis, reduces inflammatory factor levels, and decreases damage to intestinal and renal structures in adenine-induced CKD rats. Additionally, our results suggest that SSKE regulates NF-κB pathways, increases the expression of tight junction proteins, improves intestinal permeability, promotes the growth of beneficial bacteria, inhibits the proliferation of harmful bacteria, and reduces metabolic disorders. Ultimately, these effects contribute to the efficacy of SSKE in treating CKD.

Conclusion: These results indicate that SSKE restores intestinal barrier function by regulating the microbiota-gut-kidney axis, thereby treating CKD.

KEYWORDS

chronic kidney disease (CKD), shenshuaikang enema (SSKE), microbiota-gutkidney axis, NF-κB pathway, intestinal barrier

1 Introduction

Chronic kidney disease (CKD) is a chronic progressive disease characterized by abnormalities in the structure or function of the kidneys, mainly evidenced by a decrease in the estimated glomerular filtration rate (eGFR) (Chen T. K. et al., 2023; Kanbay et al., 2024). Currently, CKD has emerged as a significant threat to human health, following cardiovascular and cerebrovascular diseases, cancers, and diabetes (Liu et al., 2022). CKD represents a global public health problem because of its high incidence, low awareness rate, poor prognosis, and high medical expenses. It severely impacts the quality of life of patients and their families and increases the burden on both families and society (Banerjee et al., 2021; Jadoul et al., 2024; Luyckx et al., 2020).

In 2011, Meijers introduced the concept of the "gut-kidney axis," which innovatively linked the interaction between gut microbiota and the kidneys (Meijers and Evenepoel, 2011). In recent years, research on the gut-kidney interaction in CKD has been on the rise (Ai et al., 2023; Pan et al., 2023; Shen et al., 2023). Studies have shown that CKD leads to disruptions in intestinal flora and metabolic disorders (Holle et al., 2022; Sarakpi et al., 2023; Sohn et al., 2024). Concurrently, disorders in the intestinal flora and the accumulation of uremic toxins can damage the intestinal barrier function in CKD patients (Beker et al., 2022; Wang et al., 2024; Ye et al., 2023). Moreover, the impaired intestinal barrier further exacerbates the translocation of bacteria and toxins, worsening systemic inflammation in patients (Ji et al., 2020; Wu et al., 2022). Therefore, targeting and regulating gut microbiota holds significant potential for the treatment of CKD.

Traditional Chinese medicine (TCM) is widely used as a complementary therapy for CKD, addressing interactions between the intestines and kidneys (Zhao et al., 2020). According to the classical theory of TCM, CKD arises from the pathogenesis of "deficiency, stasis, dampness, and toxicity" (Wang et al., 2022). Based on this theory, we propose a Chinese herbal formula called Shenshuaikang Enema (SSKE), composed of Astragalus membranaceus, Rheum palmatum, Salvia miltiorrhiza, and Carthamus tinctorius, to "benefit qi, activate blood circulation, and dispel dampness and detoxification" (Lu et al., 2020). In our previous study, SSKE was shown to reduce urinary albumin excretion and inflammatory markers in CKD mice after 4 weeks of administration (Lu et al., 2020). The effective constituents of rhubarb (emodin, chrysophanol, etc.) have also been found to improve intestinal barrier function, regulate intestinal microbial disorders, and affect the expression of key proteins in the intestinal NF-κB and Keap1/Nrf2 signalling pathways to alleviate renal dysfunction and tubulointerstitial fibrosis (Gu et al., 2022; Xu et al., 2022). Based on these findings, we propose the hypothesis that SSKE is a novel approach for the treatment of CKD, with its molecular mechanism related to its ability to combat oxidative stress.

In this study, we investigated the protective effects of SSKE on CKD-related symptoms using various pharmacological approaches and CKD rat models. The results showed that SSKE treatment promoted renal protection and intestinal barrier repair by inhibiting the NF-κB pathway. Additionally, SSKE improved the intestinal microbial community in CKD patients. This study aims to provide further evidence supporting the effectiveness of SSKE in managing CKD.

2 Materials and methods

2.1 Materials and chemicals

According to the Chinese Pharmacopoeia (2020 Edition), the four Chinese herbal pieces of SSKE were identified by Dr. Yan Ye. Adenine (Sigma-Aldrich LLC.), enzyme-linked immunoassay (ELISA) kits (MultiScience (Lianke) Biotech Co., Ltd.), methanol (Sigma-Aldrich LLC.), phosphoric acid (Chengdu Chron Chemical Co., Ltd.) were used. Anti-Occludin rabbit antibody, anti-Claudin-1 rabbit antibody, anti-ZO-1occludin rabbit antibody (Protein-tech Group, Inc.), 4′,6-diamidino-2-phenylindole (DAPI, Sigma-Aldrich Co. LLC.) were used. A polyclonal rabbit antibody against Toll-like receptor 4 (TLR4), anti-Myeloid differentiation primary response 88 (MyD88), anti- Nuclear Factor-kappa B (NF-κB) (Protein-tech Group, Inc.) was used.

2.2 HPLC analysis of the alcohol extract of SSKE

2.2.1 SSKN preparation

The herbal decoction SSKE combines four medicinal herbs including *Astragalus membranaceus*, *Rheum palmatum*, *Salvia miltiorrhiza* and *Carthamus tinctorius* (detailed information provided in Supplementary Table S1) were obtained from the Hospital of Chengdu University of Traditional Chinese Medicine. The ingredients were soaked in distilled water (×8) for 1 h and decocted to 5 mL. The resulting solution was then filtered through two layers of gauze.

2.2.2 Chromatographic conditions

Agilent TC-C18 (4.6 × 250 mm, 5 μm) was performed for chromato-graphic separation. The distilled water containing 0.1% phosphoric acid was used as solvent A, and Methanol was used as solvent B. Detail in-formation of HPLC operating parameters are outlined in Supplementary Table S2.

2.3 Animal model construction and administration

Male Sprague-Dawley rats (6–8 weeks, 220–240 g) were obtained from SPF Biotechnology Co., Ltd. (Beijing, China) and kept in a standard condition (22–25°C, 50%–60% humidity, 12 h/12 h light/shadow cycle). All animal procedures were conducted in accordance with the strict Guidelines for the Care and Use of Laboratory Animals of the Ministry of Science and Technology of China.

The adenine-induced CKD rat model was performed for treatment evaluation (Hayeeawaema et al., 2023; Zhao et al., 2024). The rats were randomly separated into five groups: 1) control group (saline); 2) model group (200 mg/kg/d adenine); 3) SSKE-L group (2.5 mL/kg/d); 4) SSKE-M group (5 mL/kg/d); 5) SSKE-H group (10 mL/kg/d). The model and SSKE groups involved initial oral administration of a 2.5% adenine (Sigma-Aldrich LLC.) suspension at a dosage of 200 mg/kg for the first 4 weeks, followed by the same dose administered orally every other day for the subsequent 3 weeks. The control group received equivalent volumes of saline. During the modelling period, SSKE was administered by enema, and saline enema was given to the control and model groups.

2.4 Biochemical tests

Peripheral blood was collected from mice, and enzyme-linked immunoassay (ELISA) kits were used to determine the levels of the gut-derived endotoxin indole including sulphate (IS) and lipopolysaccharide (LPS), as well as pro-inflammatory cytokines (TNF-α, IL-6) in each group of rats. Blood urea nitrogen (BUN) was detected by automatic biochemical analyzer (Cobas C 311); urine protein concentration was detected by bicinchoninic acid colorimetric method using automatic analyzer (BS-240VET).

2.5 Transmission electron microscope

To analyze ultrastructural changes in the gut and glomerulus, fresh intestinal and renal cortical tissue (<1 mm^3) was fixed in electron microscope fixative for 2–4 h at 4°C, then, rinsed three times with 0.1 M phosphate buffer PB (pH 7.4). 1% osmium acid-0.1 M phosphate buffer (pH 7.4) was fixed for 2 h at room temperature. The samples were dehydrated, osmotic embedding, sectioned and stained, and then viewed under a transmission electron microscope (TEM) (HT7700, HITACHI) and images were collected for analysis.

2.6 Histological analysis

To examine the intestinal and renal pathological changes of SSKE on CKD rats, we used hematoxylin and eosin (H&E) staining followed by observation using an optical microscope (CX33, Olympus, Tokyo, Japan). Meanwhile, for observing the degree of improvement of SSKE on renal fibrosis in CKD model rats, we used Masson's Trichrome Staining (Masson) staining, periodic acid-Schiff (PAS) staining in accordance with the standard protocols.

2.7 Immunohistochemical staining

The paraffin sections of ileum and colon tissues were washed with PBS and fixed with 4% paraformaldehyde. Immunohistochemical staining was subjected to evaluate the expression of Occludin, Claudin-1, ZO-1 and DAPI (nuclear staining). After sealing, images were observed and captured with a fluorescence microscope (Nikon Eclipse C1, Japan).

2.8 Immunofluorescence staining

To confirm the role of SSKE in modulating the gut-kidney axis to ameliorate CKD through the NF-κB signaling pathway, we used immunofluorescence staining for correlative validation. Paraffin sections of ileum, colon and kidney were dewaxed, hydrated and antigenically repaired. After blocking with 10% normal goat serum, the sections were incubated in primary antibody at 4°C overnight. Cell nuclei were stained with DAPI. Then washed with PBS, added with autofluorescence quencher dropwise, and sealed with anti-fluorescence quenching sealer. After sealing, the slices were placed under a fluorescence microscope (Nikon Eclipse C1, Japan) for observation and acquisition of the corresponding images.

2.9 *In Vivo* biodistribution evaluation

In order to dynamically monitor the NF-κB fluorescence changes in the kidney tissues of animals *in vivo* after intervention with SSKE in a mouse CKD model. Fluorescently labelled NF-κB transgenic mice (NF-κB-RE-Luc) were used for observation after isoflurane gas anaesthesia in conjunction with a Caliper Life Sciences LIVIS Lumina Series (PerkinElmer, Waltham, MA). The CKD mouse model was induced by 2.5% adenine, and the mice were randomly separated into three groups, as follows: orally administered control, model, and SSKE. After rectal administration for 1, 2, 3, 4, 5, and 6 days, the changes in fluorescence intensity in mice were observed. The mice were sacrificed at the final endpoint after administration, and the kidney was obtained from the mice to evaluate the distribution in the damaged kidney.

2.10 16S rRNA analysis of fecal microflora

To investigate the role and significance of fecal microflora in the pathogenesis of CKD and the differences before and after treatment with SSKE. We selected 10 patients who were admitted to the Hospital of Chengdu University of Traditional Chinese Medicine with a diagnosis of CKD. The research followed guidelines of the Declaration of Helsinki and Tokyo for humans, and was approved by the institutional human experimentation committee, and that informed consent was obtained. Their fecal samples before and after treatment by SSKE were collected, and their microbial composition was analyzed by 16S rRNA sequencing. PCR amplification was performed, purified amplicons were pooled, and paired-end sequenced. Then, a library was established and the sequence was compared to the bacterial gene bank to analyze the species

10.3389/fphar.2024.1453668

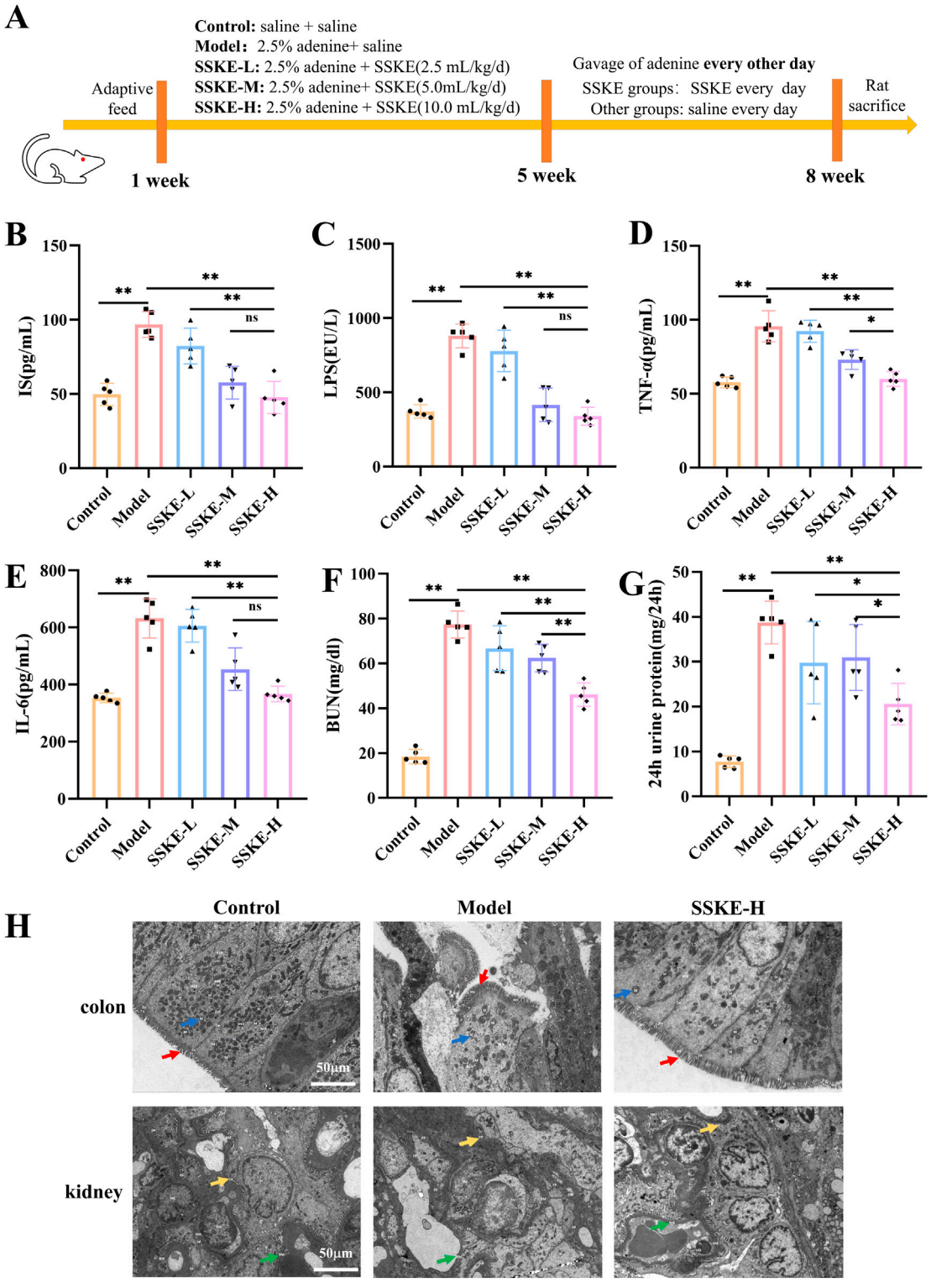


FIGURE 1
SSKE improves endotoxin, inflammation levels, renal function, and intestinal, renal ultrastructural damage in CKD rats. **(A)** Animal experimental protocol of this study. **(B–G)** The levels of IS, LPS, TNF-α, IL-6, BUN, 24 h urine protein in different groups. (n = 5). **(H)** Representative colon and kidney TEM of different groups. (n = 3). All data are presented as means ± SD. *$p < 0.05$; **$p < 0.01$.

abundance and composition of the bacterial community and observe the changes between the fecal microflora before and after treatment.

2.11 Statistical analysis

Data were presented as mean ± SD. Comparisons between two groups were made using independent samples t-test, and comparisons between three or more groups were performed using one-way ANOVA followed by the Tukey test in GraphPad Prism 8.0. It was deemed statistically significant when $p < 0.05$.

3 Results

3.1 Representative HPLC chromatograms of SSKE extract

As shown in Supplementary Figure S1, five main compounds were identified in the HPLC (246 nm) profile of the SSKE extract based on the retention time of the authentic standards. These representative components include aloe-emodin, rhein, emodin, chrysophanol, and emodin methyl ether.

3.2 SSKE improves endotoxin, inflammation levels and renal function in CKD rats

In our study, the adenine-induced CKD rat model, a widely used model, was employed to explore the role of SSKE *in vivo*. The flowchart of the animal experiments is shown in Figure 1A. Intestinal endotoxin levels were detected by ELISA, and the results showed that (Figures 1B, C) compared with the model group, the SSKE-H group significantly reduced the levels of IS and LPS ($p < 0.05$), approaching the levels of the control group. The levels of inflammatory factors are shown in Figures 1D, E. Compared with the model group, SSKE-H significantly reduced the levels of TNF-α and IL-6 ($p < 0.05$). Further analysis of renal injury indicators in serum showed that (Figures 1F, G) compared with the model group, BUN and 24-h urine protein content decreased significantly after SSKE treatment, with SSKE-H showing the most prominent improvement effect ($p < 0.05$). These results indicate that SSKE can improve intestinal endotoxin, proinflammatory cytokines, and renal function in CKD rats, with SSKE-H having the best improvement effect. Therefore, some subsequent experiments only selected the SSKE-H treatment group for further research.

3.3 SSKE attenuates intestinal and renal ultrastructural damage in CKD rats

The gut-kidney axis has been identified as an important pathway for CKD treatment (Hsu et al., 2021). In this study, we explored the role of SSKE in treating CKD by examining both the intestine and kidney. TEM results (Figure 1H) showed that the model group exhibited severe damage to the intestinal barrier, including the loss of microvilli, incomplete cellular structures such as cytoplasmic membranes, mitochondria, and nuclei, and the loss of interstitial junctions, compared with the control group. In the SSKE-H group, the intestinal barrier was repaired, with microvilli, intracellular structures, and intercellular structures showing no significant damage. Additionally, in the model group, the glomerular basement membrane, podocytes, endothelial cells, and mesangial cells were damaged compared with the control group. These structures showed improvement in the SSKE-H group compared with the model group.

3.4 SSKE alleviated the pathological damage of intestinal and renal tissues in CKD rats

When CKD occurs, both intestinal and kidney tissues undergo varying degrees of damage (Sutthasupha et al., 2022). In this study, H&E staining results (Figure 2A) showed that, compared with the control group, the model group had sparse and shortened intestinal villi, inactive glandular proliferation, and significant mucosal edema. There was severe infiltration of inflammatory cells, disorganized and missing epithelial cells, twisted and fuzzy mucosal crypts, and sparse tissue structure. SSKE treatment reduced intestinal mucosal edema and inflammatory cell infiltration, restored intestinal epithelial cell arrangement, and improved mucosal crypt structure, indicating that SSKE can improve intestinal pathological structure. Additionally, in the model group, adenine metabolite crystals were deposited in the kidney, accompanied by extensive inflammatory cell infiltration, disordered glomerular structure, renal interstitial fibrosis, cystic dilatation of renal tubules, numerous lumen obstructions, and significant mesangial proliferation, consistent with the pathological changes observed in adenine model rats. SSKE treatment significantly alleviated renal pathological changes such as tubular lumen dilatation and inflammatory cell infiltration. Masson and PAS staining results (Figures 2B,C) showed that renal fibrosis was significantly increased in the model group compared with the control group. However, SSKE treatment reduced renal fibrosis compared with the model group. These analyses illustrate the ability of SSKE to reduce damage in renal tissue.

3.5 SSKE repairs intestinal mucosa by upregulating tight junction (TJs) proteins

Modern pharmacological studies have shown that intestinal epithelial barrier dysfunction plays a crucial role in the occurrence and progression of CKD (Georgia Andriana et al., 2023; Pyatchenkov et al., 2022). TJs are an essential component of the intestinal epithelial barrier, regulating its selective permeability and preventing harmful bacteria and toxins from entering the mucosa, which can lead to intestinal and systemic inflammation. Occludin, Claudin-1, and ZO-1 are key proteins that reflect intestinal barrier function. To verify the potential therapeutic effect of SSKE on CKD by improving intestinal epithelial barrier injury, we examined the expression levels of Occludin, Claudin-1, and ZO-1 using immunohistochemistry (IHC). The results (Figures 3A, B) showed that, compared with the control group, the expression of Occludin, Claudin-1,

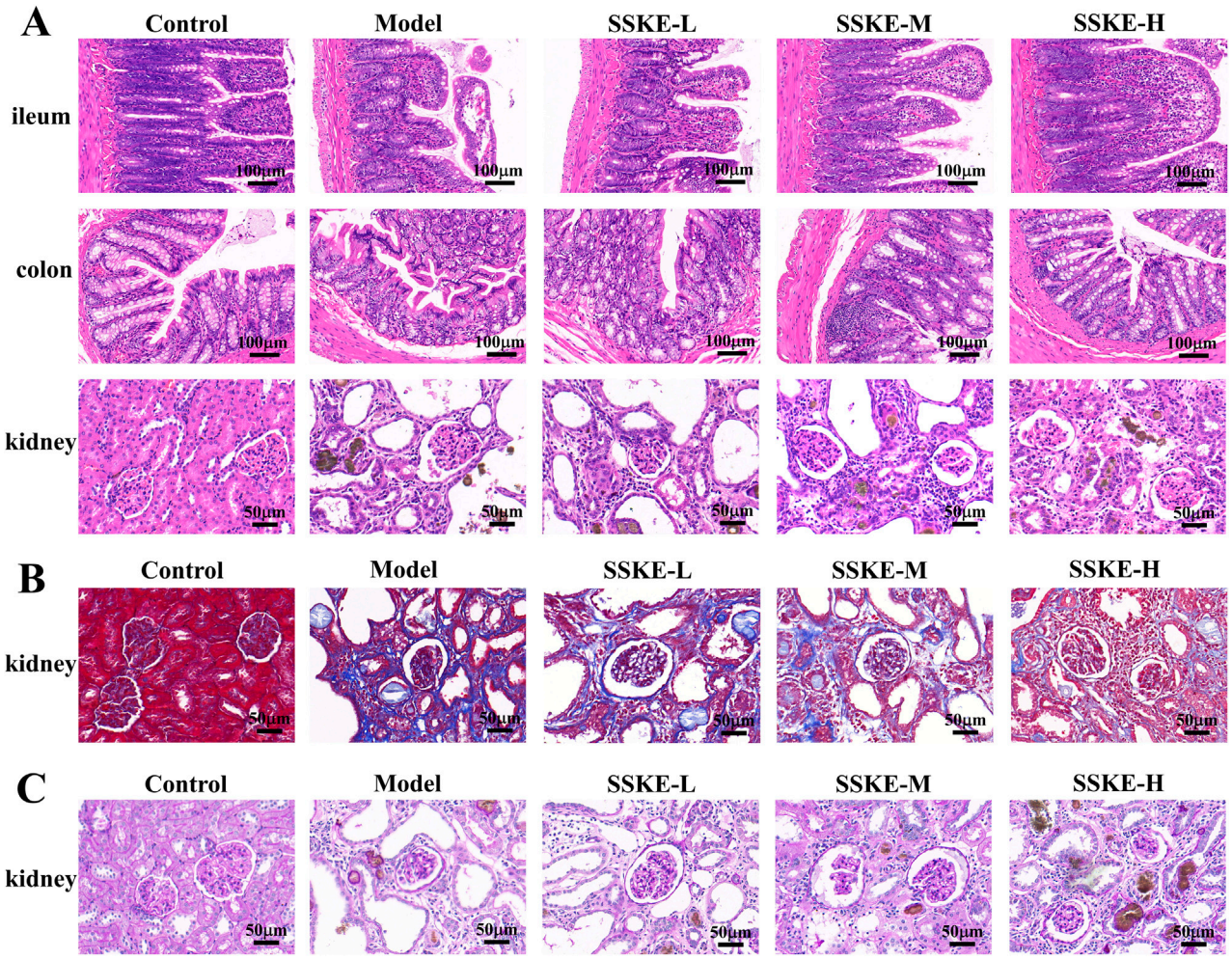


FIGURE 2
SSKE alleviated the pathological damage of intestinal and renal tissues in CKD rats. **(A)** H&E staining of ileum, colon and kidney in different groups. (n = 3). **(B, C)** Masson and PAS staining of kidney in different groups. (n = 3).

and ZO-1 decreased in the ileum and colon tissues of the model group, indicating severe damage to the intestinal barrier. However, the SSKE-H group exhibited higher expression levels of these three proteins in the ileum and colon compared to other treatment groups, suggesting a stronger protective effect on the TJs structure.

3.6 SSKE inhibits inflammatory response by inhibiting NF-κB signaling pathway

The severity of CKD is directly related to the amount of pro-inflammatory cytokines in the blood (Chen M. et al., 2023). Previous studies have confirmed that SSKE effectively reduces the production of pro-inflammatory cytokines. The activation of the NF-κB signaling pathway is crucial in the pathogenesis of CKD, promoting inflammation and regulating apoptosis and vascular remodeling. Our preliminary study suggests that SSKE's therapeutic effect on adenine-induced CKD rats is associated with the inhibition of the NF-κB signaling pathway.

To further investigate the mechanism of SSKE in treating CKD via the gut-kidney axis, we evaluated the expression levels of proteins related to the NF-κB signaling pathway using immunofluorescence (IF). As shown in Figures 4A–D, compared with the control group, the fluorescence intensity of MyD88 and NF-κB increased in the ileum and colon tissues of the model group. The SSKE-H group showed significantly lower fluorescence intensity of MyD88 and NF-κB compared to the SSKE-L and SSKE-M groups.

Furthermore, we observed the expression levels of NF-κB signaling pathway-related proteins in kidney tissues and macrophage infiltration. As shown in Figures 5A–C, the model group exhibited increased fluorescence intensities of TLR4, MyD88, and NF-κB, as well as significant macrophage infiltration, compared with the control group. After SSKE-H treatment, the fluorescence intensity of TLR4, MyD88, and NF-κB decreased, and macrophage infiltration was reduced. This indicates that SSKE has potent anti-CKD effects by regulating the NF-κB signaling pathway.

To further examine the role of the NF-κB axis in CKD development and how SSKE can improve CKD by regulating the NF-κB axis, we used a small-animal *in vivo* imaging device to

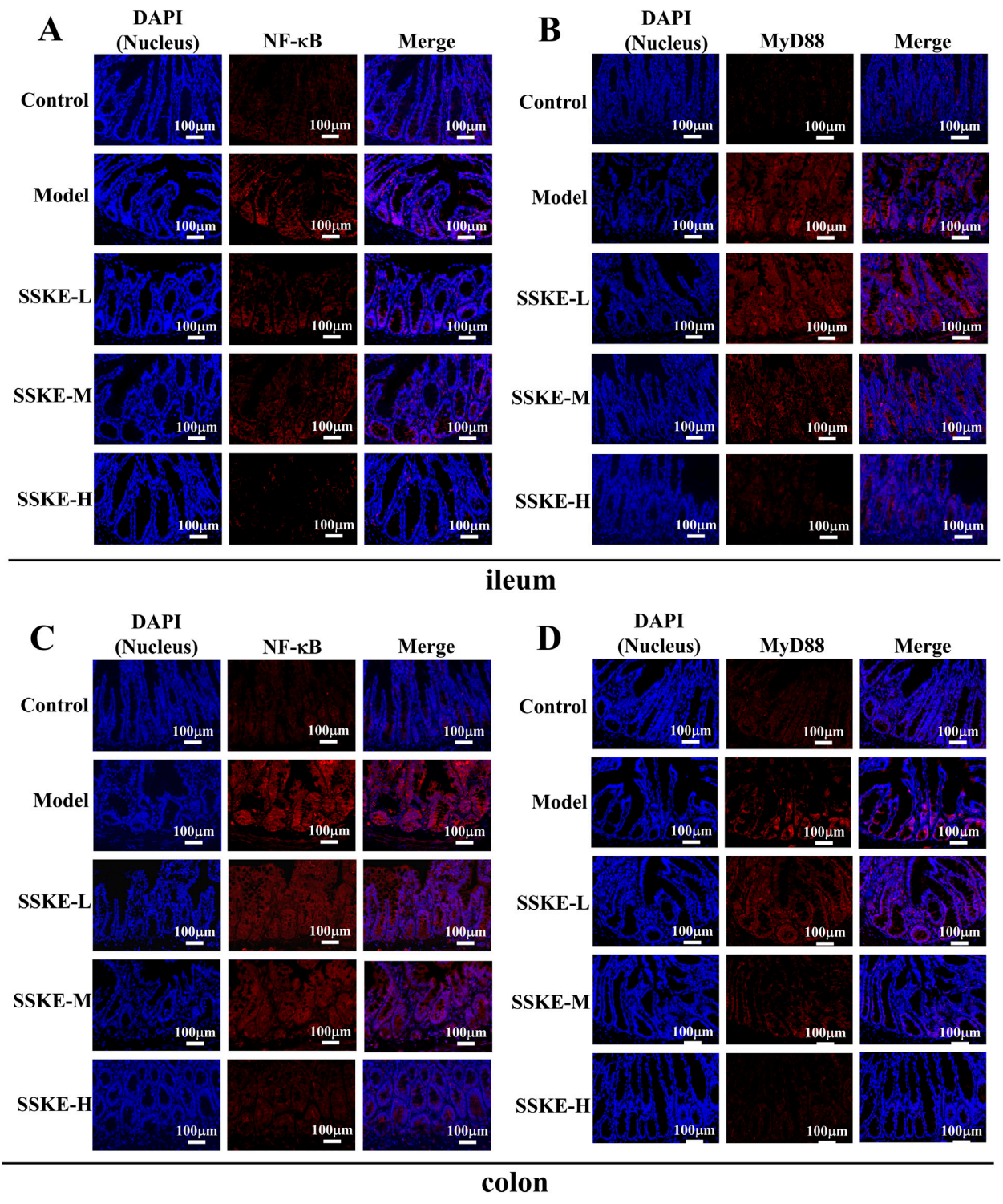


FIGURE 3
SSKE repairs intestinal mucosa by upregulating tight junction (TJs) proteins. **(A)** IHC of ileum in different groups. **(B)** IHC staining of colon in different groups. (n = 3).

monitor the fluorescence intensity of NF-κB-RE-Luc BALB/c mice *in vivo* and *in vitro* kidney tissue in real time. As shown in Figures 5D–F, compared with the control group, the model group exhibited significantly enhanced fluorescence intensity of NF-κB *in vivo*, with a diffuse distribution in the intestinal area and an accumulation of NF-κB fluorescence intensity in isolated kidneys over time. In contrast, the SSKE treatment group showed weakened fluorescence intensity of NF-κB *in vivo* and in isolated kidneys, indicating that SSKE inhibits the expression of NF-κB.

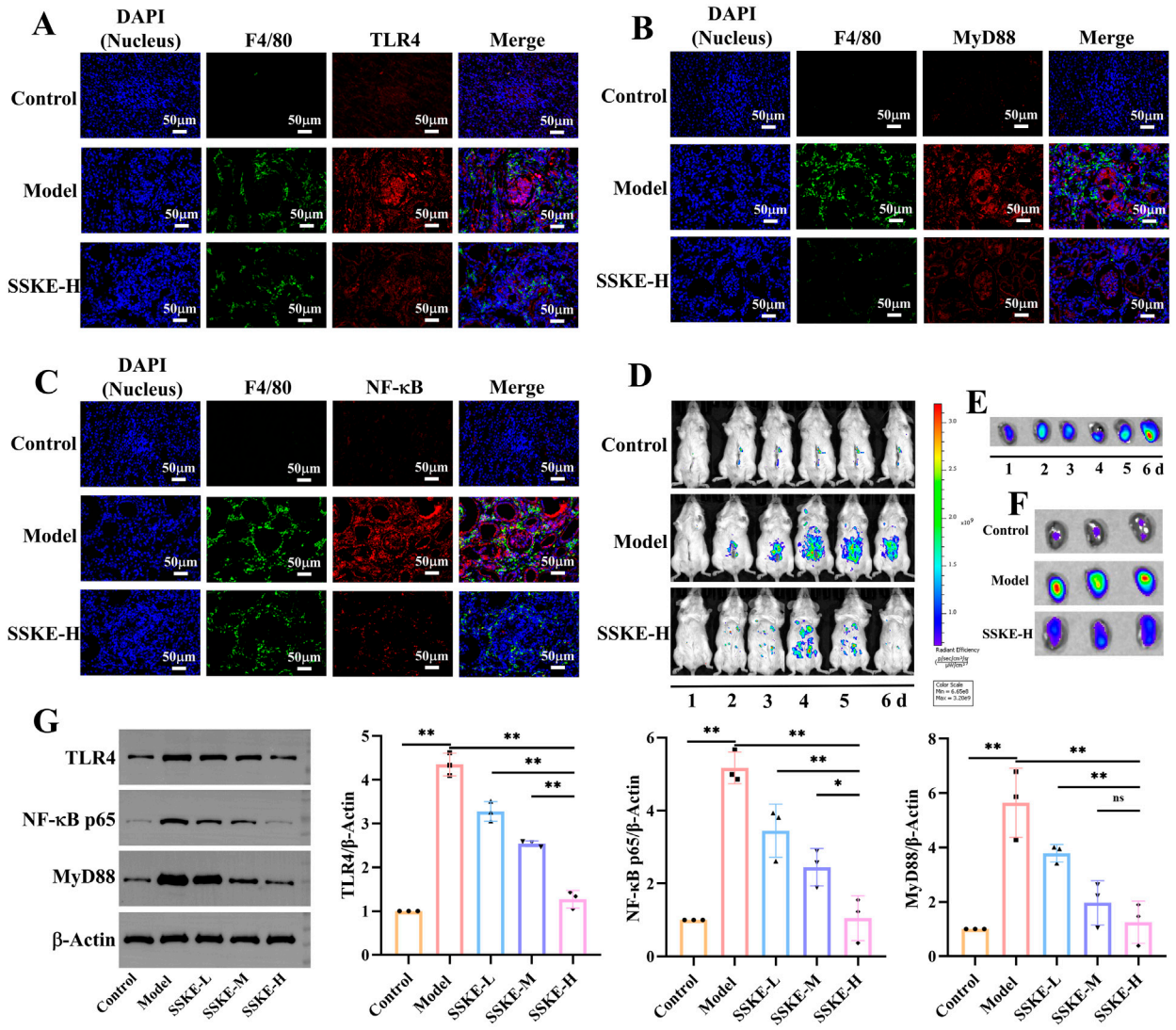


FIGURE 4
SSKE inhibits intestinal inflammatory response by inhibiting NF-κB signaling pathway. **(A, B)** IF of ileum in different groups. **(C, D)** IF of colon in different groups. (n = 3).

3.7 SSKE restores intestinal flora homeostasis by regulating flora

The gut microbiota plays a crucial role in the pathogenesis of CKD, with microbial imbalances being a key factor in the disease's onset and progression (Hsu and Tain, 2022; Mertowska et al., 2021). In this study, we used 16S rRNA gene sequencing technology to detect changes in the species composition and relative abundance of gut microbiota in CKD patients before and after SSKE treatment, evaluating SSKE's regulatory effect on the gut microbiota.

First, we analyzed the diversity of bacterial communities in each group. The α-diversity dilution curve (Figure 6A) indicated that the gut flora in both groups had good diversity. The diversity analysis (Figure 6B) showed a biological difference between the CKD patient group (ctrl) and the treatment group (drug) ($p < 0.5$). To further investigate the microbial composition between the two groups, we created a Venn diagram (Figure 6C) to identify overlapping and unique taxa in each group. The results revealed 737 overlapping OTUs in both groups, with 138 unique OTUs in the ctrl group and 124 unique OTUs in the drug group. The phylogenetic tree (Figure 6D) provided further analysis of the species composition and community structure between the two groups, showing differences in microbial composition and structure. These findings indicate that SSKE can regulate the gut microbial levels of CKD patients.

Further, we used histograms to analyze the differences in species and relative abundance of gut microbiota in different groups at the phylum, family, and genus levels. At the phylum level (Figure 6E), the dominant bacteria mainly included *Firmicutes* and *Lactobacillus*. After SSKE treatment, the relative abundance of *Lactobacillus* was downregulated, consistent with other research findings. At the family level (Figure 6F), the abundance of Ruminococcaceae and *Lachnospiraceae* in the treatment group was lower than in CKD patients. The species composition at the genus level (Figure 6G)

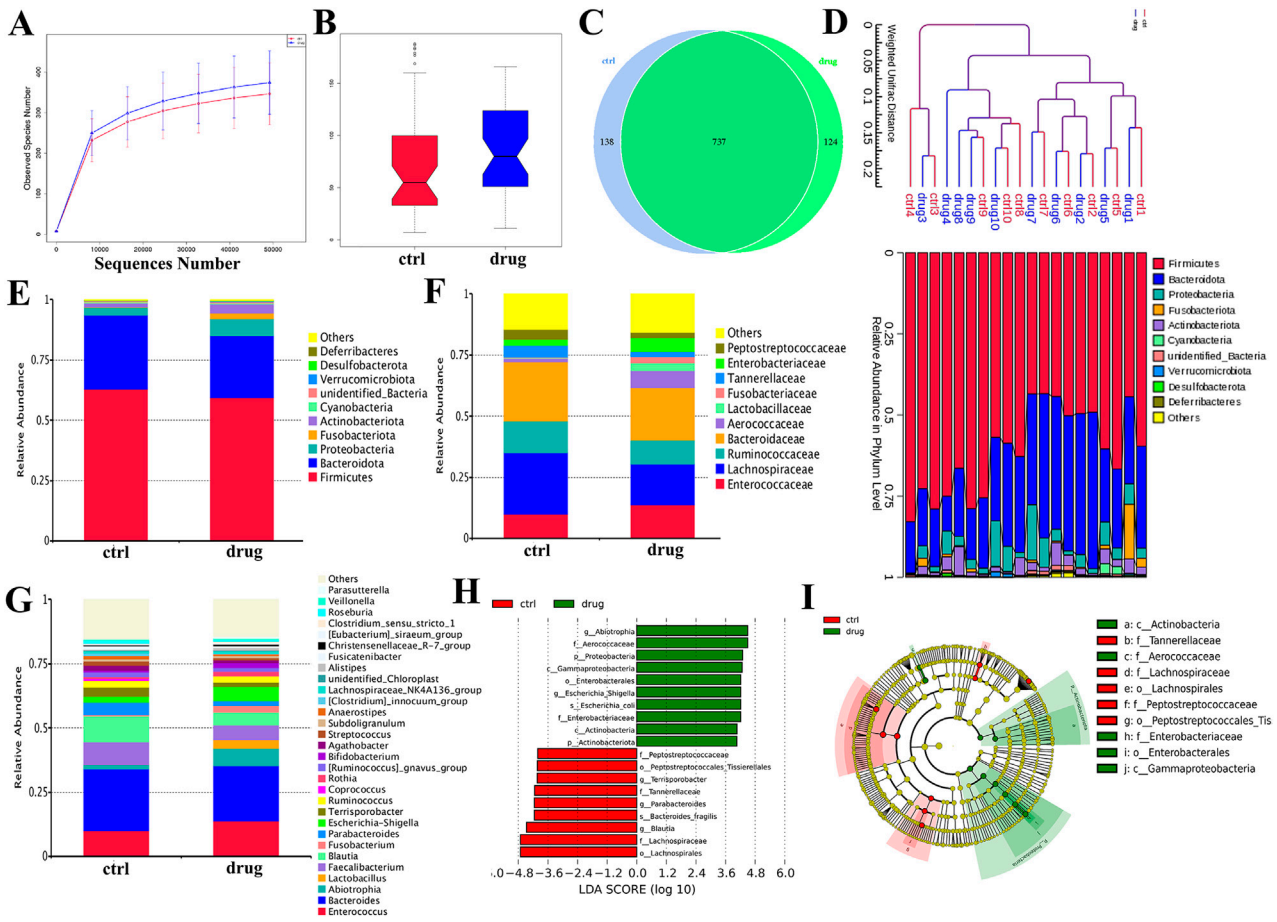


FIGURE 5
SSKE protects the kidney by inhibiting the NF-κB signalling pathway. **(A–C)** IF of kidney in different groups. **(D)** Fluorescence images of CKD mice at the different time points after SSKE administration. **(E)** The kidney fluorescence images of SSKE-H group at the different time point. **(F)** The kidney fluorescence images at the final time point.

showed that SSKE treatment increased the relative abundance of *Lactobacillus*. LEfSe analysis (Figures 6H, I) identified a total of 19 differential bacterial genera between the two groups, with 9 genera significantly regulated by SSKE, including *Tannerellaceae*, *Lachnospiraceae*, *Lachnospirales*, *Peptostreptococcaceae*, and *Peptostreptococcales*. Both species diversity analysis and relative abundance analysis indicated differences in CKD patients after SSKE treatment. After SSKE treatment, the abundance of pathogenic bacteria decreased significantly, while the abundance of probiotics increased significantly. This suggests that SSKE can reach the colon and enhance its ability to regulate intestinal microbiota, thereby treating CKD.

4 Discussion

The prevalence of CKD is increasing yearly, characterized by pathological changes such as glomerular dilatation, enlarged glomerular mesangium, accumulation of mesangial matrix, basement membrane thickening, interstitial inflammation, and eventually tubular fibrosis (Chen et al., 2019). These changes can lead to a progressive decline in renal function and, over time, the development of chronic renal failure. Current treatments offer only partial protection against the progression of CKD.

In Asian countries, Chinese herbal medicine has been widely used to treat chronic diseases (Zhao et al., 2020). Ongoing research into Chinese herbal medicine has produced increasing evidence supporting its clinical efficacy. Our proposed formulation of SSKE aligns with the principles of Chinese medicine for treating CKD. Our results showed that SSKE could reduce enteric-derived endotoxin and proteinuria levels and mitigate glomerular histological alterations in CKD rats. Additionally, SSKE can improve intestinal damage caused by CKD by repairing intestinal tight junctions. Through *in vivo* and immunofluorescence experiments, we confirmed that the therapeutic effect of SSKE on CKD is mediated by the NF-κB signaling pathway.

To determine the appropriate dose for our study, we applied low, medium, and high doses of SSKE based on the human-mouse dose conversion formula. High doses of SSKE demonstrated the most significant anti-inflammatory and tissue structure improvement effects in CKD rats, providing preliminary evidence for the therapeutic effect of SSKE on CKD and its potential mechanism. Increasing evidence supports the critical role of the gut-kidney axis

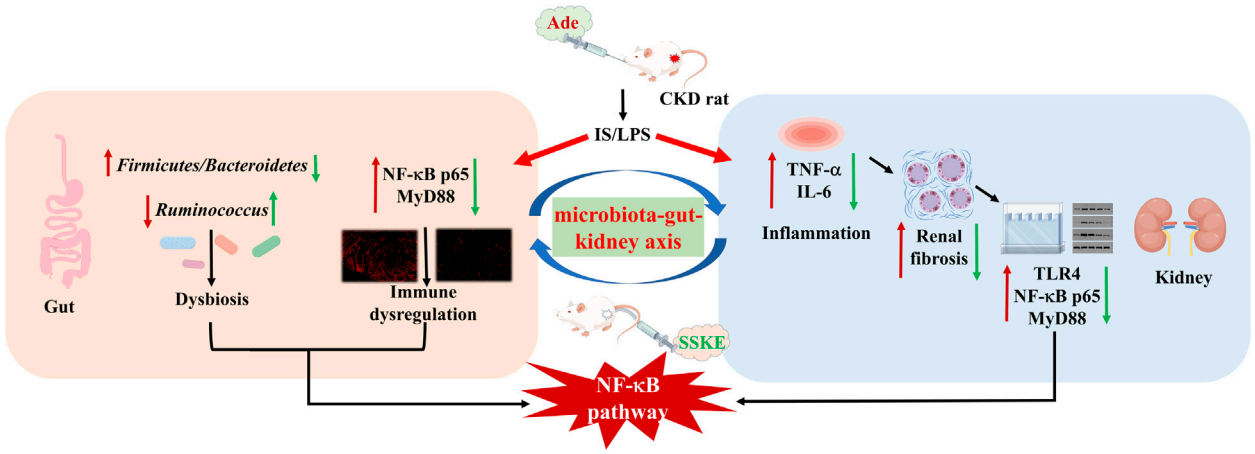


FIGURE 6
Evaluation of SSKE restoring the structure of gut microbiota in CKD patients. **(A)** α-diversity dilution curve. **(B)** β-diversity dilution curve. **(C)** Venn diagram. **(D)** Phylogenetic tree of abundance distribution. **(E)** The relative abundance of the top 10 species in each group at phylum among groups. **(F)** The relative abundance of the top 10 species in each group at family level. **(G)** The relative abundance of the top 20 species in each group at genus level. **(H, I)** Different gut microbiota composition profiles among groups and the Cladogram generated from the LEfSe analysis indicate the phylogenetic distribution from phylum to the genus of the microbiota. Histogram of LDA scores to identify differentially abundant bacterial genera (LDA score ≥3.0, Kruskal–Wallis = 0.05, Wilcoxon = 0.05).

in CKD progression. We conducted a study simultaneously investigating the gut and kidney to verify that SSKE can improve CKD by regulating the microbiota-gut-kidney axis. Our previous study showed that SSKE could influence CKD progression by targeting the intestine and restoring its physical barrier. We first observed the cell ultrastructure and pathological conditions of the intestine and kidney, finding that both suffered from tissue structure and pathological damage in CKD rats. This provided a structural basis for treating CKD based on the gut-kidney axis. The molecular mechanism involving the NF-κB signaling pathway in treating CKD through the gut-kidney axis was further examined. Results from *in vivo* and immunofluorescence studies confirmed the regulation of the NF-κB signaling pathway in the intestine and kidney and demonstrated that SSKE improves CKD by inhibiting this pathway. Additionally, substantial evidence supports that gut microbiota-derived metabolites are therapeutic targets for reducing uremic toxicity, highlighting the therapeutic potential of the gut microbiota in CKD research.

This study has some limitations. Firstly, although we have shown that SSKE plays a therapeutic role in CKD rats by regulating the NF-κB signaling pathway, further *in vitro* cell studies are needed to explore its mechanism. Secondly, each compound of SSKE has not been validated for its respective mechanism, which presents a significant challenge. Thirdly, our studies were conducted in animal experiments and clinical trials, but larger controlled clinical trials are necessary to further ensure the efficacy and safety of SSKE in treating CKD.

5 Conclusion

In summary, this study highlights gut-derived endotoxin and inflammatory responses as key factors inducing renal tubular injury and renal fibroblast activation, emphasizing the activation of the NF-κB signaling pathway via the gut microbiota-gut-kidney axis in the progression of CKD. Our findings demonstrate that SSKE improves the physical structure and pathological morphology of the intestine and kidney, attenuates the inflammatory response, and reduces the secretion of TNF-α and IL-6 cytokines in CKD through the NF-κB pathway. Additionally, SSKE decreases the level of gut-derived endotoxin and regulates the composition and abundance of intestinal microorganisms, thereby improving the progression of CKD. These results suggest that SSKE is a therapeutic agent that slows CKD progression and provides valuable insights for CKD treatment.

Data availability statement

The original contributions presented in the study are publicly available. This data can be found here: Figshare, DOI: 10.6084/m9.figshare.28062827 (https://figshare.com/articles/dataset/dx_doi_org_10_6084_m9_figshare_28062827/28062827).

Ethics statement

The studies involving humans were approved by Hospital of Chengdu University of Traditional Chinese Medicine. 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. The animal studies were approved by the Chengdu University of Traditional Chinese Medicin. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent was obtained from the owners for the participation of their animals in this study.

Author contributions

YY: Data curation, Visualization, Writing–original draft. XH: Methodology, Validation, Writing–review and editing. XL: Conceptualization, Resources, Writing–original draft. FG: Funding acquisition, Methodology, Writing–original draft. WZ: Methodology, Writing–original draft. AT: Resources, Writing–review and editing. LZ: Formal Analysis, Writing–review and editing. DX: Investigation, Writing–original draft. NY: Funding acquisition, Methodology, Project administration, Writing–original draft.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. This study was supported by the National Nature Science Foundation of China (Grant No. 82104830), the Natural Science Foundation of Sichuan, China (2022YFS0398).

Acknowledgments

The authors thank Jiayi Sun at Innovative Institute of Chinese Medicine and Pharmacy, Chengdu University of Traditional Chinese Medicine, for improving the assistance with confocal laser scanning microscopy of the 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.

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.2024.1453668/full#supplementary-material

References

Ai, S., Li, Y., Tao, J., Zheng, H., Tian, L., Wang, Y., et al. (2023). Bibliometric visualization analysis of gut-kidney axis from 2003 to 2022. *Front. Physiol.* 14, 1176894. doi:10.3389/fphys.2023.1176894

Banerjee, D., Rosano, G., and Herzog, C. A. (2021). Management of heart failure patient with CKD. *Clin. J. Am. Soc. Nephrol.* 16 (7), 1131–1139. doi:10.2215/cjn.14180920

Beker, B. M., Colombo, I., Gonzalez-Torres, H., and Musso, C. G. (2022). Decreasing microbiota-derived uremic toxins to improve CKD outcomes. *Clin. Kidney J.* 15 (12), 2214–2219. doi:10.1093/ckj/sfac154

Chen, M., Menon, M. C., Wang, W., Fu, J., Yi, Z., Sun, Z., et al. (2023). HCK induces macrophage activation to promote renal inflammation and fibrosis via suppression of autophagy. *Nat. Commun.* 14 (1), 4297. doi:10.1038/s41467-023-40086-3

Chen, T. K., Hoenig, M. P., Nitsch, D., and Grams, M. E. (2023). Advances in the management of chronic kidney disease. *Bmj* 383, e074216. doi:10.1136/bmj-2022-074216

Chen, T. K., Knicely, D. H., and Grams, M. E. (2019). Chronic kidney disease diagnosis and management: a review. *Jama* 322 (13), 1294–1304. doi:10.1001/jama.2019.14745

Georgia Andriana, G., Marios, P., Pinelopi, B., Anne-Lise de, L., Evangelos, P., Dimitrios, G., et al. (2023). #4884 Disruption of the intestinal mucosal barrier in chronic kidney disease is related to reduced expression of the tight junctions' components. *Nephrol. Dial. Transplant.* doi:10.1093/ndt/gfad063c_4884

Gu, M., Zhou, Y., Liao, N., Wei, Q., Bai, Z., Bao, N., et al. (2022). Chrysophanol, a main anthraquinone from Rheum palmatum L. (rhubarb), protects against renal fibrosis by suppressing NKD2/NF-κB pathway. *Phytomedicine* 105, 154381. doi:10.1016/j.phymed.2022.154381

Hayeeawaema, F., Muangnil, P., Jiangsakul, J., Tipbunjong, C., Huipao, N., and Khuituan, P. (2023). A novel model of adenine-induced chronic kidney disease-associated gastrointestinal dysfunction in mice: the gut-kidney axis. *Saudi J. Biol. Sci.* 30 (6), 103660. doi:10.1016/j.sjbs.2023.103660

Holle, J., Bartolomaeus, H., Löber, U., Behrens, F., Bartolomaeus, T. U. P., Anandakumar, H., et al. (2022). Inflammation in children with CKD linked to gut dysbiosis and metabolite imbalance. *J. Am. Soc. Nephrol.* 33 (12), 2259–2275. doi:10.1681/asn.2022030378

Hsu, C. N., Hou, C. Y., Chang, C. I., and Tain, Y. L. (2021). Resveratrol butyrate ester protects adenine-treated rats against hypertension and kidney disease by regulating the gut-kidney Axis. *Antioxidants (Basel).* 11 (1), 83. doi:10.3390/antiox11010083

Hsu, C. N., and Tain, Y. L. (2022). Chronic kidney disease and gut microbiota: what is their connection in early life? *Int. J. Mol. Sci.* 23 (7), 3954. doi:10.3390/ijms23073954

Jadoul, M., Aoun, M., and Masimango Imani, M. (2024). The major global burden of chronic kidney disease. *Lancet Glob. Health* 12 (3), e342–e343. doi:10.1016/s2214-109x(24)00050-0

Ji, C., Deng, Y., Yang, A., Lu, Z., Chen, Y., Liu, X., et al. (2020). Rhubarb enema improved colon mucosal barrier injury in 5/6 nephrectomy rats may associate with gut microbiota modification. *Front. Pharmacol.* 11, 1092. doi:10.3389/fphar.2020.01092

Kanbay, M., Copur, S., Bakir, C. N., Covic, A., Ortiz, A., and Tuttle, K. R. (2024). Glomerular hyperfiltration as a therapeutic target for CKD. *Nephrol. Dial. Transpl.* 39, 1228–1238. doi:10.1093/ndt/gfae027

Liu, H., Doke, T., Guo, D., Sheng, X., Ma, Z., Park, J., et al. (2022). Epigenomic and transcriptomic analyses define core cell types, genes and targetable mechanisms for kidney disease. *Nat. Genet.* 54 (7), 950–962. doi:10.1038/s41588-022-01097-w

Lu, H., Luo, X., He, Y., Qu, B., Zhao, L., and Li, M. (2020). Shenshuaikang enema, a Chinese herbal remedy, inhibited hypoxia and reoxygenation-induced apoptosis in renal tubular epithelial cells by inhibiting oxidative damage-dependent JNK/Caspase-3 signaling pathways using network pharmacology. *Evid. Based Complement. Altern. Med.* 2020, 9457101. doi:10.1155/2020/9457101

Luyckx, V. A., Cherney, D. Z. I., and Bello, A. K. (2020). Preventing CKD in developed countries. *Kidney Int. Rep.* 5 (3), 263–277. doi:10.1016/j.ekir.2019.12.003

Meijers, B. K., and Evenepoel, P. (2011). The gut-kidney axis: indoxyl sulfate, p-cresyl sulfate and CKD progression. *Nephrol. Dial. Transpl.* 26 (3), 759–761. doi:10.1093/ndt/gfq818

Mertowska, P., Mertowski, S., Wojnicka, J., Korona-Głowniak, I., Grywalska, E., Błażewicz, A., et al. (2021). A link between chronic kidney disease and gut microbiota in immunological and nutritional aspects. *Nutrients* 13 (10), 3637. doi:10.3390/nu13103637

Pan, L., Yu, H., Fu, J., Hu, J., Xu, H., Zhang, Z., et al. (2023). Berberine ameliorates chronic kidney disease through inhibiting the production of gut-derived uremic toxins in the gut microbiota. *Acta Pharm. Sin. B* 13 (4), 1537–1553. doi:10.1016/j.apsb.2022.12.010

Pyatchenkov, M. O., Rumyantsev, A. S., Sherbakov, E. V., and Alexander, G. M. (2022). Structural and functional intestinal barrier abnormalities and chronic kidney disease. literature review. Part II. Нефрология 26, 46–64. doi:10.36485/1561-6274-2022-26-2-46-64

Sarakpi, T., Mesic, A., and Speer, T. (2023). Leukocyte-endothelial interaction in CKD. *Clin. Kidney J.* 16 (11), 1845–1860. doi:10.1093/ckj/sfad135

Shen, Z., Cui, T., Liu, Y., Wu, S., Han, C., and Li, J. (2023). Astragalus membranaceus and Salvia miltiorrhiza ameliorate diabetic kidney disease via the gut-kidney axis. *Phytomedicine* 121, 155129. doi:10.1016/j.phymed.2023.155129

Sohn, M. B., Gao, B., Kendrick, C., Srivastava, A., Isakova, T., Gassman, J. J., et al. (2024). Targeting gut microbiome with prebiotic in patients with CKD: the TarGut-CKD study. *Kidney Int. Rep.* 9 (3), 671–685. doi:10.1016/j.ekir.2023.12.017

Sutthasupha, P., Promsan, S., Thongnak, L., Pengrattanachot, N., Phengpol, N., Jaruan, O., et al. (2022). Chitosan oligosaccharide mitigates kidney injury in prediabetic rats by improving intestinal barrier and renal autophagy. *Carbohydr. Polym.* 288, 119405. doi:10.1016/j.carbpol.2022.119405

Wang, M., Tang, W. H. W., Li, X. S., de Oliveira Otto, M. C., Lee, Y., Lemaitre, R. N., et al. (2024). The gut microbial metabolite trimethylamine N -oxide, incident CKD, and kidney function decline. *J. Am. Soc. Nephrol.* 35 (6), 749–760. doi:10.1681/asn.0000000000000344

Wang, Y., Feng, Y., Li, M., Yang, M., Shi, G., Xuan, Z., et al. (2022). Traditional Chinese medicine in the treatment of chronic kidney diseases: theories, applications, and mechanisms. *Front. Pharmacol.* 13, 917975. doi:10.3389/fphar.2022.917975

Wu, H., Huang, R., Fan, J., Luo, N., and Yang, X. (2022). Low potassium disrupt intestinal barrier and result in bacterial translocation. *J. Transl. Med.* 20 (1), 309. doi:10.1186/s12967-022-03499-0

Xu, Z., Hou, Y., Sun, J., Zhu, L., Zhang, Q., Yao, W., et al. (2022). Deoxycholic acid-chitosan coated liposomes combined with *in situ* colonic gel enhances renal fibrosis therapy of emodin. *Phytomedicine* 101, 154110. doi:10.1016/j.phymed.2022.154110

Ye, N., Zhao, P., Ayue, S., Qi, S., Ye, Y., He, H., et al. (2023). Folic acid-modified lactoferrin nanoparticles coated with a laminarin layer loaded curcumin with dual-targeting for ulcerative colitis treatment. *Int. J. Biol. Macromol.* 232, 123229. doi:10.1016/j.ijbiomac.2023.123229

Zhao, J., Zhao, C., Xun, T., Wang, X., Wei, S., Ye, C., et al. (2024). Huang Gan formula alleviates systemic inflammation and uremia in adenine-induced chronic kidney disease rats may associate with modification of gut microbiota and colonic microenvironment. *Drug Des. Devel Ther.* 18, 13–28. doi:10.2147/dddt.S421446

Zhao, M., Yu, Y., Wang, R., Chang, M., Ma, S., Qu, H., et al. (2020). Mechanisms and efficacy of Chinese herbal medicines in chronic kidney disease. *Front. Pharmacol.* 11, 619201. doi:10.3389/fphar.2020.619201

frontiers | Frontiers in Pharmacology

TYPE Review
PUBLISHED 22 January 2025
DOI 10.3389/fphar.2025.1511083

Check for updates

OPEN ACCESS

EDITED BY
Swayam Prakash Srivastava,
University of Michigan, United States

REVIEWED BY
Zhi-Lin Luan,
Dalian Medical University, China
Nisha Sharma,
National Institute of Pharmaceutical Education and Research, India

*CORRESPONDENCE
Zhengzhen Tang,
tangzz957150@163.com
Jun Tan,
juntan_zmu@126.com
Jidong Zhang,
jdzhang0851@163.com

RECEIVED 14 October 2024
ACCEPTED 06 January 2025
PUBLISHED 22 January 2025

CITATION
Li X, Xu R, Zhang D, Cai J, Zhou H, Song T, Wang X, Kong Q, Li L, Liu Z, He Z, Tang Z, Tan J and Zhang J (2025) Baicalin: a potential therapeutic agent for acute kidney injury and renal fibrosis.
Front. Pharmacol. 16:1511083.
doi: 10.3389/fphar.2025.1511083

COPYRIGHT
© 2025 Li, Xu, Zhang, Cai, Zhou, Song, Wang, Kong, Li, Liu, He, Tang, Tan and Zhang. 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.

Baicalin: a potential therapeutic agent for acute kidney injury and renal fibrosis

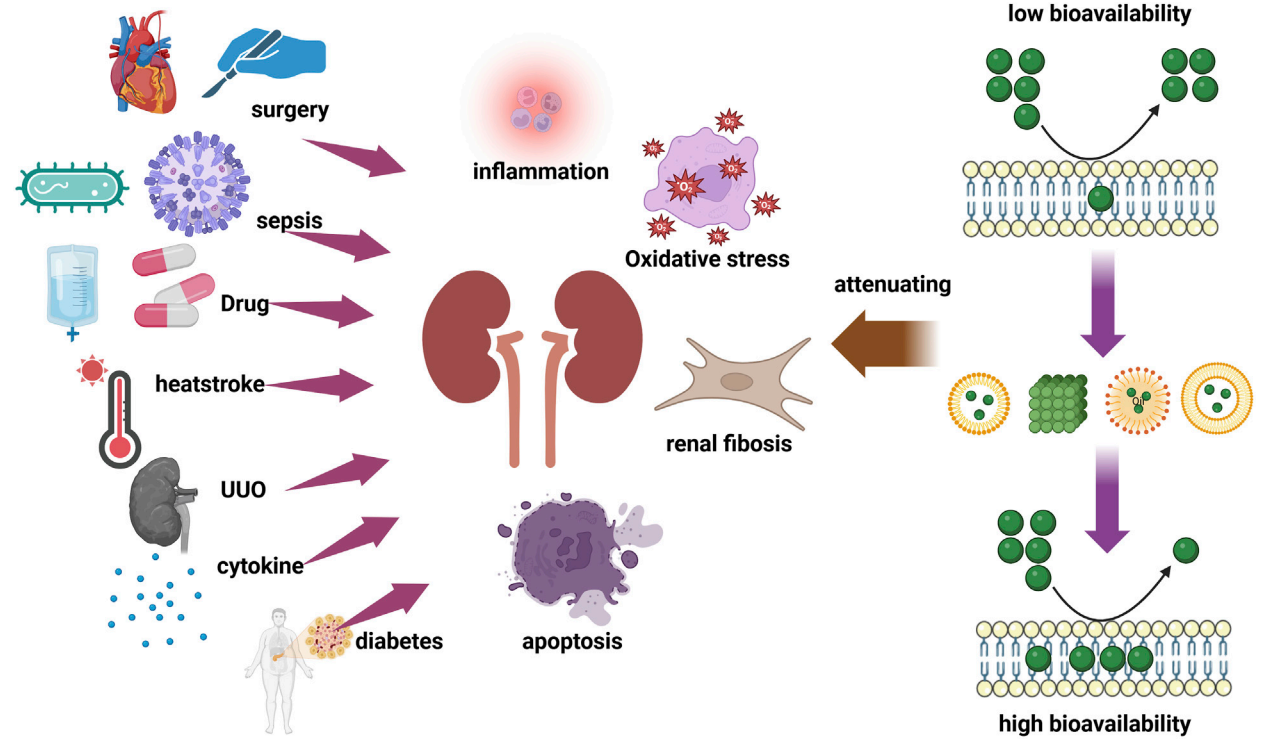
Xiaoming Li[1,2], Rui Xu[1,2], Dan Zhang[3], Ji Cai[1,2], He Zhou[1,2], Tao Song[1,2], Xianyao Wang[1,2], Qinghong Kong[4], Liujin Li[5], Zhaohui Liu[5], Zhixu He[6], Zhengzhen Tang[7]*, Jun Tan[8]* and Jidong Zhang[1,2,6]*

[1]Department of Immunology, Zunyi Medical University, Zunyi, China, [2]Special Key Laboratory of Gene Detection and Therapy of Guizhou Province, Zunyi Medical University, Zunyi, China, [3]Zunyi Medical University Library Administrative Office, Zunyi, China, [4]Guizhou Provincial College-Based Key Lab for Tumor Prevention and Treatment with Distinctive Medicines, Zunyi Medical University, Zunyi, China, [5]Department of Otolaryngology, Affiliated Hospital of Zunyi Medical University, Zunyi, China, [6]Collaborative Innovation Center of Tissue Damage Repair and Regeneration Medicine, Zunyi Medical University, Zunyi, China, [7]Department of Pediatrics, The First People's Hospital of Zunyi, Third Affiliated Hospital of Zunyi Medical University, Zunyi, China, [8]Department of Histology and Embryology, Zunyi Medical University, Zunyi, China

Acute kidney injury (AKI) is a common critical clinical disease that is linked to significant morbidity, recurrence, and mortality. It is characterized by a fast and prolonged loss in renal function arising from numerous etiologies and pathogenic pathways. Renal fibrosis, defined as the excessive accumulation of collagen and proliferation of fibroblasts within renal tissues, contributes to the structural damage and functional decline of the kidneys, playing a pivotal role in the advancement of Chronic Kidney Disease (CKD). Until now, while continuous renal replacement therapy (CRRT) has been utilized in the management of severe AKI, there remains a dearth of effective targeted therapies for AKI stemming from diverse etiologies. Similarly, the identification of specific biomarkers and pharmacological targets for the treatment of renal fibrosis remains a challenge. Baicalin, a naturally occurring compound classified within the flavonoid group and commonly found in the Chinese herb Scutellaria baicalensis, has shown a range of pharmacological characteristics, such as antioxidant, anti-inflammatory, antifibrotic, antitumor and antiviral effects, as evidenced by research studies. Research shows that Baicalin has potential in treating kidney diseases like AKI and renal fibrosis. This review aims to summarize Baicalin's progress in these areas, including its molecular mechanism, application in treatment, and absorption, distribution, metabolism, and excretion. Baicalin's therapeutic effects are achieved through various pathways, including antioxidant, anti-inflammatory, antifibrosis, and regulation of apoptosis and cell proliferation. Besides, we also hope this review may give some enlightenment for treating AKI and renal fibrosis in clinical practice.

KEYWORDS

acute kidney injury, renal fibrosis, herbal medicine, baicalin, mechanism



GRAPHICAL ABSTRACT

1 Introduction

Acute kidney injury (AKI) is defined by a sudden drop in renal function, including an increase in serum creatinine (SCr) (≥0.3 mg/dL within 48 h, or ≥1.5 times baseline), or a urine volume of <0.5 mL/kg/h for 6 h (Turgut et al., 2023). 30%–60% of critically sick patients worldwide suffer from AKI, which is linked to acute morbidity and mortality (Hoste et al., 2018). AKI is a clinical syndrome caused by multiple etiologic factors and can result from various kinds of injuries, such as exposure to nephrotoxins, decreased renal perfusion, urinary obstruction, or nephrogenic renal disease, causing a dramatic decrease in renal function in the short term, manifested as a decrease in the glomerular filtration rate (GFR) with retention of nitrogen products, such as SCr and blood urea nitrogen (BUN), and disturbances in the water, electrolyte, and acid-base balances, which can be severe and can lead to multiple Systemic complications can occur in severe cases (Ronco et al., 2019; Erfurt et al., 2023). Failure to recognize, intervened and treated early, it can cause serious damage to the kidneys, progress to chronic kidney disease (CKD), and increase the risk of cardiovascular events, and even lead to death (See et al., 2019; Kurzhagen et al., 2020). However, the current treatment of AKI is only supportive, with limited therapeutic options, and short-term mortality remains high.

CKD is usually caused by an assortment of factors, including hypertension, diabetes, and glomerulonephritis (Rapa et al., 2019). These pathologies lead to glomerular and tubular damage, which triggers an inflammatory response and cellular damage. Over time, the degree of kidney damage increases and the fibrotic process begins to develop as collagen begins to be deposited, gradually replacing the healthy kidney tissue, and this will eventually lead to further decline in renal function and deterioration of CKD (Hung et al., 2021; Huang et al., 2023).

Currently, the main means of clinical treatment for AKI is to correct reversible etiological and pre-renal factors as soon as possible at the onset of AKI, including volume expansion, maintenance of hemodynamic stability, discontinuation of drugs affecting renal perfusion and so on, whereas glucocorticoids and/or immunosuppressant therapy are commonly used for AKI secondary to glomerulonephritis. Drug usage is not risk-free, though. Oral glucocorticoids have been shown in a cohort study to substantially increase the risk of adrenal insufficiency, Cushing syndrome, and death (Mebrahtu et al., 2019).

Endothelin receptor antagonists (ERAs), sodium-glucose transporter-2 (SGLT-2) inhibitors, dipeptidase transferase-4 (DPP-4) inhibitors, salt-corticosteroid receptor antagonists, and N-acetyl-sericyl-aspartate-lysine (Ac-SDKP) are among the numerous pharmaceutical agents presently undergoing clinical trials for the treatment of diabetic nephropathy. These substances have been shown to offer defense against increased renal inflammatory and fibrotic responses in addition to preclinical decrease in renal function (Bhatia and Srivastava, 2023). Traditional treatments for diabetic nephropathy (DN) include renin angiotensin aldosterone system (RAAS) inhibitors such as angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin II receptor blockers (ARBs), which are first-line agents that are effective in reducing the incidence of end-stage renal disease (Srivastava et al., 2020a). In DN animal models, ACEIs and

ARBs fulfill distinct roles. Specifically, ACEIs mitigate renal fibrosis by attenuating dipeptidyl peptidase-4 (DPP-4) and transforming growth factor-beta (TGFβ) signaling pathways, a function not observed with ARBs (Srivastava et al., 2020b). Heart failure, coronary artery disease, essential hypertension, and CKD are among the clinical conditions for which ACEI and ARBs can be utilized as antihypertensive medications. However, ACEI and ARBs have slightly different mechanisms of action. By decreasing the generation of angiotensin II, vasoconstriction, and aldosterone secretion, ACEI lower blood pressure and cardiac burden. ARBs, on the other hand, directly prevent angiotensin II from binding to its receptors, which may occasionally lead to less adverse effects, such as coughing (Matchar et al., 2008), angioedema (Makani et al., 2012). Therefore, the search for an effective therapeutic drug is imperative.

One of the most widely used multipurpose herbs or medicinal plants for treating bacterial and viral infections, allergies, and inflammation is Scutellaria baicalensis (Yoon et al., 2009; Mir-Palomo et al., 2016; Jung et al., 2012). Baicalin (C21H18O11), a flavonoid extracted from Scutellaria baicalensis (SR) dried roots, has been evaluated in multiple types of AKI. The pharmaceutical industry has become increasingly interested in baicalin due to its superior biological effect. Baicalin has also been reported to exhibit various biological properties, including anti-inflammatory (Fu et al., 2021), antioxidant (Ganguly et al., 2022), antiviral (Feng H. et al., 2022) and anti-tumor (Song et al., 2022). Other pharmacological effects of this compound include antibacterial activity, purgative and detoxification (Bai et al., 2018). Experimental studies have demonstrated that baicalin is protective against renal damage caused by various stimulation, including renal ischemia, nephrotoxic drugs and sepsis while being able to effectively intervene in the occurrence of renal fibrosis (Wang et al., 2015; Le et al., 2021; Shi et al., 2019; Zhang S. et al., 2020). Therefore, this article mainly reviews its renoprotective effects of baicalin. We believe that baicalin may be a promising drug to the treat patients with AKI and renal fibrosis. Currently, most clinical therapeutic drugs cause further kidney damage to varying degrees. In order to prevent AKI from occurring and to create successful preventive and therapeutic methods, it is imperative that safe and effective natural active components be found, given the current clinical status of AKI treatment.

2 Baicalin

Baicalin (C21H18O11) is a natural herbal compound of flavonoids. It is utilized extensively in traditional Chinese medicine and is mostly found in Scutellaria baicalensis, a traditional Chinese medication. 6 polysaccharides and 126 small molecule compounds have been identified from SR thus far (Wang Z. L. et al., 2018). Among them, baicalin, wogonoside, baicalein and wogonin are recognized as the most effective drug candidates. The chemical symbol for baicalin is 7-glucuronic acid, 5,6-dihydroxyflavone, and its molecular weight is 446.4 g/mol. The structure of baicalin has a number of peculiarities, containing a glycosidic group (glycoside) and a flavonoid backbone. First, the glycosidic portion of baicalin is formed by linking glucose and baicalein. This linkage is known as the glycosidic bond, which makes baicalin highly stable and easy to store. For example, one study showed that eight flavonol O-glycosides exhibited antioxidant activity (Kimura H. et al., 2017). In addition, the flavonoid backbone of baicalin is composed of two benzene rings connected by three carbons. This special backbone structure confers many pharmacological and biological activities to baicalin. The phenolic hydroxyl group and carbonyl group of baicalin play important roles in pharmacological activities. For instance, a study showed that the phenolic hydroxyl group of flavonoids, including baicalin, can have a scavenging effect on hydroxyl radicals (Chen et al., 2002). Due to its unique chemical structure, baicalin has many pharmacological activities. It is considered to have anti-inflammatory, antioxidant, antibacterial, antitumor, and antiallergic effects, making baicalin a natural pharmaceutical compound that has been widely studied and applied.

2.1 Absorption

Baicalin itself cannot be directly absorbed by the intestinal tract and is first hydrolyzed by intestinal bacteria to its glycoside baicalein (Akao et al., 2000). Using the *in situ* absorption approach, a preliminary assessment of the absorption sites of baicalin in rats' stomachs and various intestine segments revealed two distinct sites of absorption in one research. Baicalin was absorbed in two different ways: first, it was absorbed in the upper intestine, probably directly; and second, it was absorbed as glycosides in the colon (Lu et al., 2007). One of the most significant techniques for researching medication absorption in rats is *in situ* perfusion. In a rat study, the authors performed *in situ* perfusion in rats with and without ligated bile ducts, and the experiments' findings from 2006 demonstrated that baicalin absorbed badly in the small intestine and colon and somewhat in the stomach, suggesting that baicalin was unable to effectively pass through the intestinal epithelium (Taiming and Xuehua, 2006).

2.2 Distribution

Baicalin can accumulate in various tissues. The largest quantity of baicalin was detected in the kidneys, and the drug's subsequent concentration in each of the main organs in the following order: kidneys > lungs > liver > spleen, according to a tissue distribution study conducted after injection. Following an intravenous injection of liposomal baicalin, the lungs exhibited the highest concentration of the medication. Subsequently, the concentration of the drug declined in the following sequence in the major organs and tissues: kidney > spleen > liver > lungs (Wei et al., 2016). Another finding was that baicalin in the preparation of Huanglian Xieyu Tang was rapidly distributed in the lungs and accumulated at high levels in the lungs (Zhu et al., 2013). In the kidney, the distribution of scutellarin and its metabolite scutellarein isolated from Scutellaria baicalensis showed that the two were mainly distributed in the renal cortex and medullary regions with high abundance, suggesting that scutellarin may have the potential to prevent and treat kidney diseases (Wang T. et al., 2021).

2.3 Metabolism

With regard to gastrointestinal hydrolysis, enterohepatic circulation, carrier-mediated transport, and complex metabolism,

baicalin possesses a distinct pharmacokinetic profile. Once baicalin is absorbed, the interaction between baicalin and its aglycone baicalein will occur in the body. Studies in rats have demonstrated that baicalin is rapidly converted to baicalein in the gut through the enzymatic activity of β-glucuronidase, a process contingent upon the presence of gut microbiota. Then, once baicalin enters the systemic circulation, it is converted back to baicalein by Uridine diphosphate glucuronosyltransferase (UGT) (Akao et al., 2000; Noh et al., 2016). 18.7% of the baicalin that was administered orally entered the enterohepatic circulation. These findings demonstrate that a substantial amount of baicalin is subjected to first-pass glucuronidation and that the enterohepatic circulation significantly influences the amount of baicalin that is exposed to rats (Xing et al., 2005a). Wistar rats' plasma contained baicalein 6-O-β-dlucopyranuronoside (B6G) as the major metabolite after oral baicalin treatment at 20 mg/kg of dosage, and its level was greater than that of baicalin (Akao et al., 2013). Furthermore, the existence of baicalin isomers in plasma following oral treatment suggests that baicalin is at least partially converted to baicalein prior to absorption and then to its conjugated metabolite in rats (Xing et al., 2005b). In recent years, in order to obtain more metabolites, the metabolites of baicalin were studied by linear ion trap-orbitrap mass spectrometry, and 32 metabolites in all were found and described. Also, The corresponding reactions of baicalin *in vivo* were also found, such as methylation, methoxylation, hydrolysis, hydroxylation, glucuronic acid binding, sulfate binding and complex reactions (Zhang et al., 2015), indicating that baicalin has a complicated metabolism and that these isomers could greatly enhance baicalin's benefits if they have biological activity.

2.4 Excretion

In the body, baicalin is first absorbed as baicalein and subsequently converted to baicalin. It is possible for the colon to excrete some of the baicalein that is generated from absorbed or intravenously administered baicalein (Taiming and Xuehua, 2006). Meanwhile, in a previous study, using the *in situ* jejunal loop technique and *in vitro* jejunal ectopic capsule assay, It was shown that multidrug resistance binding protein 2 (MRP2) significantly processed baicalein in intestinal mucosal cells to produce baicalin, which was then eliminated into the intestinal lumen (Akao et al., 2004). It is important to note that rats and humans dispose of baicalin quite differently. Notably, the UGT activity of rat jejunum microsomes against baicalin is comparable to that of liver microsomes, even though the liver is traditionally regarded as the primary site for xenobiotic metabolism (Akao et al., 2004). Furthermore, an *in vitro* absorption experiment utilizing everted rat jejunal sacs revealed that baicalin emerged outside the sac rather than inside it, whereas very little of the baicalein absorbed was transported to the inner (serosal) side of the sac. Eisai hyperbilirubinemia rats (EHBR) with hereditary MRP2 deficiency exhibited a considerably decreased jejunal sac effection rate (56.4%) in comparison to wild-type Sprague-Dawley rats, indicating that baicalin efflux may be mediated by MRP2. The human colonic adenocarcinoma cell line Caco-2 is used to study the absorption and disposition of drugs in the human gut, although it is limited in the expression of certain transporters and metabolic enzymes. Baicalein can be transported bidirectionally (from the apex to the basal side and from the basal side to the apex) in a single-layer model of Caco-2 cells, while the levels of the glucuronides formed are very low (Ng et al., 2004). In addition, baicalin was mainly accumulated on the basal side, suggesting that MRP3 may be involved in baicalin transport (Zhang et al., 2005). Furthermore, research has demonstrated that the primary transport medium for the body's absorption and utilization of flavonoids (including baicalin, catechin, quercetin, etc.) is human serum albumin (HSA). The degree to which baicalin binds to human serum albumin determines its transit rate and volume distribution inside the host. Specifically, the relative absorption rate of baicalin is approximately 65%, as indicated by the area under the time-concentration curve (AUC_{∞}) (Wen et al., 2023). This difference may be related to differences in intestinal flora composition, metabolic enzyme activity, and transporter expression between humans and rats, which together affect drug absorption and metabolism processes. It has also been reported that enzymes in the digestive tract (e.g., β-glucosidase or lactase chlorin hydrolase (LPH) are involved in the hydrolysis of flavonoids, which suggests to us that this enzyme may be involved in the hydrolysis of baicalin (Murota et al., 2018). A study that included ten healthy male volunteers also discovered that baicalin was recovered from urine at doses of 5.0, 10.0, and 20.0 mg/mL, with baicalin recoveries from urine of 105.9%, 101.1%, and 108.4%, respectively, after oral administration of the drug. Correspondingly. Baicalin was recovered in 105.9%, 101.1%, and 108.4% of the urine samples, suggesting that urine is another route by which baicalin can be eliminated (Lai et al., 2003).

3 Protective effect of baicalin on AKI

AKI has multiple causes, which fall into three primary categories: pre-renal, renal, and post-renal, according to the anatomical site where the cause occurs. In terms of causes, the causes include drugs (Privratsky et al., 2018), sepsis (Huang J. et al., 2019), obstruction, and Renal hypoperfusion, etc. Regardless of the cause of AKI, the specific pathomechanisms include inflammatory response, oxidative stress, immune dysregulation, apoptosis, and mitochondrial dysfunction, among others (Kumar, 2018; Sureshbabu et al., 2015; Kimura T. et al., 2017; Thomas et al., 2022). The main manifestation is ischemic damage to the kidney's proximal tubules, with detachment of tubular epithelial cells and brush border and eventual loss of proximal tubular function (Makris and Spanou, 2016). The absence of a "gold standard" for calculating AKI and the use of various benchmarks to define the condition contribute to the varying incidence of AKI (Chang-Panesso, 2021). An abrupt (within hours to days) drop in glomerular filtration rate that causes the retention of nitrogenous waste products (such as BUN and SCr) in the blood plasma is referred to AKI, which replaces early acute renal failure. It occurs in 5.0%–7.5% of hospitalised patients and 50%–60% of critically ill patients (Rodrigues et al., 2013;

TABLE 1 The studies of baicalin intervention AKI *in vivo* and *in vitro*.

Type	Animal/ Cell	Experimental model	Dosages	Pharmacological effect	Mechanism (marker)	References
AKI Induced by nephrotoxic drugs (drug-induced)	HK-2 cells	H_2O_2-induced AKI	Baicalin (100 μmol/L)	Anti-apoptosis, antioxidation	BiP↓, CHOP↓, Nrf2↑, ROS↓, GSH/GSSG↓, Caspase-3↓	Zhang et al. (2020b)
	Mice	Aristolochic acid I-induced AKI	Baicalin (80 and 160 mg/kg)	Reduced BUN and SCr, improved the extensive tubular necrosis and tubular dilatation	BUN↓, SCr↓, CYP1A1↑, CYP1A2↑	Fu et al. (2021)
	Mice	Lead-induced AKI	Baicalin (12.5, 25, 50 mg/kg)	Anti-inflammatory, anti-apoptosis	SOD↑, GSH-Px↑, MDA↓, Bax↓, Bcl-2↑	Kelley et al. (2019)
	HK-2 cells	Contrast-induced AKI	Baicalin (10 mg/mL)	Antioxidant, anti-inflammatory	NLRP3↓, IL-1β↓, IL-18↓, ASC↓, Caspase-1↓, GSDMD↓, LDH↓, SOD↑, MDA↓, ROS↓	Rodrigues et al. (2013)
AKI Induced by Sepsis (infection induction)	ICR mice	LPS-induced AKI	Baicalin (74 mg/kg/d)	Reduced histopathological changes, anti-inflammatory, antioxidant	SCr↓, TNF-α↓, IL-1β↓, IL-6↓, IL-10↓, NO↓, MDA↓, ALT↓, prolylate synthase CS↑, ketoacid kinase PK↑	Gameiro et al. (2020)
	HK-2 cells	LPS-induced AKI	Baicalin (5, 15, 25, 50, 75 μmol/L)	Inhibited inflammation response	TNF-α↓, IL-6↓, IL-1β↓, iNOS↓, COX-2↓, NF-κB65↓, TXNIP↓, NLRP3↓	Lim et al. (2012)
	C57BL/ 6 mice	cLP-induced AKI	Baicalin (200 mg/kg/d)	Reduced BUN and SCr, anti-apoptosis	BUN↓, SCr↓, Bax↓, Bcl2↑	Li et al. (2020)
	ICR mice	cLP-induced AKI	Baicalin (50 and 100 mg/kg)	Anti-apoptosis, decreased the pathological injury of renal tissue	BUN↓, SCr↓, c-FLIP↑, pNGAL↓,pKIM-1↓	Ganguly et al. (2022)
AKI Induced by Ischemia	Wistar rats	Renal ischemia-reperfusion injury	Baicalin (10 and 100 mg/kg)	Reduced BUN and SCr, improved renal histopathology scores, Anti-apoptosis, antioxidant, anti-inflammatory	BUN↓, SCr↓, MDA↓,SOD↑, TLR2/4↓, MyD88↓, p-NF-κB↓, p-IκB↓, IκB↑, caspase-9↓, caspase-3↓, Bax,↓, Bcl-2↑	Ramesh and Reeves (2002)
	Sprague-Dawley rats	75-min cardiopulmonary bypass (CPB) and 45-min cardiac arrest (CA) to establish an AKI model in rats	Baicalin (100 and 200 mg/kg)	Reduced BUN and SCr, antioxidant, anti-inflammatory	BUN↓, SCr↓, Kim1↓, NGAL↓, MDA↓, MPO↓, SOD↑, GSH↑ IL-18↓, iNOS↓, Nrf2↑, HO-1↑	Feng et al. (2022a)
AKI Induced by other way	Sprague Dawley rats	Preeclampsia (PE) rat disease model	Baicalin (50, 100, 150 mg/kg/d)	Attenuated acute injury symptoms, anti-apoptosis	Bcl-2↑, XIAP↑, Caspase-3↓, Caspase-6↓, Caspase-9↓, AT1↓	Lin et al. (2014b)
	Sprague-Dawley rats	Sodium taurocholate –introduce SAP	Baicalin (100 mg/kg/h)	Reduced BUN and SCr, improved renal, histopathological scoring, anti-inflammatory	BUN↓, SCr↓, NO↓, TNF-α↓, IL-6↓, ET-1↓, Bax↑,Bcl-2↓, PLA_2↓	Zhao et al. (2021)
	Sprague-Dawley rats	Sodium taurocholate –introduce SAP	Baicalin (100 mg/kg/h)	Improved renal histopathological scoring	AMY↓, NO↓, MDA↓, TNF-α↓	Jang et al. (2017)
	Sprague-Dawley rats	Heatstroke induced AKI model	Baicalin (10, 20, 40 mg/kg)	Reduced BUN and SCr, anti-inflammatory	BUN↓, SCr↓, IL-1β↓, TNF-β↓	Kønig et al. (2019)
	Fischer 344 aged rats	LPS-induced AKI	Baicalin (10 mg/kg/d)	Anti-inflammatory	VCAM-1↓, IL-1β↓, IL-6↓, PPARγ↑, NF-κB↓	Hoste et al. (2015)

Gameiro et al., 2020; Hoste et al., 2015). Throughout the past few years, a number of experimental models of AKI have been used to examine the nephroprotective benefits of baicalin (Le et al., 2021; Shi et al., 2019).

As shown in Table 1, the studies *in vivo* and *in vitro* of the herbal medicine baicalin for the treatment of AKI are listed. The studies include AKI caused by nephrotoxic drugs and toxins, ischemia/ reperfusion contrast agents, sepsis, etc. Mechanistically, Baicalin may lessen oxidative stress, damage to mitochondria and other organelles, inflammation, and apoptosis, among other cytoprotective mechanisms, to lessen AKI (Figure 1). A more thorough explanation of these mechanisms can be found below.

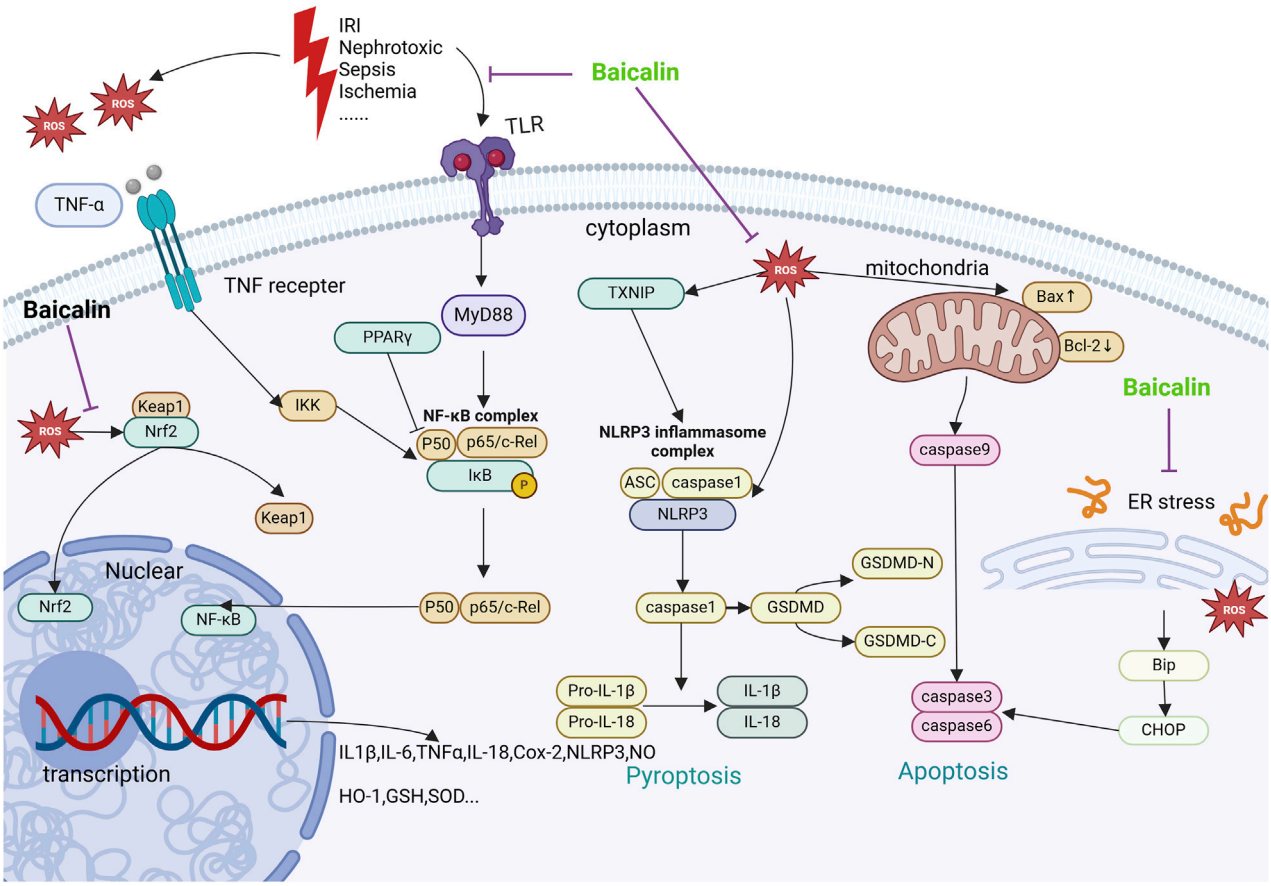


FIGURE 1
Mechanisms by which baicalin intervenes in AKI. This figure summarizes the molecular pathways by which baicalin treats AKI. Receptors such as TNFR, TLR are subjected to nephrotoxic stimuli such as lipopolysaccharide I/R and inflammatory factors. Furthermore, mitochondrial quality control mechanisms and membrane potential are directly impacted by hypoxia and ischemia-reperfusion, which results in the generation of ROS. The aforementioned receptors' activation along with the creation of intracellular ROS might set off subsequent cascades that worsen inflammation, apoptosis, and renal damage. Baicalin targets Nrf2 and activates the expression of anti-aging chemokines to enhance cellular tolerance to oxidative stress. By blocking the NF-κB pathway via TLR2/4 and TNFR, baicalin inhibits the inflammatory response and prevents the synthesis of pro-inflammatory proteins. Baicalin also decreases apoptosis by preventing endoplasmic reticulum stress, which is known to alleviate AKI through ROS/NLRP3/Caspase-1/GSDMD Pathway-Mediated proptosis.

3.1 Baicalin prevents AKI by reducing the inflammatory response

The physiological process of inflammation serves to shield the body against acutely harmful stimuli like ischemia, poisons, or viruses, and plays a defensive role in injury or infection (Saade et al., 2021). Inflammation is closely related to AKI, an inflammatory state can cause AKI, which in turn can exacerbate the inflammatory response. Indeed, a number of ischemia, toxic, or immune disorders can harm renal cells by causing inflammation and cell death, which subsequently damages other organs and ultimately results in total failure (Feng Q. et al., 2022). Pro-inflammatory cytokines, chemokines, adhesion molecules, different growth factors, and nuclear factors are some of the molecular characteristics of inflammation that are linked to the inflammatory response. Minimizing the detrimental effects of cytokines on the kidneys is crucial since the decline in renal function during AKI typically causes cytokine concentrations to rise in tandem with a decrease in plasma cytokine clearance (Andres-Hernando et al., 2012). AKI trauma patients had much greater levels of IL-1, IL-8, IL-6 and MCP-1 early in life compared to trauma patients without AKI (Ma et al., 2021). Inflammatory mediators such as inflammatory factors (TNF-α, TGF-β, IL-6, IL-1β, IL-18), chemokines (CCL2), and adhesion factors (ICAM-1,MCP-1 and P-selectin) are induced when kidney injury occurs, which facilitates the recruitment of leukocytes to the kidney and the arrival of macrophages, neutrophils, and lymphocytes at the site of injury (McWilliam et al., 2021). According to recent research, TNF-α, IL-6, and IL-1β have been found to be significant participants in the pathogenesis of renal injury (Ramesh and Reeves, 2002). And inhibition of these inflammatory cytokines attenuates renal tissue damage. Therefore, early anti-inflammatory treatment can improve renal function. By inhibiting the manufacture of inflammatory factors and preventing the inflammatory pathway, baicalin can provide protection against AKI. As evidenced by a notable decrease in IL-10 levels and a considerable increase in TNF-α, IL-8, and IL-6 levels in renal tissue, cisplatin was found to induce a strong pro-inflammatory response in rat renal tissue in one investigation (Kumar et al., 2017). And in

several studies, Baicalin demonstrated its ability to suppress ROS release, which raised oxidative stress in cells, released more malondialdehyde (MDA) and superoxide dismutase (SOD), and reduced TNF-α, IL-6, IL-8, and IL-1β levels (Li et al., 2022; Liao et al., 2016; Lim et al., 2012). Academic circles largely recognize the nuclear transcription factor κB (NF-κB) pathway as a significant pro-inflammatory signaling pathway, mostly because of NF-κB's critical function in controlling the expression of pro-inflammatory genes such adhesion molecules, cytokines, and chemokines (Lawrence, 2009). By coordinating inflammatory processes that aid in the onset and progression of AKI, NF-κB plays a essential role in the pathophysiology of the condition (Liu and Dong, 2021). NF-κB binds to IκB protein in an inactivated state under normal conditions. When cells are stimulated internally and externally, activation of proximal signal bridging proteins leads to activation of IκB kinase and degradation of IκB proteins by phosphorylation ubiquitination, and thus NF-κB is released, translocated to the nucleus and binds to particular target genes, initiating transcription of the target genes and further inducing an acute inflammatory response. The activation of classical NF-κB causes the active immune system to create pro-inflammatory cytokines, which in turn trigger an inflammatory response (Oeckinghaus et al., 2011). Conversely, these pro-inflammatory cytokines activate classical NF-κB (Yu et al., 2020). This leads to a vicious circle. Studies have shown that baicalin significantly reduced the number of $CD68^+$ macrophages and $CD3^+$ lymphocytes, in addition to affecting NF-κB signaling by downregulating IκB expression and upregulating TNF-α, NLRP3 and P65 expression (Zhang X. T. et al., 2020). Baicalin inhibits the TLR4/MyD88/NF-κB signaling cascade in an ischemia-reperfusion injury (IRI) model, hence reducing the synthesis of pro-inflammatory proteins brought on by renal ischemia-reperfusion injury (IRI). Furthermore, it suppresses apoptosis by reducing cleaved caspase-9 levels (Lin et al., 2014a). An essential component of the innate immune system, the NLRP3 inflammasome promotes caspase-1 activation and the release of pro-inflammatory cytokines, such as IL-1β and IL-18, in response to microbial infections and cellular injury (Kelley et al., 2019). In cisplatin-induced AKI (CI-AKI) models that are both *in vivo* and *in vitro*, renal damage and apoptosis have been shown to be aggravated by the activation of the NLRP3 inflammasome and the ensuing generation of IL-1β and IL-18 (Shen et al., 2016; Lin et al., 2019). In contrast, following baicalin therapy, the pro-inflammatory factors IL-1β and IL-18 as well as the amount of active caspase-1 were reduced due to the inhibition of NLRP3 inflammasome activation (Li et al., 2022; Sun et al., 2020). In conclusion, baicalin has a potent anti-inflammatory action that may prevent AKI by lowering immune cell activation and infiltration as well as the synthesis and release of inflammatory mediators.

3.2 Baicalin prevents AKI by reducing oxidative stress

An imbalance in the body between oxidation and antioxidants is identified as oxidative stress. The overabundance of ROS is acknowledged as a critical element contributing to the development of oxidative stress. Differential intracellular oxidative stress levels cause intracellular oxidative components to be misregulated, which in turn causes the production of oxidative markers like SOD and MDA, which promotes the process of apoptosis (Hajam et al., 2022). In renal cells, oxidative stress causes a number of harmful outcomes, including DNA damage, lipid peroxidation, protein modification, pro-inflammatory and pro-fibrotic pathway activation, and apoptosis induction (Emma et al., 2016). A range of antioxidant defense mechanisms exist within cells for scavenging free radicals and maintaining oxidation-reduction balance. These include antioxidant enzymes (e.g., SOD, GSH) and non-enzymatic antioxidant substances (e.g., glutathione, vitamin C, vitamin E, etc.). Research has indicated a significant correlation between oxidative stress and AKI. The aetiology of increased oxidative stress and consequent ischemic renal cell injury is linked to the direct nephrotoxic effects of contrast agents and cisplatin on renal tubular epithelial cells as well as the physiological alterations brought on by the release of vasoactive molecules (Chandiramani et al., 2020; Holditch et al., 2019). ROS disrupt a variety of signaling pathways, such as PI3K, MAPK, Nrf2, iron metabolism, and cell death, if they are not properly neutralized and strong antioxidant baicalin controls oxidative stress in cells through both direct and indirect means, including scavenging ROS and raising the activity of antioxidant enzymes, which prevents AKI (Li et al., 2022; Lin et al., 2014b). Large-scale production of ROS in the AKI model causes direct oxidative damage to the lipids and proteins in the mitochondria, which reduces the permeability of the mitochondrial membrane by interfering with electron transfer chain (ETC) function and raising mitochondrial bioenergetics (Zhao et al., 2021). Significantly reduced lipid peroxidation MDA increased superoxide dismutase SOD levels after baicalin treatment (Lin et al., 2014a; Zhang et al., 2017). In contrast-induced AKI, it also showed strong antioxidant effects, including lowering ROS, MDA, and increasing SOD levels (Li et al., 2022). Research has indicated that exposure to lead increases oxidative stress (Lin et al., 2019; Sun et al., 2020) and that SOD, GSH-Px, and MDA are targets of lead (Hsu and Guo, 2002). Exposure to lead affects the levels and activity of antioxidant enzymes, thereby damaging cells and tissues. In addition, mice develop a dose-dependent accumulation of lead, primarily in the kidneys (Yu et al., 2022). In contrast, following baicalin therapy, the baicalin group's SOD and GSH-Px activity rose, and baicalin dose-dependently reduced the elevated MDA levels. Nrf2, a transcription factor, is typically located in the cytoplasm where it binds to the actin-associated Keap1 protein and undergoes normal degradation. This relationship is broken under oxidative stress circumstances, which leads to the translocation of Nrf2 into the nucleus and the subsequent upregulation of the synthesis of cytoprotective enzymes, including HO-1 and SOD (Li et al., 2020). It is well established that the Nrf2-associated signaling pathway provides protection against acute kidney injury brought on by a variety of circumstances (Wang Z. et al., 2021). In rats with AKI from cardiac surgery, The Nrf2 defense mechanism was significantly activated by baicalin pretreatment, as evidenced by increased kidney levels of Nrf2 and downstream aging inhibitor enzymes (e.g., HO-1) (Shi et al., 2019). This implies that baicalin activates Nrf2 oxidative stress, which impairs the

defense mechanism against acute kidney injury caused by cardiac surgery. Thus, baicalin has therapeutic potential to antagonize oxidative stress in AKI of various etiologies.

3.3 Baicalin prevents AKI by reducing apoptosis

Given that tubular damage can result in a sharp reduction in renal function, it has been observed that tubular epithelial cells injury in sepsis-associated AKI(SA-AKI) (Zhang et al., 2024). Notably, when renal tubular injury is severe, this damage will be irreversible. Although preventive mechanisms of oxidative stress and apoptosis in renal tubular epithelial cells are essential for the treatment of sepsis-associated AKI, little progress has been made in pharmacologic therapy. Renal tubular epithelial cells undergo necrosis and apoptosis *in vivo* and *in vitro* due to a variety of conditions, and this result was shown by cell morphology, apoptotic activation of cysteine asparaginase, and TUNEL assay for DNA damage (Thomas et al., 2022; Ren et al., 2020; Wang et al., 2020). And in several studies, baicalin is able to attenuate apoptosis in renal tubular epithelial cells through several pathways, such as mitochondria-dependent pathway, cell death receptor pathway (Wang Y. et al., 2018; Zhang et al., 2007). The caspase cascade triggers apoptosis in response to different injuries. Cacpase3 serves as the downstream effector in this cascade and initiates apoptosis directly upon activation by various upstream signals. Therefore, caspase3 is considered a key indicator (Yang et al., 2014; Yang et al., 2006). Bax and Bcl-2 are pro-apoptotic and anti-apoptotic proteins, respectively, in the mitochondrial apoptotic pathway. Because of their abnormal expression, the outer mitochondrial membrane becomes more permeable, allowing cytochrome c to pass through the membrane and into the cytoplasm (Spitz and Gavathiotis, 2022). In sepsis model mice, baicalin inhibits apoptosis by regulating Bax and Bcl-2 (Zhu et al., 2016). Cell FLICE like inhibitory protein (c-FLIP) is an apoptosis inhibitory protein that reduces lipopolysaccharide-mediated apoptosis in endothelial cells (Shen et al., 2017; Jang et al., 2017). Upon binding to FADD, the activation and recruitment of caspase-8 are inhibited by c-FLIP, thereby suppressing the cascade activation of downstream caspases and ultimately preventing apoptosis. A study demonstrated that via downregulating the expression of the negative regulator c-FLIP, c-Myc accelerates FasL/Fas-mediated apoptosis in renal tubular epithelial cells (Xu D. et al., 2019). In contrast, baicalin inhibited the apoptosis of renal tubular epithelial cells in SA-AKI mice, thereby considerably protecting the kidneys, and this protective effect may have been caused by enhanced c-FLIP protein expression (Le et al., 2021). Increased organ damage results from IRI, which is the stopping of blood flow to a specific organ and then resuming blood flow and oxygenation. Owing to its unique tissue structure and function, as well as its increased dependence on oxygen delivery, the kidney is especially susceptible to ischemia-reperfusion injury (Van Avondt et al., 2019). In a renal IRI model, baicalin lowered caspase-3 activity and decreased the Bax/Bcl-2 ratio, showing a favorable anti-apoptotic effect, thereby protecting the kidney and attenuating renal pathological changes (Lin et al., 2014a). However, the regulation of apoptotic signaling by baicalin is significantly more complex. For example, in a cellular model of arsenic-induced nephrotoxicity, baicalin elevated the Bcl-2/Bax ratio, thereby protecting the kidney and attenuating renal pathologic changes. The coexistence of necrosis and apoptosis in the pancreas, with necrosis being predominant, may explain why inducing pancreatic apoptosis results in a protective effect.

In AKI, cell death pathways cause necroinflammation (von Mässenhausen et al., 2018). Notably, unlike necrosis, apoptosis does not cause inflammation (Kønig et al., 2019). The term "pyroptosis" was first coined by Cookson and Brennan (2001) to refer to a cysteine-aspartate-specific protease 1 (caspase-1)-dependent cell death pattern. During pyroptosis, NLRP3 binds to the cysteine protease cysteine aspartate proteasome-1 to form inflammasome. Inflammasome can process pre-cysteine aspartyl protease-1 into mature cysteine aspartyl protease-1, leading to inflammatory death of a large number of somatic cells. Thus, "pyroptosis" is inflammatory death (Huang et al., 2021). Previous studies have identified IL-8 and IL-1β as significant markers of pyroptosis. Additionally, the cleavage and activation of the pore-forming effector protein gasdermin D (GSDMD) by activated cysteine asparaginase is a crucial step that triggers pyroptosis, leading to the compromised cells releasing IL-1β and IL-18 (Fu and Wu, 2023; Zhang X. et al., 2020). Apoptosis may result from pro-inflammatory cytokines activating immune cells, creating a detrimental cycle. Research has demonstrated that pyroptosis can be induced by contrast agents via the ROS/NLRP3/Caspase-1/GSDMD pathway, emphasizing baicalin's capacity to block this mechanism. Intervention with baicalin attenuated the associated inflammation and oxidative levels (Li et al., 2022). Overall, Baicalin exerts an inhibitory influence on renal cell apoptosis by suppressing the activation of apoptotic signaling pathways. This protection shields renal cells from apoptosis triggered by diverse factors.

3.4 Baicalin prevents AKI by improving damage markers

In AKI, renal pathological changes include tubular injury, interstitial oedema, inflammatory cell infiltration, and more (Huang et al., 2020). Interestingly, baicalin attenuated renal pathological changes and improved renal histopathological scores in several studies, including models of sepsis-induced AKI (Le et al., 2021), or acute pancreatitis-induced AKI (Zhang et al., 2007; Zhang X. P. et al., 2008). For the most effective treatment to be given and additional kidney damage to be prevented, early identification of AKI is essential. In clinical practice, SCr, BUN and urine output are usually considered as the main indicators of renal function. SCr is a product of muscle metabolism, produced by muscle creatine metabolism, and SCr levels are readily available; however, Considering that creatinine concentrations are impacted by muscle mass, proteolytic metabolic rate, age, sex, and race, this is not a suitable marker. In addition, after AKI, SCr is a sluggish surrogate for decreased glomerular filtration rate, and it can take up to 72 h to return to a stable state. BUN is a waste product of protein metabolism, mainly converted from the conversion of amino acids to urea by the liver, both of which are excreted through the kidneys. When the glomerular filtration rate (GFR) decreases, the excretion of these two substances decreases, and the SCr and BUN levels in the blood increase accordingly (Macedo et al., 2011). Urine output and

SCr are often the criteria for detecting AKI, but changes in his two are often inconsistent. Nonetheless, they are used as keys to diagnosing AKI (Kellum et al., 2013). A clinical study showed that when urea is reduced in patients with AKI, it reduces mortality (Chávez-Íñiguez et al., 2023). In studies on baicalin, it has been able to restore SCr and BUN to normal levels in multiple causes of AKI models (Wang et al., 2015; Chang et al., 2007). Notably, Clinical trials have demonstrated the considerable impact of baicalin. However, based on the pathogenesis and etiology of AKI, the original renal markers are limited. For example, volume expansion reduces SCr and delays recognition, whereas volume contraction induces an increase in serum concentrations, despite the absence of renal damage (Liu et al., 2011). Acute diseases (e.g., sepsis) and disease complications (e.g., severe malnutrition and sarcopenia) also reduce creatinine production. Neutrophil gelatinase-associated lipid carrier protein (NGAL), which is abundantly expressed and substantially upregulated in the kidney, is thought to be a novel biomarker with a high sensitivity for the detection of AKI (Shang and Wang, 2017). Although multiple renal injury markers can serve as important indicators for the detection of AKI, however, the markers due to different causes of AKI as well as different periods of AKI remain different. In critically ill patients and those with multiple comorbidities, identifying the underlying cause of AKI remains challenging. As an AKI biomarker, urinary NGAL, can help facilitate the differential diagnosis of chronic disease and intrinsic, pre-renal or post-renal etiologies at an early stage. As damage to the renal tubules is gradually repaired, the expression level of NGAL may decline (Romejko et al., 2023). Therefore, a drop in NGAL may indicate both a recovery of renal function and an improvement in renal tubular function. Research has demonstrated that baicalin can reduce NGAL level (Le et al., 2021). It is more sensitive than SCr, and When kidney cells are under stress or are damaged, this gene is expressed quickly. It is essential to identify AKI as soon as possible. Normal kidneys and other organs typically express the KIM-1 protein at low levels. However, its expression is notably upregulated in IRI rat kidneys and in rodent models of drug-induced AKI (Schrezenmeier et al., 2017). According to some authors, patients with AKI may experience histologic alterations prior to elevated Kim-1 levels (Zhang P. L. et al., 2008), implying that Kim1 could be one of the indicators for preferential detection of renal injury. In two studies on baicalin in the treatment of AKI, the authors detected changes in Kim1 reduction (Le et al., 2021; Shi et al., 2019). Although the above markers can be used to assess a certain degree of acute kidney injury, they all have limitations in diagnostic sensitivity and specificity, are susceptible to interference by multiple factors, and are still limited in early diagnosis and accurate assessment of the degree of injury. Hence, the pursuit of more precise and sensitive biomarkers remains a crucial objective in the investigation of AKI.

In conclusion, the anti-inflammatory, anti-oxidative stress and anti-apoptotic effects of baicalin in the treatment of AKI may be related to each other and jointly promote kidney protection. There is a close interaction between these three mechanisms, forming a complex network. For example, inflammatory responses can promote oxidative stress, and *vice versa*. After the release of inflammatory factors, ROS generation will be induced and the oxidative damage of cells will be aggravated. In addition, oxidative stress can also further activate inflammatory pathways, leading to apoptosis. Therefore, Baicalin can simultaneously reduce the occurrence of apoptosis by inhibiting inflammation and oxidative stress. Baicalin reduced ROS production by inhibiting inflammatory mediators, suggesting a positive feedback relationship between inflammatory response and oxidative stress (Li et al., 2022; Chen et al., 2024). Since oxidative stress can induce cell apoptosis, Baicalin reduces the risk of cell apoptosis by enhancing the activity of antioxidant enzymes, forming a virtuous cycle of antioxidant and anti-apoptosis (Li et al., 2022). The release of inflammatory mediators not only causes oxidative stress, but also directly promotes cell apoptosis. By inhibiting the expression of pro-inflammatory factors, Baicalin can effectively reduce the activation of apoptosis signal, thereby protecting the survival of kidney cells (Li et al., 2022; Zhu et al., 2016).

4 Protective effect of baicalin on DN and its renal fibrosis

Renal fibrosis can arise from CKD. The most typical route for the development of progressive renal disease is renal fibrosis (Martínez-Klimova et al., 2019; Nastase et al., 2018). The final stage of the development of many chronic kidney disorders is renal fibrosis, ultimately resulting in end-stage renal failure (Djudjaj and Boor, 2019). DN, a kidney disease brought on by consistently high blood glucose levels and a common side effect of diabetes mellitus, is a major contributor to the progress of end-stage renal disease (ESRD) (Association et al., 2003). In addition to kidney damage, people with diabetes often suffer from multiple kinds of complications such as neuropathy, retinopathyand cardiovascular disease. They are all at high risk of death (Rao Kondapally Seshasai et al., 2011). DN occurs in about 40% of diabetic patients and is characterized by proteinuria or reduced GFR (Viberti et al., 1982; Eknoyan et al., 2003; Levey et al., 2020). The exact mechanism of DN is unclear, and it has been show that uncontrolled hyperglycemia promotes oxidative stress, inflammation, reactivity, and fibrosis. The pathological definition of DN is the accumulation of extracellular matrix (ECM) in the glomerular mesangium, which thickens the basement membrane and eventually causes glomerulosclerosis (Samsu, 2021). At present, it is known that a variety of pathways play an important role in the occurrence and development of DN, such as TGF-β (Sharma and McGowan, 2000), Notch (Lin et al., 2010), Wnt (Rooney et al., 2011) pathway, and FGFR1, SIRT3, and dipeptidyl peptidase-4 (DPP-4) mediated signaling mechanisms also play an important role in the occurrence and development of DN (Liu Y. et al., 2022). Research findings have demonstrated that baicalin has the capacity to enhance renal function in DN patients and to mitigate the advancement of this condition by engaging multiple pathways, including anti-inflammatory and antioxidant mechanisms (Yang et al., 2019). In the latter case, the kidneys undergo an inadequate healing process; while they can recover from modest injuries, chronic or severe injury causes scarring (fibrosis) and renal function gradually declines (Hewitson et al., 2017). In turn, one of the key processes facilitating the shift from AKI to CKD is renal fibrosis (Romagnani et al., 2017). The main pathologic features of AKI and CKD are inflammation and fibrosis of the kidneys (Wu et al., 2022). Fibrosis is a warning that

TABLE 2 The studies of baicalin intervention renal fibrosis *in vivo*.

Type	Animal/ Cell	Experimental model	Dosages	Reno-protective effect	Mechanism	References
In vivo	Wistar rats	STZ-induced DN	Baicalin (75 mg/kg) and chrysin (10 mg/kg) combined	Antioxidant, anti-inflammatory	BUN↓, SCr↓, ROS↓, RAGE↓, CAT↑, GOx↑, SOD↑, GSH↑, GR↑, iNOS↓, PKC↓, p-IκB↓	Singh et al. (2017)
In vivo	Sprague-Dawley rats	UUO-induced renal fibrosis	Baicalin (20 and 40 mg/kg)	Anti-inflammatory	TGF-β1↓, Notch1↓, Jagged1↓	Tan et al. (2016)
In vivo	Sprague-Dawley rats	UUO-induced renal fibrosis	Baicalin (20 and 100 mg/kg)	Inhibits EMT	N- calmodulin↑, E– calmodulin↑, α-SMA↓, vimentin↓, TGF-β1↓, p-SMAD2↓, p-SMAD3↓	Zheng et al. (2017)
In vivo	Sprague-Dawley rats	STZ -induced DN	Baicalin (160 mg/kg/d)	Reduced SCr and BUN, anti-inflammatory	BUN↓, SCr↓, UPr↓, MDA↓, NF-κB↓, p-NF-κBp65↓, IL-1β↓, IL-6↓, IGF-R↓, mTOR↓, SIRT1↑, caspase-3↓, caspase-9↓, p-p38 MAPK↓	Zheng et al. (2020)
In vivo	Kunming mice	STZ -induced DN	Baicalin (40 mg/kg)	Reduced BUN and SCr and UACR, anti-inflammatory, anti-apoptosis, antioxidant, inhibits EMT	BUN↓, SCr↓, UACR↓, SOD↑, CAT↑, GPX↑, MDA↓, Nrf2↑, P65↓, TNF-α↓, NLRP3↓, CD3↓, CD68↓, IκB↑, Nrf2↑, caspase-9↓, TGF-β1↓, α-SMA↓, SMAD2/3↓, MMP13↑, β-catenin↓, E-Cadherin↑, Dnmt3a↓, Dnmt3b↓, Klotho↑	Zhang et al. (2020b)
In vivo	Sprague-Dawley rats	High-fat and STZ -induced DN	Baicalin (50, 100, 200 mg/kg)	Reduced BUN and SCr, anti-inflammatory	BUN↓, SCr↓, TC↓, TG↓,TNF-α↓, IL-1β↓, IL-6↓, LDH↓, SOD↑, MDA↓, p-PI3K/PI3K↓, p-Akt/Akt↓, p-mTOR/mTOR↓	Ou et al. (2021)
In vivo	db/db and db/m mice	Spontaneous DN model	Baicalin (40 mg/kg)	Antioxidant, anti-inflammatory	GSH-PX↑, SOD↑, CAT↑, MDA↓, TNF-α↓, IL-1β↓, IL-6↓, MCP-1↓, Nrf2↑, HO-1↑, NQO-1↑, U—Alb↓, ACR↓, AER↓, Bax↓, Bcl-2↑, Cleaved caspase-3↓, P38↓, Erk1/2↓, JNK↓	Ma et al. (2021)
In vivo	db/db and db/m mice	Spontaneous DN model	Baicalin (100 mg/kg/d)	Improves lipid metabolism	GLU↓, Alb↓, SIRT1↑, p-AMPKα↑, HNF4A↓	Zhang et al. (2022)
In vivo	C57BL/ 6 male mice	STZ -induced DN	Baicalin (15, 30, 45 mg/kg/d)	Reduced SCr and BUN, anti-fibrogenic, anti-inflammatory	BUN↓, SCr↓, ACR↓,COLIV↓, Fibronectin↓, miR-124↑	Zhang et al. (2020a)
In vivo	C57BL/ 6 male mice	UUO-induced renal fibrosis	Baicalin (10, 20, 40 mg/kg)	Reduced SCr and BUN, anti-inflammatory, anti-fibrogenic	BUN↓, SCr↓, α-SMA↓, COLA1↓, Fibronectin↓, TNF-α↓, IL-1β↓, IL-6↓, U—Alb↓	Wang et al. (2022)
In vivo	db/db and db/m mice	Spontaneous DN model	Baicalin (50 mg/kg/d)	Reduced BUN and SCr and UACR, improves lipid metabolism, anti-inflammatory	BUN↓, SCr↓, UACR↓, TCH↓, TG↓, HDL-C↑, LDL-C↓, ALB↓, α-SMA↓, Fibronectin↓, CPT1α↑, MCAD↑, Hmgcs2↑, PPARα↑	Hu et al. (2024)

AKI is turning into CKD, so interrupting fibrosis is especially important.

As shown in Tables 2, 3, the studies of the baicalin for treating renal fibrosis are listed. From the perspective of mechanism, baicalin can lessen oxidative stress, mitochondrial damage, inflammation and apoptosis, and reverse matrix deposition and other cell protective mechanisms to alleviate renal fibrosis. These mechanisms are described in more detail below (Figure 2).

4.1 Antifibrotic effects of baicalin

Damage to tubular epithelial cells, glomerulosclerosis, inflammatory infiltrates, apoptosis, and interstitial fibrosis are typical pathologic characteristics of DN. The correlation between oxidative stress and DN has been well-documented in research. Research has demonstrated that mitigating oxidative stress can alleviate the manifestations linked to streptozotocin (STZ)-induced DN (Thallas-Bonke et al., 2008). Pathological and histological observations revealed that rats in the DN group had severe inflammatory lesions, glycogen deposition, and renal fibrosis, and treatment with baicalin had a significant ameliorating effect on these pathological changes. TGF-β has gained clinical recognition as a vital factor in the pathogenesis of ESRD (Meng et al., 2016) and has attracted widespread attention. Renal tubular epithelial cells can be differentiated into fibroblasts during the process of DN through various pathways (including autocrine and paracrine), accelerating tubulointerstitial fibrosis. As a major component of the cytoskeleton, α-SMA is important in the diagnosis and differential diagnosis of fibrosis. According to certain research, α-SMA is significantly expressed in the DN rats' renal tissues, which can be used as an indicator of fibrosis detection (Zeng et al., 2019). Baicalin treatment

10.3389/fphar.2025.1511083

TABLE 3 The studies of baicalin intervention renal fibrosis *in vitro*.

Type	Animal/ Cell	Experimental model	Dosages	Reno-protective effect	Mechanism	References
In vitro	NRK-49F cells	TGF-β1-induced renal fibrosis	Baicalin (20, 40, 80 μM)	Anti-apoptosis, inhibits EMT	COLA1↓, COLA2↓, TGF-β1↓, SMAD3↓, SEAP↓,α-SMA↓	Hu et al. (2017)
in vitro	C57BL/ 6 male mice	HG-treated HK-2	Baicalin (100 μM)	Anti-inflammatory, anti-fibrogenic	COLIV↓, Fibronectin↓, p-IκBα↓, p-p65↓, TLR4↓, miR-124↑	Zhang et al. (2020a)
in vitro	HK-2 cells	0 mM glucose, and/or 100 ng/mL TNFα, and/or 100 μg/mL AGEs	Baicalin (5 μM, 10 μM)	Antioxidant, anti-inflammatory, anti-fibrogenic	FN↓, COL4A↓, TGFβ↓, ICAM1↓, VCAM1↓, IL1β↓, MCP1↓, pIκB↓, pJAK2↓, pSTAT3↓, cAMP↓, cGMP↓	Nam et al. (2020)
in vitro	HK-2 cells	HG-treated HK-2	Baicalin (50 μM)	Improves lipid metabolism, anti-inflammatory	TNF-α↓, IL-1β↓, IL-6↓, α-SMA↓, Fibronectin↓, CPT1α↑	Hu et al. (2024)

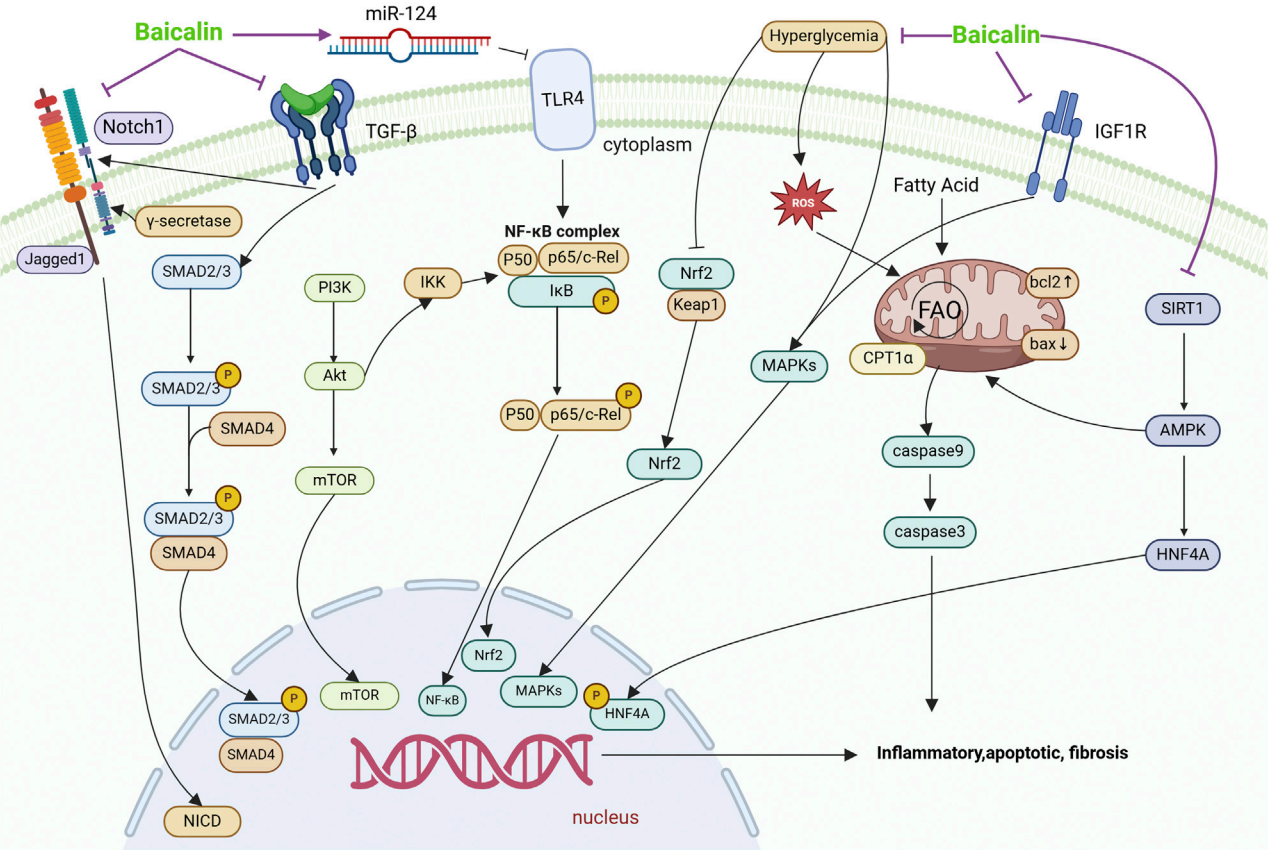


FIGURE 2
Mechanisms by which baicalin intervenes in renal fibrosis. This figure summarises the molecular pathways by which baicalin treats renal fibrosis. EMT, effector cell activation, local ischemia and hypoxia, and their effects on different signaling pathways cause renal fibrosis, leading to renal parenchymal damage, decreased glomerular filtration rate, and progression to chronic and ESRD. Baicalin affects fibrosis through TGF-β, Notch, IGF1R and TLR4 signalling pathways, which are involved in processes such as inhibition of inflammation, apoptosis, lipid metabolism and EMT.

significantly improved both renal fibrosis and renal function in this animal model by reducing TGF-β1 and α-SMA protein expression (Hu et al., 2017). The progression of DN is facilitated by TGF-β1 through its regulation of glomerular and tubulointerstitial fibrosis, a process that relies on Smad2 and Smad3 phosphorylation and activation. There is growing evidence that in the context of kidney injury, epithelial-mesenchymal transformation (EMT) occurs, in which renal tubular epithelial cells lose their phenotypic markers (including n-cadherin and e-cadherin) and transform into fibroblasts, myofibroblasts, or mesenchymal cells, acquiring mesenchymal phenotypic markers. Includes vimentin and α-SMA (Lan, 2003). In UUO-induced renal fibrosis model, baicalin significantly decreased the expression levels of α-SMA and vimentin, and increased the expression levels of N- and e-cadherin (Zheng

et al., 2017). In STZ-induced DN, baicalin can inhibit EMT by partially regulating the methylation of klotho promoter, thus alleviating renal fibrosis (Zhang X. T. et al., 2020). In addition to canonical signaling pathways (Xianyuan et al., 2019). The ECM constitutes a fundamental element of the stromal and epithelial vascular matrix, encompassing collagen fibers (types I, III, and IV), non-collagenous glycoproteins (e.g., laminin, fibronectin), proteoglycans, elastin, and aminoglycans. TGF-β1 participates in both the synthesis and degradation of the ECM, thereby exerting a significant impact on the development of renal interstitial fibrosis (Yuan et al., 2022). Baicalin inhibits ECM accumulation by targeting the TGF-β1/Smad3 pathway (Zheng et al., 2017) and Notch signaling pathway (Tan et al., 2016) in rats with renal fibrosis. It also suppresses the p38 MAPK inflammatory signaling pathway and its downstream mediator NF-κB, thereby retarding the advancement of renal fibrosis (Zheng et al., 2020). Interestingly, though, one study found that via stimulating the TGF-β/Smad signaling pathway, high dosages of baicalin could instead cause kidney damage and fibrosis (Cai et al., 2017). The reason for this may be that it leads to accumulation of the drug, where the drug acts at other, non-specific targets, triggering unintended side effects or toxic reactions. Therefore, attention needs to be paid not only to individual differentiation but also to dosage issues during treatment.

4.2 Anti-apoptotic effects of baicalin

Research has demonstrated that DN patients who have prolonged hyperglycemia experience apoptosis and a deterioration in renal function. Additionally, renal tubular epithelial cells may undergo apoptosis in a hyperglycemic milieu (Ding et al., 2021). Tubular epithelial cells apoptosis is one of the main indicators of interstitial fibrosis and tubular atrophy (Johnson and DiPietro, 2013). Renal tubular epithelial cells from DN patients have been shown to exhibit apoptosis; however, fibrosis is delayed when renal tubular cell apoptosis is blocked (Huang F. et al., 2019). The processes of cell development, differentiation, death, environmental stress response, and inflammatory response are all impacted by activated p38 MAPK signaling (Coulthard et al., 2009). Research has demonstrated that renal inflammation and apoptosis are regularly regulated and promoted by p38 MAPK (Ren et al., 2020). In one study, Following treatment with STZ, there was a rise in caspase-3 and caspase-9 levels as well as hyperphosphorylation of p38 (Zheng et al., 2020). On the other hand, baicalin therapy decreased the production of apoptotic proteins in DN because these proteins strongly prevent apoptosis from starting in nephrotic tissues. This was accompanied by increased levels of insulin production with baicalin treatment, suggesting that baicalin may modulate the activation of the IGF-1/IGF-1R/p38 signalling pathway ameliorating STZ-induced renal fibrosis in DN rats (Zheng et al., 2020). Additionally, it has been discovered that JNK and p38, two members of the MAPK family, are upstream promoters of the mitochondrial apoptotic pathway. They can disrupt dimer formation and cellular localization to induce apoptosis through the mitochondrial pathway, or they can increase the Bax/Bcl-2 ratio (Chen et al., 2010). According to these research, the development of apoptosis and fibrosis in the kidneys is significantly influenced by p38 signaling.

4.3 Anti-inflammatory and antioxidant effects of baicalin

Numerous studies have demonstrated the critical roles that the development of diabetes is mostly influenced by inflammation and oxidative stress (Wang et al., 2019). Studies show that renal inflammation has a major role in the development and progression of fibrosis (Lv et al., 2018). Baicalin decreases renal fibrosis in DN patients by upregulating miR-124 and inhibiting the downstream TLR4/NF-κB pathway in STZ-induced mouse model and HG-induced HK-2 cell model (Zhang S. et al., 2020). In STZ-induced DN and UUO-induced fibrosis models, baicalin was found to significantly reduce inflammation levels, including TNF-α↓, IL-6, IL-1β↓and iNOS (Ou et al., 2021; Wang et al., 2022; Singh et al., 2017). Furthermore, in a model of spontaneous DN, baicalin attenuates aberrant lipid metabolism in the kidneys of db/db mice and may exert nephroprotective effects through the SIRT1/AMPK/hNF4A pathway (Zhang et al., 2022). Baicalin was found to decrease MAPK signaling in the same model, which enhanced Nrf2 activity and HO-1 production, lowering oxidative stress and slowing the progression of the disease (Ma et al., 2021). Other results together suggest that baicalin may protect against renal fibrosis by reducing inflammation-induced IκB phosphorylation, JAK2 phosphorylation, and subsequent NF-κB and STAT3 activation and oxidative stress in HK-2 (Nam et al., 2020).

4.4 Regulation of metabolic pathway of baicalin

In diabetes and other CKD, renal fibrosis is often associated with metabolic disturbances that may involve abnormalities in glucose, lipid and protein metabolism (Qu and Jiao, 2023). Baicalin may modulate the course of renal fibrosis by affecting these metabolic pathways. Currently, controlling the metabolism of lipids and glucose can help treat DN (Wang et al., 2023). However, these clinically standard therapies only slow the progression of the disease, not stop it. Therefore, another approach to the treatment of DN may include using drugs to activate the body's cytoprotective pathways. Under normal conditions, diabetic kidney injury gradually develops in db/db mice on its own. A prolonged high glucose environment can lead to excessive proliferation and thickening of glomerular mesangial cells (GMC) and glomerular basement membrane (GBM) as well as damage to podocytes (Barutta et al., 2022; Tung et al., 2018; Tervaert et al., 2010). Proximal tubular epithelial cells are very important in renal function, They are in charge of various substances' secretion and reabsorption, and an environment with high glucose levels may cause these cells to dysfunction, which can lead to renal fibrosis (Zhang et al., 2023; Liu L. et al., 2022). In STZ-induced mouse model and HG-induced HK-2 cell model, investigations were conducted into the effects of baicalin on renal fibrosis and its molecular mechanisms. Baicalin therapy boosted insulin production and ameliorated renal fibrosis via activating the IGF-1/IGF-1R/p38 signaling pathway (Zheng et al., 2020). Disorders of lipid metabolism, in addition to those of glucose metabolism, play a vital part in the onset and progression of DN (Xu et al., 2021). The transcription of lipid genes is regulated by the HNF4 family; among these, hNF4α controls several metabolic pathways in the kidney.

Using molecular docking and network pharmacology, baicalin was discovered to bind to HNF4A and SIRT1 with efficiency (Zhang Q. et al., 2020), which indicates that baicalin may play a significant role in glucose and lipid metabolism. Further experiments showed that baicalin reduced triglyceride levels, and more importantly, baicalin was reported to attenuate lipid accumulation and MPC-5 in db/db mice by the SIRT1/AMPK/hNF4A pathway, suggesting that baicalin has a potentially attractive and potent therapeutic role in DN (Zhang et al., 2022). A vital enzyme in lipid metabolism, CPT1α, also known as carnitine palmitoyltransferase 1α, is a part of the fatty acid oxidation process. Long-chain fatty acids need to be converted to their corresponding carnitine derivatives before crossing the outer mitochondrial membrane, and this conversion is facilitated by the enzyme CPT1α (Lin et al., 2024). In the context of Diabetic kidney disease (DKD), according to mRNA sequencing, CPT1α activity is significantly suppressed, which causes a series of cellular disruptions that are essential for the development of renal fibrosis (Lin et al., 2024). According to a recent study, renal tubular cell damage was reversed and mitochondrial respiration was enhanced by up-regulating CPT1α, while down-regulating CPT1α increased renal tubular injury and interstitial fibrosis (Yuan et al., 2021). Intriguingly, Studies suggest that increasing CPT1α activity can prevent renal fibrosis from developing (Miguel et al., 2021). A study shows that baicalin targets CPT1α and enhances its expression to ameliorate impaired lipid peroxidation, thereby attenuating renal fibrosis in DKD (Hu et al., 2024). In addition, apoptosis of proximal tubular cells was also reported to be prevented by promoting CPT1α expression (Sun et al., 2018), therefore the relationship between baicalin and CPT1α, apoptosis deserves further investigation.

Similarly, multiple mechanisms play an important role in the occurrence and development of renal fibrosis. Anti-inflammation, anti-oxidative stress, anti-apoptosis and the regulation of material metabolism are related to each other in the treatment of renal fibrosis. Due to their correlation, baicalin can affect one pathway and then other pathways, such as inhibiting inflammation, inhibiting oxidative stress, and inhibiting apoptosis, which is extremely important in the treatment of renal fibrosis.

5 Constraints in utilizing baicalin clinically and strategies for enhancing its efficacy

Baicalin's pharmacological effects have been verified through a variety of ways. However, baicalin has poor water and fat solubility, while generally speaking, well-absorbed chemicals have strong water and fat solubility. One study showed that baicalin belongs to Class IV of the biotherapeutic classification scheme due to its very low hydrophilicity (solubility in water 0.052 mg/mL) and lipophilicity (Papp = 0.037×10^{-6} cm/s), resulting in very low bioavailability of baicalin due to poor solubility (Wu et al., 2014). This could be due to flavones and glucuronide could compose intramolecular hydrogen bonds that lead to the low solubility, which results in low oral bioavailability (2.2% ± 0.2% in rats) (Wu et al., 2018). Similarly, baicalin has poor lipophilicity, so it is not easily absorbed as a mother. In addition, baicalin also has unique characteristics of gastrointestinal absorption and biotransformation, including hydrolysis of intestinal bacteria, undergoing enterohepatic circulation, and gluconaldehyde acidification (Chen et al., 2014). Its bioavailability is low due to the aforementioned characteristics, and a higher dose is frequently needed to produce a favorable therapeutic effect. At the same time, its toxicity should also be taken into consideration. Its clinical usefulness is occasionally limited by its low bioavailability. As a result, increasing baicalin's bioavailability is crucial. Newly created baicalin preparations with improved absorption and increased bioavailability are the result of advancements in preparation processes. In Figure 3, we have enumerated the innovative tactics and provided an overview of the study effort toward enhancing baicalin's bioavailability. Drugs can be made into nano-emulsions, nano-suspensions, nano-solid lipid nanoparticles and nano-lipid carriers by nanotechnology. When the drug particle size reaches nanoscale, due to the effect of quantum size and surface effect, nanoparticles show new physical and biological properties, thereby improving the biological activity and bioavailability of drugs. It can overcome many defects of traditional medicine. For medications like baicalin that are poorly soluble, liposomes are an effective drug delivery mechanism because they increase the solubility and stability of the molecule. Baicalin-liposomes enhanced the tissue distribution and oral bioavailability of baicalin in comparison to baicalin solutions (Huang T. et al., 2019). The optimal prescription was as follows: phospholipid drug ratio 3.81:1, phospholipid cholesterol ratio 5.70:1, hydration volume 1.02 mL, ultrasonic power 60W, ultrasonic time 10 min, ultrasonic temperature 20°C ± 5°C successfully prepared liposomes. A study on the development of BA-loaded liposomes (BAI-LP) showed a significant increase in drug concentrations of BAI-LP in the liver, kidney, and lungs (Li et al., 2018), indicating that BAI-LP might be a viable oral medication administration method to increase baicalin's bioavailability. In addition to improve utilization, liposome-encapsulated baicalin also improves drug targeting. A study successfully used lipids/cholesterol (L), long circulating invisible liposomes (L-PEG) and folate receptor (FR) -targeted liposomes (L-FA) as drug carriers for baicalin. By modifying folic acid on the surface of the liposomes, it can improve its affinity to specific cells, thereby enhancing its efficacy. The finding demonstrated increased cytotoxicity and intracellular absorption of baicalin liposomes with folate receptor (FR) targeting in comparison to non-targeted liposomes, indicating that baicalin loaded FR-targeted liposomes (L-FA-BAI) could improve the anti-tumor impact (Chen et al., 2016). Baicalin's oral bioavailability can be greatly increased by baicalin-loaded nanoemulsions because of their sustained-release properties (Zhao et al., 2013). However, liposomes may degrade while being used and stored, which could reduce the drug's efficacy. One significant problem is figuring out how to regulate the rate and timing of drug release. To attain the intended drug release characteristics, researchers must adjust the liposomes' structure and content. Nanoemulsion is a transparent, thermodynamically stable mixture of water, oil, co-surfactant, and surfactant. Nanoemulsion is a dispersion with droplets smaller than 100 nm that is stabilized by an interfacial layer (Wu et al., 2009). A novel nanoemulsion improves systemic exposure to baicalin. Study using celiac flow blockage and *in situ* single-

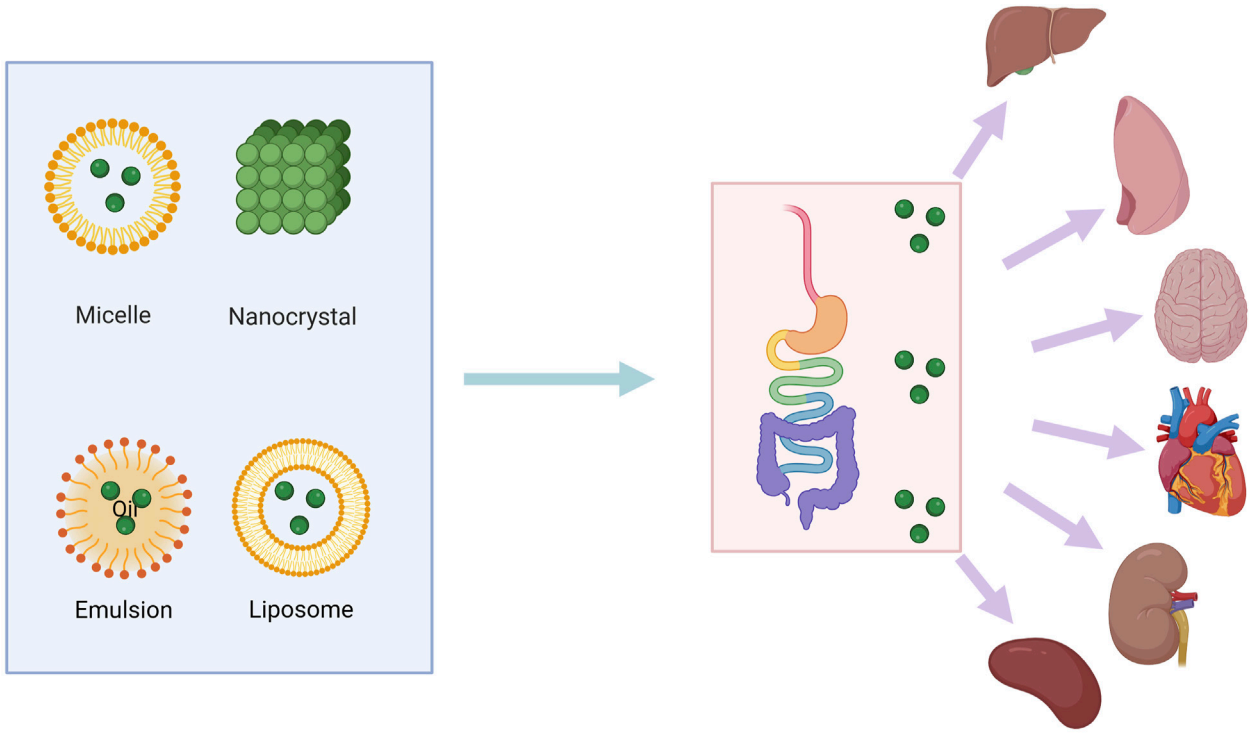


FIGURE 3
Various methods for increasing bioavailability of baicalin. The green dots represent the natural drug baicalin, which enters the body in liposomes or other carriers. It is digested and absorbed through the intestines into various parts of the body, including the liver, kidneys, lungs, and spleen.

pass enteral perfusion indicate that intestine absorption and lymphatic transport play a role in systemic exposure to baicalin (Wu et al., 2018). Baicalin's lymphatic system concentration can be increased using nanoemulsions. A baicalin nanoemulsion demonstrated enhanced bioavailability in the transfer of baicalin to the lymphatic system, according to an *in vivo* investigation (Xu Q. et al., 2019). The mixed micelle system is a micron-scale structure composed of two or more different surfactants self-assembled in the water phase, and its characteristics give it special properties. For example, the outside of the micelle is covered by water-soluble polar groups, while the hydrophobic alkyl group portion is hidden inside. This structure makes the mixed micelles exhibit good stability and biocompatibility in the interaction with the water phase (Carey and Small, 1970). By coating drug molecules, the mixed micelle can improve the bioavailability and therapeutic effect of drugs (Ezhilrani et al., 2021). After oral administration, the BAI-loaded mixed micellar system containing sodium taurocholate and Pluronics P123 copolymer as carrier materials exhibited a sustained release effect. Oral bioavailability was also enhanced, and compared to free baicalin solution, Its absorption through the intestinal tract was noticeably greater (Zhang et al., 2016). Nonetheless, research on the biocompatibility and intracellular absorption of mixed micelles is still crucial for drug delivery applications. Current biodegradable micelles' therapeutic efficacy is impacted by issues such sluggish intracellular drug release and limited tumor cell uptake (Zhong et al., 2013). The self-emulsifying drug delivery system (SMDDS) can make lipophilic drugs spontaneously form a stable microparticle system in water, improve their dissolution and dispersion ability, and promote drugs to pass through intestinal epithelial cell mucosa to improve bioavailability. SMDDS dissolve hydrophobic drugs in the oil phase and spontaneously form emulsions upon contact with the water phase. To enhance baicalin oral absorption, phospholipid complex (PC) and SMDDS can work in concert. Single-pass intestinal perfusion models and Caco-2 cell uptake studies shown that this formulation might greatly increase baicalin transit and relative bioavailability (Wu et al., 2014). It is noteworthy that the properties of compounds can be improved by structural modification (Yao et al., 2017). According to two recent research, the substitution and esterification of baicalin by halogenated hydrocarbons can lead to the synthesis of baicalin-2-ethoxyethyl ester, a new compound that significantly improves the low bioavailability and low solubility of baicalin, and has been discovered to significantly ameliorate AKI and renal fibrosis in mice *in vivo* experiments (Chen et al., 2024; Li et al., 2024). Although structural modification can increase baicalin's bioavailability, there are drawbacks to this as well. A large modification of a structure may prevent its binding to the target molecule, which may affect its biological activity and efficacy (Wen et al., 2023). There are two challenges in this area that need further exploration. First, while a large portion of research has focused on improving nanotechnology-based bioavailability, few studies have provided scientific explanations or explanations for how baicalin's bioavailability is genuinely enhanced by nanoformulations. Secondly, although some have reported significant enhancements or modifications to ADME

characteristics, comparatively little study has been conducted on further uses of nanomaterials.

6 Clinical prospects

Clinical trials are very important for drugs to provide key information on efficacy, safety and dosage to provide a scientific foundation for clinical application of drugs. Clinical practice makes extensive use of baicalin, which is isolated from Scutellaria baicalensis. In one study, Baicalin was administered orally to 374 patients suffering from rheumatoid arthritis and coronary artery disease at a dosage of 500 mg/d. The patients' blood lipid levels and inflammation were found to be reduced by baicalin (Hang et al., 2018). In addition, baicalin reduced the survival of peripheral blood leukocytes (PBLs) in individuals suffering from acute lymphoblastic leukemia (ALL), increased IFNγ production in PBLs and decreased the production of TNFα and IL-10 in bone marrow cells (BMCs) in ALL patients. Importantly, baicalin did not induce PBLs in healthy controls to undergo apoptosis (Orzechowska et al., 2014). In addition, baicalin can be combined with other herbal extracts to form a drug to reduce the severity of postoperative pain after impacted mandibular third molar surgery (Isola et al., 2019). In AKI, a study of SCr and BUN levels in 50 pediatric patients with or without baicalin-assisted therapy found that baicalin-assisted therapy significantly reduced SCr and BUN levels in children with sepsis (Zhu et al., 2016). However, it is significant to note that further research is needed to completely evaluate the safety, effectiveness, and ideal dosage of baicalin in these indications. This research should include longer-term studies as well as larger clinical trials.

Drugs similar to baicalin, such as baicalein, have been shown to be safe in a single oral dose of 100–2,800 mg of baicalein in healthy people (Li et al., 2014). In dose range of 200–800 mg, multiple-dose oral baicalein administration was safe and well tolerated, dose proportionality was inconclusive, and no serious accumulation of baicalein was observed (Pang et al., 2016). Breviscapine is a flavonoid that also has a favorable protective impact on the kidney, can lower blood pressure, and enhance renal function and urine microalbuminuria (Wei and Tan, 2005). In addition, Breviscapine has many clinical indications, mainly used in the treatment of coronary heart disease, angina pectoris, hypertension, hyperviscoemia and cerebral ischemia. Therefore, we speculate that baicalin, which is also a flavonoid, can enter the clinic and has certain safety.

Currently, multi-drug combination therapy is now common in many diseases and can lead to drug interactions (Cheng et al., 2019). The interaction between baicalin and other drugs is also noteworthy. In kidney disease, commonly used drugs are antibiotics, blood pressure drugs such as nifedipine, immunosuppressants such as cyclosporin A and so on. The study found that gut flora plays a key role in drug absorption and utilization (Feng et al., 2019). The effectiveness of baicalin can be impacted by gut bacteria and β-glucosidase structurally converting it to baicalein (Xing et al., 2005b). Therefore, when baicalin is combined with antibiotics, the effect of antibiotics on the pharmacokinetic properties of baicalin by inhibiting intestinal bacteria should be considered. As natural carriers of many endogenous and exogenous drugs, plasma proteins are responsible for determining the pharmacokinetic properties of many drugs. The plasma protein binding rate of baicalin was found to be between 86% and 92%, suggesting that baicalin is a competitive substitute for some drugs and plasma protein (Tang et al., 2006). In a randomised, three-period crossover study, researcher evaluated the pharmacokinetics of nifedipine and baicalin (0.225 or 0.45 g/kg) after intravenous administration in rats. The results showed that the pharmacokinetics of nifedipine could be significantly changed: at low doses, the total concentration maximum (C_{max}) of nifedipine was decreased by 40%, the area under the concentration–time curve (AUC_{∞}) was decreased by 41%, the volume of distribution (Vd) was increased by 85%, and the clearance rate (CL) was increased by 97%. At high doses, Cmax was reduced by 65%, AUC_{∞} by 63%, Vd by 224%, and CL by 242%. At the same time, C_{max} of free nifedipine increased by 25% at low doses and 44% at high doses, suggesting that baicalin may regulate the pharmacokinetics of nifedipine by affecting plasma protein binding and inhibiting cytochrome P450 3A (CYP3A) activity (Cheng et al., 2014). CYP3A subfamily and P-glycoprotein (P-gp) exist in human intestinal epithelial cells at high levels as metabolic enzymes and efflux transporters, respectively. At the same time, the substrate of P-gp overlaps that of CYP3A4 (Morisaki et al., 2013). As a substrate of P-gp and CYP3A4, the C_{max} and $AUC_{0\text{-}540}$ of cyclosporine increased significantly by 408.1% and 685.3%, respectively, after it was combined with baicalin. The pharmacokinetic parameters of rats treated with baicalein (112 μmol/kg) were also increased by 87.5% and 150.2%, respectively. However, there was no difference in the clearance stage of cyclosporin regardless of baicalin or baicalein treatment (Lai et al., 2004). These results indicate that it is necessary to consider the effects of drugs on each other, although both drugs have positive effects on the disease.

7 Conclusion and future perspective

Baicalin, as a potential herbal active ingredient for the tre atment of AKI and renal fibrosis, possesses wide range of pharmacological activities and multiple mechanisms of action. Baicalin exhibits a protective effect on the kidney in AKI by lowering oxidative stress, decreasing the inflammatory response, and preventing the release of inflammatory cytokines. It also reduces necrosis and apoptosis of renal tubular epithelial cells and promotes tubular repair. For DN and its renal fibrosis, baicalin can act through several pathways. It can lessen the synthesis and accumulation of ECM and suppress the fibrotic and inflammatory reactions of renal tubular epithelial cells brought on by hyperglycemia. In addition, baicalin can intervene in diabetes-related signaling pathways, such as TGF-β1, thereby reducing the process of renal fibrosis. In clinical trials, baicalin was able to show better results. However, there are some limitations in the current research on Baicalin, including insufficient pharmacokinetic studies and limited clinical trial data.

Author contributions

XL: Writing–original draft, Writing–review and editing. RX: Methodology, Writing–review and editing. DZ: Software, Writing–review and editing. JC: Formal Analysis, Writing–review and editing. HZ: Formal Analysis, Writing–review and editing. TS: Methodology, Writing–review and editing. XW: Methodology, Writing–review and editing. QK: Supervision, Writing–review and editing. LL: Methodology, Writing–review and editing. ZL: Software, Writing–review and editing. ZH: Conceptualization,

Writing–review and editing. ZT: Methodology, Writing–review and editing. JT: Methodology, Writing–review and editing. JZ: Funding acquisition, Investigation, 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 work was supported by the National Nature Science Foundation of China (NSFC 82460290, 31960156, 31660338, and 32270848); the Collaborative Innovation Center of Chinese Ministry of Education (2020-39); the Science and Technology Support Program of Guizhou Province [QKH (2020) 4Y192, QKH (2023) 506].

Acknowledgments

We thank Biorender (https://www.biorender.com/) for providing us with the image material

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

Akao, T., Kawabata, K., Yanagisawa, E., Ishihara, K., Mizuhara, Y., Wakui, Y., et al. (2000). Baicalin, the predominant flavone glucuronide of scutellariae radix, is absorbed from the rat gastrointestinal tract as the aglycone and restored to its original form. *J. Pharm. Pharmacol.* 52 (12), 1563–1568. doi:10.1211/0022357001777621

Akao, T., Sakashita, Y., Hanada, M., Goto, H., Shimada, Y., and Terasawa, K. (2004). Enteric excretion of baicalein, a flavone of Scutellariae Radix, via glucuronidation in rat: involvement of multidrug resistance-associated protein 2. *Pharm. Res.* 21 (11), 2120–2126. doi:10.1023/b:pham.0000048205.02478.b5

Akao, T., Sato, K., He, J. X., Ma, C. M., and Hattori, M. (2013). Baicalein 6-O-β-D-glucopyranuronoside is a main metabolite in the plasma after oral administration of baicalin, a flavone glucuronide of scutellariae radix, to rats. *Biol. and Pharm. Bull.* 36 (5), 748–753. doi:10.1248/bpb.b12-00850

Andres-Hernando, A., Dursun, B., Altmann, C., Ahuja, N., He, Z., Bhargava, R., et al. (2012). Cytokine production increases and cytokine clearance decreases in mice with bilateral nephrectomy. *Nephrol. Dial. Transplant.* 27 (12), 4339–4347. official publication of the European Dialysis and Transplant Association - European Renal Association 27. doi:10.1093/ndt/gfs256

Association, A. D., DeFronzo, R. A., Franz, M. J., Keane, W. F., Mogensen, C. E., Parving, H. H., et al. (2003). Diabetic nephropathy. *Diabetes care* 26 (Suppl. l_1), s94–s98. doi:10.2337/diacare.26.2007.S94

Bai, C., Li, T., Sun, Q., Xin, Q., Xu, T., Yu, J., et al. (2018). Protective effect of baicalin against severe burn-induced remote acute lung injury in rats. *Mol. Med. Rep.* 17 (2), 2689–2694. doi:10.3892/mmr.2017.8120

Barutta, F., Bellini, S., and Gruden, G. (2022). Mechanisms of podocyte injury and implications for diabetic nephropathy. *Clin. Sci. Lond. Engl. 1979* 136 (7), 493–520. doi:10.1042/cs20210625

Bhatia, D., and Srivastava, S. P. (2023). Editorial: diabetic kidney disease: routes to drug development, pharmacology and underlying molecular mechanisms. *Front. Pharmacol.* 14, 1252315. doi:10.3389/fphar.2023.1252315

Cai, Y., Ma, W., Xiao, Y., Wu, B., Li, X., Liu, F., et al. (2017). High doses of baicalin induces kidney injury and fibrosis through regulating TGF-β/Smad signaling pathway. *Toxicol. Appl. Pharmacol.* 333, 1–9. doi:10.1016/j.taap.2017.08.003

Carey, M. C., and Small, D. M. (1970). The characteristics of mixed micellar solutions with particular reference to bile. *Am. J. Med.* 49, 590–608. doi:10.1016/s0002-9343(70)80127-9

Chandiramani, R., Cao, D., Nicolas, J., and Mehran, R. (2020). Contrast-induced acute kidney injury. *Cardiovasc. intervention Ther.* 35 (3), 209–217. doi:10.1007/s12928-020-00660-8

Chang, C. P., Huang, W. T., Cheng, B. C., Hsu, C. C., and Lin, M. T. (2007). The flavonoid baicalin protects against cerebrovascular dysfunction and brain inflammation in experimental heatstroke. *Neuropharmacology* 52 (3), 1024–1033. doi:10.1016/j.neuropharm.2006.10.018

Chang-Panesso, M. (2021). Acute kidney injury and aging. *Pediatr. Nephrol. Berl. Ger.* 36 (10), 2997–3006. doi:10.1007/s00467-020-04849-0

Chávez-Íñiguez, J. S., Maggiani-Aguilera, P., González-Barajas, D., Rizo-Topete, L., Galindo, P., Rifkin, B., et al. (2023). Urea reduction in acute kidney injury and mortality risk. *Kidney and blood Press. Res.* 48 (1), 357–366. doi:10.1159/000530237

Chen, H., Gao, Y., Wu, J., Chen, Y., Chen, B., Hu, J., et al. (2014). Exploring therapeutic potentials of baicalin and its aglycone baicalein for hematological malignancies. *Cancer Lett.* 354 (1), 5–11. doi:10.1016/j.canlet.2014.08.003

Chen, J. W., Zhu, Z. Q., Hu, T. X., and Zhu, D. Y. (2002). Structure-activity relationship of natural flavonoids in hydroxyl radical-scavenging effects. *Acta Pharmacol. Sin.* 23 (7), 667–672.

Chen, W., Zhang, K., Cui, H., Li, J., Yao, D., Wang, S., et al. (2024). Baicalin-2-ethoxyethyl ester alleviates gentamicin-induced acute kidney injury via NF-κB signaling pathway. *Biomed. and Pharmacother. = Biomedecine and Pharmacother.* 172, 116276. doi:10.1016/j.biopha.2024.116276

Chen, Y., Minh, L. V., Liu, J., Angelov, B., Drechsler, M., Garamus, V. M., et al. (2016). Baicalin loaded in folate-PEG modified liposomes for enhanced stability and tumor targeting. *Colloids surfaces. B, Biointerfaces* 140, 74–82. doi:10.1016/j.colsurfb.2015.11.018

Chen, Y. J., Liu, W. H., Kao, P. H., Wang, J. J., and Chang, L. S. (2010). Involvement of p38 MAPK- and JNK-modulated expression of Bcl-2 and Bax in Naja nigricollis CMS-9-induced apoptosis of human leukemia K562 cells. *Toxicon* 55 (7), 1306–1316. doi:10.1016/j.toxicon.2010.01.024

Cheng, F., Kovács, I. A., and Barabási, A. L. (2019). Publisher Correction: network-based prediction of drug combinations. *Nat. Commun.* 10 (1), 1806. doi:10.1038/s41467-019-09692-y

Cheng, Z. Y., Tian, X., Gao, J., Li, H. M., Jia, L. J., and Qiao, H. L. (2014). Contribution of baicalin on the plasma protein binding displacement and CYP3A activity inhibition to the pharmacokinetic changes of nifedipine in rats *in vivo* and *in vitro*. *PloS one* 9 (1), e87234. doi:10.1371/journal.pone.0087234

Cookson, B. T., and Brennan, M. A. (2001). Pro-inflammatory programmed cell death. *Trends Microbiol.* 9 (3), 113–114. doi:10.1016/s0966-842x(00)01936-3

Coulthard, L. R., White, D. E., Jones, D. L., McDermott, M. F., and Burchill, S. A. (2009). p38(MAPK): stress responses from molecular mechanisms to therapeutics. *Trends Mol. Med.* 15 (8), 369–379. doi:10.1016/j.molmed.2009.06.005

Ding, M., Tang, Z., Liu, W., Shao, T., Yuan, P., Chen, K., et al. (2021). Burdock fructooligosaccharide attenuates high glucose-induced apoptosis and oxidative stress injury in renal tubular epithelial cells. *Front. Pharmacol.* 12, 784187. doi:10.3389/fphar.2021.784187

Djudjaj, S., and Boor, P. (2019). Cellular and molecular mechanisms of kidney fibrosis. *Mol. aspects Med.* 65, 16–36. doi:10.1016/j.mam.2018.06.002

Eknoyan, G., Hostetter, T., Bakris, G. L., Hebert, L., Levey, A. S., Parving, H. H., et al. (2003). Proteinuria and other markers of chronic kidney disease: a position statement of the national kidney foundation (NKF) and the national institute of diabetes and digestive and kidney diseases (NIDDK). *Am. J. kidney Dis. official J. Natl. Kidney Found.* 42 (4), 617–622. doi:10.1016/s0272-6386(03)00826-6

Emma, F., Montini, G., Parikh, S. M., and Salviati, L. (2016). Mitochondrial dysfunction in inherited renal disease and acute kidney injury. *Nat. Rev. Nephrol.* 12 (5), 267–280. doi:10.1038/nrneph.2015.214

Erfurt, S., Lehmann, R., Matyukhin, I., Marahrens, B., Patschan, S., and Patschan, D. (2023). Stratification of acute kidney injury risk, disease severity, and outcomes by electrolyte disturbances. *J. Clin. Med. Res.* 15 (2), 59–67. doi:10.14740/jocmr4832

Ezhilrani, V. C., Karunanithi, P., Sarangi, B., Joshi, R. G., and Dash, S. (2021). Hydrophilic-hydrophilic mixed micellar system: effect on solubilization of drug. *SN Appl. Sci.* 3 (3), 371. doi:10.1007/s42452-021-04250-y

Feng, H., Zhang, K., Zhang, K., Guo, Z., Liu, Q., Wang, L., et al. (2022a). Antiviral activity and underlying mechanisms of baicalin against avian infectious bronchitis virus *in vitro*. *Avian pathology J. W.V.P.A* 51 (6), 574–589. doi:10.1080/03079457.2022.2109453

Feng, Q., Yu, X., Qiao, Y., Pan, S., Wang, R., Zheng, B., et al. (2022b). Ferroptosis and acute kidney injury (AKI): molecular mechanisms and therapeutic potentials. *Front. Pharmacol.* 13, 858676. doi:10.3389/fphar.2022.858676

Feng, W., Ao, H., Peng, C., and Yan, D. (2019). Gut microbiota, a new frontier to understand traditional Chinese medicines. *Pharmacol. Res.* 142, 176–191. doi:10.1016/j.phrs.2019.02.024

Fu, J., and Wu, H. (2023). Structural mechanisms of NLRP3 inflammasome assembly and activation. *Annu. Rev. Immunol.* 41, 301–316. doi:10.1146/annurev-immunol-081022-021207

Fu, Y. J., Xu, B., Huang, S. W., Luo, X., Deng, X. L., Luo, S., et al. (2021). Baicalin prevents LPS-induced activation of TLR4/NF-κB p65 pathway and inflammation in mice via inhibiting the expression of CD14. *Acta Pharmacol. Sin.* 42 (1), 88–96. doi:10.1038/s41401-020-0411-9

Gameiro, J., Fonseca, J. A., Outerelo, C., and Lopes, J. A. (2020). Acute kidney injury: from diagnosis to prevention and treatment strategies. *J. Clin. Med.* 9 (6), 1704. doi:10.3390/jcm9061704

Ganguly, R., Kumar, R., and Pandey, A. K. (2022). Baicalin provides protection against fluoxetine-induced hepatotoxicity by modulation of oxidative stress and inflammation. *World J. hepatology* 14 (4), 729–743. doi:10.4254/wjh.v14.i4.729

Hajam, Y. A., Rani, R., Ganie, S. Y., Sheikh, T. A., Javaid, D., Qadri, S. S., et al. (2022). Oxidative stress in human pathology and aging: molecular mechanisms and perspectives. *Cells* 11 (3), 552. doi:10.3390/cells11030552

Hang, Y., Qin, X., Ren, T., and Cao, J. (2018). Baicalin reduces blood lipids and inflammation in patients with coronary artery disease and rheumatoid arthritis: a randomized, double-blind, placebo-controlled trial. *Lipids health Dis.* 17 (1), 146. doi:10.1186/s12944-018-0797-2

Hewitson, T. D., Holt, S. G., and Smith, E. R. (2017). Progression of tubulointerstitial fibrosis and the chronic kidney disease phenotype - role of risk factors and epigenetics. *Front. Pharmacol.* 8, 520. doi:10.3389/fphar.2017.00520

Holditch, S. J., Brown, C. N., Lombardi, A. M., Nguyen, K. N., and Edelstein, C. L. (2019). Recent advances in models, mechanisms, biomarkers, and interventions in cisplatin-induced acute kidney injury. *Int. J. Mol. Sci.* 20 (12), 3011. doi:10.3390/ijms20123011

Hoste, E. A., Bagshaw, S. M., Bellomo, R., Cely, C. M., Colman, R., Cruz, D. N., et al. (2015). Epidemiology of acute kidney injury in critically ill patients: the multinational AKI-EPI study. *Intensive care Med.* 41 (8), 1411–1423. doi:10.1007/s00134-015-3934-7

Hoste, E. A. J., Kellum, J. A., Selby, N. M., Zarbock, A., Palevsky, P. M., Bagshaw, S. M., et al. (2018). Global epidemiology and outcomes of acute kidney injury. *Nat. Rev. Nephrol.* 14 (10), 607–625. doi:10.1038/s41581-018-0052-0

Hsu, P. C., and Guo, Y. L. (2002). Antioxidant nutrients and lead toxicity. *Toxicology* 180 (1), 33–44. doi:10.1016/s0300-483x(02)00380-3

Hu, H., Li, W., Hao, Y., Peng, Z., Zou, Z., and Liang, W. (2024). Baicalin ameliorates renal fibrosis by upregulating CPT1α-mediated fatty acid oxidation in diabetic kidney disease. *Phytomedicine Int. J. phytotherapy Phytopharm.* 122, 155162. doi:10.1016/j.phymed.2023.155162

Hu, Q., Gao, L., Peng, B., and Liu, X. (2017). Baicalin and baicalein attenuate renal fibrosis *in vitro* via inhibition of the TGF-β1 signaling pathway. *Exp. Ther. Med.* 14 (4), 3074–3080. doi:10.3892/etm.2017.4888

Huang, F., Wang, Q., Guo, F., Zhao, Y., Ji, L., An, T., et al. (2019b). FoxO1-mediated inhibition of STAT1 alleviates tubulointerstitial fibrosis and tubule apoptosis in diabetic kidney disease. *EBioMedicine* 48, 491–504. doi:10.1016/j.ebiom.2019.09.002

Huang, G., Bao, J., Shao, X., Zhou, W., Wu, B., Ni, Z., et al. (2020). Inhibiting pannexin-1 alleviates sepsis-induced acute kidney injury via decreasing NLRP3 inflammasome activation and cell apoptosis. *Life Sci.* 254, 117791. doi:10.1016/j.lfs.2020.117791

Huang, J., Li, J., Lyu, Y., Miao, Q., and Pu, K. (2019a). Molecular optical imaging probes for early diagnosis of drug-induced acute kidney injury. *Nat. Mater.* 18 (10), 1133–1143. doi:10.1038/s41563-019-0378-4

Huang, R., Fu, P., and Ma, L. (2023). Kidney fibrosis: from mechanisms to therapeutic medicines. *Signal Transduct. Target. Ther.* 8 (1), 129. doi:10.1038/s41392-023-01379-7

Huang, T., Liu, Y., and Zhang, C. (2019c). Pharmacokinetics and bioavailability enhancement of baicalin: a review. *Eur. J. drug metabolism Pharmacokinet.* 44 (2), 159–168. doi:10.1007/s13318-018-0509-3

Huang, Y., Xu, W., and Zhou, R. (2021). NLRP3 inflammasome activation and cell death. *Cell. and Mol. Immunol.* 18 (9), 2114–2127. doi:10.1038/s41423-021-00740-6

Hung, P. H., Hsu, Y. C., Chen, T. H., and Lin, C. L. (2021). Recent advances in diabetic kidney diseases: from kidney injury to kidney fibrosis. *Int. J. Mol. Sci.* 22 (21), 11857. doi:10.3390/ijms222111857

Isola, G., Matarese, M., Ramaglia, L., Iorio-Siciliano, V., Cordasco, G., and Matarese, G. (2019). Efficacy of a drug composed of herbal extracts on postoperative discomfort after surgical removal of impacted mandibular third molar: a randomized, triple-blind, controlled clinical trial. *Clin. oral Investig.* 23 (5), 2443–2453. doi:10.1007/s00784-018-2690-9

Jang, J. H., Kim, E. A., Park, H. J., Sung, E. G., Song, I. H., Kim, J. Y., et al. (2017). Methylglyoxal-induced apoptosis is dependent on the suppression of c-FLIP(L) expression via down-regulation of p65 in endothelial cells. *J. Cell. Mol. Med.* 21 (11), 2720–2731. doi:10.1111/jcmm.13188

Johnson, A., and DiPietro, L. A. (2013). Apoptosis and angiogenesis: an evolving mechanism for fibrosis. *FASEB J.* 27 (10), 3893–3901. official publication of the Federation of American Societies for Experimental Biology. doi:10.1096/fj.12-214189

Jung, H. S., Kim, M. H., Gwak, N. G., Im, Y. S., Lee, K. Y., Sohn, Y., et al. (2012). Antiallergic effects of Scutellaria baicalensis on inflammation *in vivo* and *in vitro*. *J. Ethnopharmacol.* 141 (1), 345–349. doi:10.1016/j.jep.2012.02.044

Kelley, N., Jeltema, D., Duan, Y., and He, Y. (2019). The NLRP3 inflammasome: an overview of mechanisms of activation and regulation. *Int. J. Mol. Sci.* 20 (13), 3328. doi:10.3390/ijms20133328

Kellum, J. A., Lameire, N., and KDIGO AKI Guideline Work Group (2013). Diagnosis, evaluation, and management of acute kidney injury: a KDIGO summary (Part 1). *Crit. care London, Engl.* 17 (1), 204. doi:10.1186/cc11454

Kimura, H., Ogawa, S., Ishihara, T., Maruoka, M., Tokuyama-Nakai, S., Jisaka, M., et al. (2017a). Antioxidant activities and structural characterization of flavonol O-glycosides from seeds of Japanese horse chestnut (Aesculus turbinata BLUME). *Food Chem.* 228, 348–355. doi:10.1016/j.foodchem.2017.01.084

Kimura, T., Isaka, Y., and Yoshimori, T. (2017b). Autophagy and kidney inflammation. *Autophagy* 13 (6), 997–1003. doi:10.1080/15548627.2017.1309485

Kønig, S. M., Rissler, V., Terkelsen, T., Lambrughi, M., and Papaleo, E. (2019). Alterations of the interactome of Bcl-2 proteins in breast cancer at the transcriptional, mutational and structural level. *PLoS Comput. Biol.* 15 (12), e1007485. doi:10.1371/journal.pcbi.1007485

Kumar, P., Sulakhiya, K., Barua, C. C., and Mundhe, N. (2017). TNF-α, IL-6 and IL-10 expressions, responsible for disparity in action of curcumin against cisplatin-induced nephrotoxicity in rats. *Mol. Cell. Biochem.* 431 (1-2), 113–122. doi:10.1007/s11010-017-2981-5

Kumar, S. (2018). Cellular and molecular pathways of renal repair after acute kidney injury. *Kidney Int.* 93 (1), 27–40. doi:10.1016/j.kint.2017.07.030

Kurzhagen, J. T., Dellepiane, S., Cantaluppi, V., and Rabb, H. (2020). AKI: an increasingly recognized risk factor for CKD development and progression. *J. Nephrol.* 33 (6), 1171–1187. doi:10.1007/s40620-020-00793-2

Lai, M. Y., Hsiu, S. L., Chen, C. C., Hou, Y. C., and Chao, P. D. (2003). Urinary pharmacokinetics of baicalein, wogonin and their glycosides after oral administration of Scutellaria Radix in humans. *Biol. and Pharm. Bull.* 26 (1), 79–83. doi:10.1248/bpb.26.79

Lai, M. Y., Hsiu, S. L., Hou, Y. C., Tsai, S. Y., and Chao, P. D. (2004). Significant decrease of cyclosporine bioavailability in rats caused by a decoction of the roots of Scutellaria baicalensis. *Planta medica.* 70 (2), 132–137. doi:10.1055/s-2004-815489

Lan, H. Y. (2003). Tubular epithelial-myofibroblast transdifferentiation mechanisms in proximal tubule cells. *Curr. Opin. Nephrol. Hypertens.* 12 (1), 25–29. doi:10.1097/00041552-200301000-00005

Lawrence, T. (2009). The nuclear factor NF-kappaB pathway in inflammation. *Cold Spring Harb. Perspect. Biol.* 1 (6), a001651. doi:10.1101/cshperspect.a001651

Le, J., Fan, H., Sun, M., and Zhu, J. (2021). Protective effect and mechanism of Baicalin on acute kidney injury in septic mice. *Zhonghua wei zhong bing ji jiu yi xue* 33 (7), 866–870. doi:10.3760/cma.j.cn121430-20201204-00756

Levey, A. S., Gansevoort, R. T., Coresh, J., Inker, L. A., Heerspink, H. L., Grams, M. E., et al. (2020). Change in albuminuria and GFR as end points for clinical trials in early stages of CKD: a scientific workshop sponsored by the national kidney foundation in collaboration with the us food and drug administration and European medicines agency. *Am. J. kidney Dis. official J. Natl. Kidney Found.* 75 (1), 84–104. doi:10.1053/j.ajkd.2019.06.009

Li, B., Nasser, M. I., Masood, M., Adlat, S., Huang, Y., Yang, B., et al. (2020). Efficiency of Traditional Chinese medicine targeting the Nrf2/HO-1 signaling pathway. *Biomed. and Pharmacother. = Biomedecine and Pharmacother.* 126, 110074. doi:10.1016/j.biopha.2020.110074

Li, J., Zhang, K., Xu, M., Cui, H., Guo, Y., Yao, D., et al. (2024). Baicalin - 2-ethoxyethyl ester alleviates renal fibrosis by inhibiting PI3K/AKT/NF-κB signaling pathway. *Toxicol. Appl. Pharmacol.* 483, 116827. doi:10.1016/j.taap.2024.116827

Li, M., Shi, A., Pang, H., Xue, W., Li, Y., Cao, G., et al. (2014). Safety, tolerability, and pharmacokinetics of a single ascending dose of baicalein chewable tablets in healthy subjects. *J. Ethnopharmacol.* 156, 210–215. doi:10.1016/j.jep.2014.08.031

Li, N., Feng, L., Tan, Y., Xiang, Y., Zhang, R., and Yang, M. (2018). Preparation, characterization, pharmacokinetics and biodistribution of baicalin-loaded liposome on cerebral ischemia-reperfusion after i.v. Administration in rats. *v. Adm. Rats, Mol. Basel, Switz.* 23 (7), 1747. doi:10.3390/molecules23071747

Li, Y., Wang, J., Huang, D., and Yu, C. (2022). Baicalin alleviates contrast-induced acute kidney injury through ROS/NLRP3/Caspase-1/GSDMD pathway-mediated proptosis *in vitro*. *Drug Des. Dev. Ther.* 16, 3353–3364. doi:10.2147/dddt.S379629

Liao, S., Li, P., Wang, J., Zhang, Q., Xu, D., Yang, M., et al. (2016). Protection of baicalin against lipopolysaccharide induced liver and kidney injuries based on (1)H NMR metabolomic profiling. *Toxicol. Res.* 5 (4), 1148–1159. doi:10.1039/c6tx00082g

Lim, H. A., Lee, E. K., Kim, J. M., Park, M. H., Kim, D. H., Choi, Y. J., et al. (2012). PPARγ activation by baicalin suppresses NF-κB-mediated inflammation in aged rat kidney. *Biogerontology* 13 (2), 133–145. doi:10.1007/s10522-011-9361-4

Lin, C. L., Wang, F. S., Hsu, Y. C., Chen, C. N., Tseng, M. J., Saleem, M. A., et al. (2010). Modulation of notch-1 signaling alleviates vascular endothelial growth factor-mediated diabetic nephropathy. *Diabetes* 59 (8), 1915–1925. doi:10.2337/db09-0663

Lin, M., Li, L., Li, L., Pokhrel, G., Qi, G., Rong, R., et al. (2014a). The protective effect of baicalin against renal ischemia-reperfusion injury through inhibition of inflammation and apoptosis. *BMC complementary Altern. Med.* 14, 19. doi:10.1186/1472-6882-14-19

Lin, M., Li, L., Zhang, Y., Zheng, L., Xu, M., Rong, R., et al. (2014b). Baicalin ameliorates H2O2 induced cytotoxicity in HK-2 cells through the inhibition of ER stress and the activation of Nrf2 signaling. *Int. J. Mol. Sci.* 15 (7), 12507–12522. doi:10.3390/ijms150712507

Lin, Q., Li, S., Jiang, N., Shao, X., Zhang, M., Jin, H., et al. (2019). PINK1-parkin pathway of mitophagy protects against contrast-induced acute kidney injury via decreasing mitochondrial ROS and NLRP3 inflammasome activation. *Redox Biol.* 26, 101254. doi:10.1016/j.redox.2019.101254

Lin, S., Wang, L., Jia, Y., Sun, Y., Qiao, P., Quan, Y., et al. (2024). Lipin-1 deficiency deteriorates defect of fatty acid β-oxidation and lipid-related kidney damage in diabetic kidney disease. *J. laboratory Clin. Med.* 266, 1–15. doi:10.1016/j.trsl.2023.07.004

Liu, K. D., Thompson, B. T., Ancukiewicz, M., Steingrub, J. S., Douglas, I. S., Matthay, M. A., et al. (2011). Acute kidney injury in patients with acute lung injury: impact of fluid accumulation on classification of acute kidney injury and associated outcomes. *Crit. care Med.* 39 (12), 2665–2671. doi:10.1097/CCM.0b013e318228234b

Liu, L., Bai, F., Song, H., Xiao, R., Wang, Y., Yang, H., et al. (2022b). Upregulation of TIPE1 in tubular epithelial cell aggravates diabetic nephropathy by disrupting PHB2 mediated mitophagy. *Redox Biol.* 50, 102260. doi:10.1016/j.redox.2022.102260

Liu, Y., Zheng, J. Y., Wei, Z. T., Liu, S. K., Sun, J. L., Mao, Y. H., et al. (2022a). Therapeutic effect and mechanism of combination therapy with ursolic acid and insulin on diabetic nephropathy in a type I diabetic rat model. *Front. Pharmacol.* 13, 969207. doi:10.3389/fphar.2022.969207

Liu, Z., and Dong, Z. (2021). A cross talk between HIF and NF-κB in AKI. *Am. J. physiology. Ren. physiology* 321 (3), F255–f256. doi:10.1152/ajprenal.00256.2021

Lu, T., Song, J., Huang, F., Deng, Y., Xie, L., Wang, G., et al. (2007). Comparative pharmacokinetics of baicalin after oral administration of pure baicalin, Radix scutellariae extract and Huang-Lian-Jie-Du-Tang to rats. *J. Ethnopharmacol.* 110 (3), 412–418. doi:10.1016/j.jep.2006.09.036

Lv, W., Booz, G. W., Wang, Y., Fan, F., and Roman, R. J. (2018). Inflammation and renal fibrosis: recent developments on key signaling molecules as potential therapeutic targets. *Eur. J. Pharmacol.* 820, 65–76. doi:10.1016/j.ejphar.2017.12.016

Ma, L., Wu, F., Shao, Q., Chen, G., Xu, L., and Lu, F. (2021). Baicalin alleviates oxidative stress and inflammation in diabetic nephropathy via Nrf2 and MAPK signaling pathway. *Drug Des. Dev. Ther.* 15, 3207–3221. doi:10.2147/dddt.S319260

Macedo, E., Malhotra, R., Claure-Del Granado, R., Fedullo, P., and Mehta, R. L. (2011). Defining urine output criterion for acute kidney injury in critically ill patients. *Nephrol. Dial. Transplant.* 26 (2), 509–515. official publication of the European Dialysis and Transplant Association - European Renal Association 26. doi:10.1093/ndt/gfq332

Makani, H., Messerli, F. H., Romero, J., Wever-Pinzon, O., Korniyenko, A., Berrios, R. S., et al. (2012). Meta-analysis of randomized trials of angioedema as an adverse event of renin-angiotensin system inhibitors. *Am. J. Cardiol.* 110 (3), 383–391. doi:10.1016/j.amjcard.2012.03.034

Makris, K., and Spanou, L. (2016). Acute kidney injury: definition, pathophysiology and clinical phenotypes. *Clin Biochem Rev*. 37 (2), 85–98.

Martínez-Klimova, E., Aparicio-Trejo, O. E., Tapia, E., and Pedraza-Chaverri, J. (2019). Unilateral ureteral obstruction as a model to investigate fibrosis-attenuating treatments. *Biomolecules* 9 (4), 141. doi:10.3390/biom9040141

Matchar, D. B., McCrory, D. C., Orlando, L. A., Patel, M. R., Patel, U. D., Patwardhan, M. B., et al. (2008). Systematic review: comparative effectiveness of angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers for treating essential hypertension. *Ann. Intern. Med.* 148 (1), 16–29. doi:10.7326/0003-4819-148-1-200801010-00189

McWilliam, S. J., Wright, R. D., Welsh, G. I., Tuffin, J., Budge, K. L., Swan, L., et al. (2021). The complex interplay between kidney injury and inflammation. *Clin. kidney J.* 14 (3), 780–788. doi:10.1093/ckj/sfaa164

Mebrahtu, T. F., Morgan, A. W., Keeley, A., Baxter, P. D., Stewart, P. M., and Pujades-Rodriguez, M. (2019). Dose dependency of iatrogenic glucocorticoid excess and adrenal insufficiency and mortality: a cohort study in england. *J. Clin. Endocrinol. metabolism* 104 (9), 3757–3767. doi:10.1210/jc.2019-00153

Meng, X. M., Nikolic-Paterson, D. J., and Lan, H. Y. (2016). TGF-β: the master regulator of fibrosis. *Nephrology* 12 (6), 325–338. doi:10.1038/nrneph.2016.48

Miguel, V., Tituaña, J., Herrero, J. I., Herrero, L., Serra, D., Cuevas, P., et al. (2021). Renal tubule Cpt1a overexpression protects from kidney fibrosis by restoring mitochondrial homeostasis. *J. Clin. investigation* 131 (5), e140695. doi:10.1172/jci140695

Mir-Palomo, S., Nácher, A., Díez-Sales, O., Ofelia Vila Busó, M. A., Caddeo, C., Manca, M. L., et al. (2016). Inhibition of skin inflammation by baicalin ultradeformable vesicles. *Int. J. Pharm.* 511 (1), 23–29. doi:10.1016/j.ijpharm.2016.06.136

Morisaki, T., Hou, X. L., Takahashi, K., and Takahashi, K. (2013). Baicalin pharmacokinetic profile of absorption process using novel *in-vitro* model: cytochrome P450 3A4-induced Caco-2 cell monolayers combined with rat intestinal rinse fluids. *J. Pharm. Pharmacol.* 65 (10), 1526–1535. doi:10.1111/jphp.12127

Murota, K., Nakamura, Y., and Uehara, M. (2018). Flavonoid metabolism: the interaction of metabolites and gut microbiota. *Biosci. Biotechnol. Biochem.* 82 (4), 600–610. doi:10.1080/09168451.2018.1444467

Nam, J. E., Jo, S. Y., Ahn, C. W., and Kim, Y. S. (2020). Baicalin attenuates fibrogenic process in human renal proximal tubular cells (HK-2) exposed to diabetic milieu. *Life Sci.* 254, 117742. doi:10.1016/j.lfs.2020.117742

Nastase, M. V., Zeng-Brouwers, J., Wygrecka, M., and Schaefer, L. (2018). Targeting renal fibrosis: mechanisms and drug delivery systems. *Adv. drug Deliv. Rev.* 129, 295–307. doi:10.1016/j.addr.2017.12.019

Ng, S. P., Wong, K. Y., Zhang, L., Zuo, Z., and Lin, G. (2004). Evaluation of the first-pass glucuronidation of selected flavones in gut by Caco-2 monolayer model. *J. Pharm. Pharm. Sci.* 8 (1), 1–9.

Noh, K., Kang, Y., Nepal, M. R., Jeong, K. S., Oh, D. G., Kang, M. J., et al. (2016). Role of intestinal microbiota in baicalin-induced drug interaction and its pharmacokinetics. *Mol. Basel, Switz.* 21 (3), 337. doi:10.3390/molecules21030337

Oeckinghaus, A., Hayden, M. S., and Ghosh, S. (2011). Crosstalk in NF-κB signaling pathways. *Nat. Immunol.* 12 (8), 695–708. doi:10.1038/ni.2065

Orzechowska, B., Chaber, R., Wiśniewska, A., Pajtasz-Piasecka, E., Jatczak, B., Siemieniec, I., et al. (2014). Baicalin from the extract of Scutellaria baicalensis affects the innate immunity and apoptosis in leukocytes of children with acute lymphocytic leukemia. *Int. Immunopharmacol.* 23 (2), 558–567. doi:10.1016/j.intimp.2014.10.005

Ou, Y., Zhang, W., Chen, S., and Deng, H. (2021). Baicalin improves podocyte injury in rats with diabetic nephropathy by inhibiting PI3K/Akt/mTOR signaling pathway. *Open Med. Wars. Pol.* 16 (1), 1286–1298. doi:10.1515/med-2021-0335

Pang, H., Xue, W., Shi, A., Li, M., Li, Y., Cao, G., et al. (2016). Multiple-Ascending-dose pharmacokinetics and safety evaluation of baicalein chewable tablets in healthy Chinese volunteers. *Clin. drug Investig.* 36 (9), 713–724. doi:10.1007/s40261-016-0418-7

Privratsky, J. R., Zhang, J., Lu, X., Rudemiller, N., Wei, Q., Yu, Y. R., et al. (2018). Interleukin 1 receptor (IL-1R1) activation exacerbates toxin-induced acute kidney injury. *Am. J. physiology. Ren. physiology* 315 (3), F682-F691–f691. doi:10.1152/ajprenal.00104.2018

Qu, L., and Jiao, B. (2023). The interplay between immune and metabolic pathways in kidney disease. *Cells* 12 (12), 1584. doi:10.3390/cells12121584

Ramesh, G., and Reeves, W. B. (2002). TNF-alpha mediates chemokine and cytokine expression and renal injury in cisplatin nephrotoxicity. *J. Clin. investigation* 110 (6), 835–842. doi:10.1172/jci15606

Rao Kondapally Seshasai, S., Kaptoge, S., Thompson, A., Di Angelantonio, E., Gao, P., Sarwar, N., et al. (2011). Diabetes mellitus, fasting glucose, and risk of cause-specific death. *N. Engl. J. Med.* 364 (9), 829–841. doi:10.1056/NEJMoa1008862

Rapa, S. F., Di Iorio, B. R., Campiglia, P., Heidland, A., and Marzocco, S. (2019). Inflammation and oxidative stress in chronic kidney disease-potential therapeutic role of minerals, vitamins and plant-derived metabolites. *Int. J. Mol. Sci.* 21 (1), 263. doi:10.3390/ijms21010263

Ren, Q., Guo, F., Tao, S., Huang, R., Ma, L., and Fu, P. (2020). Flavonoid fisetin alleviates kidney inflammation and apoptosis via inhibiting Src-mediated NF-κB p65 and MAPK signaling pathways in septic AKI mice. *Biomed. and Pharmacother. = Biomedecine and Pharmacother.* 122, 109772. doi:10.1016/j.biopha.2019.109772

Rodrigues, F. B., Bruetto, R. G., Torres, U. S., Otaviano, A. P., Zanetta, D. M., and Burdmann, E. A. (2013). Incidence and mortality of acute kidney injury after

myocardial infarction: a comparison between KDIGO and RIFLE criteria. *PloS one* 8 (7), e69998. doi:10.1371/journal.pone.0069998

Romagnani, P., Remuzzi, G., Glassock, R., Levin, A., Jager, K. J., Tonelli, M., et al. (2017). Chronic kidney disease. *Nat. Rev. Dis. Prim.* 3, 17088. doi:10.1038/nrdp.2017.88

Romejko, K., Markowska, M., and Niemczyk, S. (2023). The review of current knowledge on neutrophil gelatinase-associated lipocalin (NGAL). *Int. J. Mol. Sci.* 24 (13), 10470. doi:10.3390/ijms241310470

Ronco, C., Bellomo, R., and Kellum, J. A. (2019). Acute kidney injury. *Lancet London, Engl.* 394 (10212), 1949–1964. doi:10.1016/s0140-6736(19)32563-2

Rooney, B., O'Donovan, H., Gaffney, A., Browne, M., Faherty, N., Curran, S. P., et al. (2011). CTGF/CCN2 activates canonical Wnt signalling in mesangial cells through LRP6: implications for the pathogenesis of diabetic nephropathy. *FEBS Lett.* 585 (3), 531–538. doi:10.1016/j.febslet.2011.01.004

Saade, M., Araujo de Souza, G., Scavone, C., and Kinoshita, P. F. (2021). The role of GPNMB in inflammation. *Front. Immunol.* 12, 674739. doi:10.3389/fimmu.2021.674739

Samsu, N. (2021). Diabetic nephropathy: challenges in pathogenesis, diagnosis, and treatment. *BioMed Res. Int.* 2021, 1497449. doi:10.1155/2021/1497449

Schrezenmeier, E. V., Barasch, J., Budde, K., Westhoff, T., and Schmidt-Ott, K. M. (2017). Biomarkers in acute kidney injury - pathophysiological basis and clinical performance. *Acta physiol. Oxf. Engl.* 219 (3), 554–572. doi:10.1111/apha.12764

See, E. J., Jayasinghe, K., Glassford, N., Bailey, M., Johnson, D. W., Polkinghorne, K. R., et al. (2019). Long-term risk of adverse outcomes after acute kidney injury: a systematic review and meta-analysis of cohort studies using consensus definitions of exposure. *Kidney Int.* 95 (1), 160–172. doi:10.1016/j.kint.2018.08.036

Shang, W., and Wang, Z. (2017). The update of NGAL in acute kidney injury. *Curr. protein and peptide Sci.* 18 (12), 1211–1217. doi:10.2174/1389203717666160909125004

Sharma, K., and McGowan, T. A. (2000). TGF-beta in diabetic kidney disease: role of novel signaling pathways. *Cytokine and growth factor Rev.* 11 (1-2), 115–123. doi:10.1016/s1359-6101(99)00035-0

Shen, J., Wang, L., Jiang, N., Mou, S., Zhang, M., Gu, L., et al. (2016). NLRP3 inflammasome mediates contrast media-induced acute kidney injury by regulating cell apoptosis. *Sci. Rep.* 6, 34682. doi:10.1038/srep34682

Shen, L., Sun, Z., Zhao, F., Wang, W., Zhang, W., and Zhu, H. (2017). Expression of c-FLIP in a rat model of sepsis and its effects on endothelial apoptosis. *Mol. Med. Rep.* 16 (1), 231–237. doi:10.3892/mmr.2017.6564

Shi, J., Wu, G., Zou, X., Jiang, K., Baicalin, E., and Flavone Glycoside, A. (2019). Enteral baicalin, a flavone glycoside, reduces indicators of cardiac surgery-associated acute kidney injury in rats. *Cardiorenal Med.* 9 (1), 31–40. doi:10.1159/000492159

Singh, J., Chaudhari, B. P., and Kakkar, P. (2017). Baicalin and chrysin mixture imparts cyto-protection against methylglyoxal induced cytotoxicity and diabetic tubular injury by modulating RAGE, oxidative stress and inflammation. *Environ. Toxicol. Pharmacol.* 50, 67–75. doi:10.1016/j.etap.2017.01.013

Song, L., Zhu, S., Liu, C., Zhang, Q., and Liang, X. (2022). Baicalin triggers apoptosis, inhibits migration, and enhances anti-tumor immunity in colorectal cancer via TLR4/NF-κB signaling pathway. *J. food Biochem.* 46 (3), e13703. doi:10.1111/jfbc.13703

Spitz, A. Z., and Gavathiotis, E. (2022). Physiological and pharmacological modulation of BAX. *Trends Pharmacol. Sci.* 43 (3), 206–220. doi:10.1016/j.tips.2021.11.001

Srivastava, S. P., Goodwin, J. E., Kanasaki, K., and Koya, D. (2020a). Metabolic reprogramming by N-acetyl-seryl-aspartyl-lysyl-proline protects against diabetic kidney disease. *Br. J. Pharmacol.* 177 (16), 3691–3711. doi:10.1111/bph.15087

Srivastava, S. P., Goodwin, J. E., Kanasaki, K., and Koya, D. (2020b). Inhibition of angiotensin-converting enzyme ameliorates renal fibrosis by mitigating DPP-4 level and restoring antifibrotic MicroRNAs. *Genes.* 11 (2), 211. doi:10.3390/genes11020211

Sun, J., Chen, X., Liu, T., Jiang, X., Wu, Y., Yang, S., et al. (2018). Berberine protects against palmitate-induced apoptosis in tubular epithelial cells by promoting fatty acid oxidation. *Med. Sci. Monit. Int. Med. J. Exp. Clin. Res.* 24, 1484–1492. doi:10.12659/msm.908927

Sun, Y., Liu, M. W., Zhao, Y. H., Lu, Y. X., Wang, Y. A., and Tong, C. W. (2020). Baicalin attenuates lipopolysaccharide-induced renal tubular epithelial cell injury by inhibiting the TXNIP/NLRP3 signalling pathway via increasing miR-223-3p expression. *J. Biol. Regul. Homeost. agents* 34 (1), 69–82. doi:10.23812/19-502-a

Sureshbabu, A., Ryter, S. W., and Choi, M. E. (2015). Oxidative stress and autophagy: crucial modulators of kidney injury. *Redox Biol.* 4, 208–214. doi:10.1016/j.redox.2015.01.001

Taiming, L., and Xuehua, J. (2006). Investigation of the absorption mechanisms of baicalin and baicalein in rats. *J. Pharm. Sci.* 95 (6), 1326–1333. doi:10.1002/jps.20593

Tan, Y. J., Zhu, C. L., and Mao, H. X. (2016). Therapeutic effect of baicalin in treatment of renal interstitial fibrosis in rats with unliateral ureteral obstruction and related mechanisms. *Zhongguo dang dai er ke za zhi = Chin. J. Contemp. Pediatr.* 18 (4), 365–371. doi:10.7499/j.issn.1008-8830.2016.04.017

Tang, Y., Zhu, H., Zhang, Y., and Huang, C. (2006). Determination of human plasma protein binding of baicalin by ultrafiltration and high-performance liquid chromatography. *Biomed. Chromatogr. BMC* 20 (10), 1116–1119. doi:10.1002/bmc.655

Tervaert, T. W., Mooyaart, A. L., Amann, K., Cohen, A. H., Cook, H. T., Drachenberg, C. B., et al. (2010). Pathologic classification of diabetic nephropathy. *J. Am. Soc. Nephrol. JASN* 21 (4), 556–563. doi:10.1681/asn.2010010010

Thallas-Bonke, V., Thorpe, S. R., Coughlan, M. T., Fukami, K., Yap, F. Y., Sourris, K. C., et al. (2008). Inhibition of NADPH oxidase prevents advanced glycation end product-mediated damage in diabetic nephropathy through a protein kinase C-alpha-dependent pathway. *Diabetes* 57 (2), 460–469. doi:10.2337/db07-1119

Thomas, K., Zondler, L., Ludwig, N., Kardell, M., Lüneburg, C., Henke, K., et al. (2022). Glutamine prevents acute kidney injury by modulating oxidative stress and apoptosis in tubular epithelial cells. *JCI insight* 7 (21), e163161. doi:10.1172/jci.insight.163161

Tung, C. W., Hsu, Y. C., Shih, Y. H., Chang, P. J., and Lin, C. L. (2018). Glomerular mesangial cell and podocyte injuries in diabetic nephropathy. *Nephrol. Carlt. Vic.* 23 (Suppl. 4), 32–37. doi:10.1111/nep.13451

Turgut, F., Awad, A. S., and Abdel-Rahman, E. M. (2023). Acute kidney injury: medical causes and pathogenesis. *J. Clin. Med.* 12 (1), 375. doi:10.3390/jcm12010375

Van Avondt, K., Nur, E., and Zeerleder, S. (2019). Mechanisms of haemolysis-induced kidney injury. *Nat. Rev. Nephrol.* 15 (11), 671–692. doi:10.1038/s41581-019-0181-0

Viberti, G. C., Hill, R. D., Jarrett, R. J., Argyropoulos, A., Mahmud, U., and Keen, H. (1982). Microalbuminuria as a predictor of clinical nephropathy in insulin-dependent diabetes mellitus. *Lancet London, Engl.* 1 (8287), 1430–1432. doi:10.1016/s0140-6736(82)92450-3

von Mässenhausen, A., Tonnus, W., and Linkermann, A. (2018). Cell death pathways drive necroinflammation during acute kidney injury. *Nephron* 140 (2), 144–147. doi:10.1159/000490807

Wang, H., Jiang, Q., and Zhang, L. (2022). Baicalin protects against renal interstitial fibrosis in mice by inhibiting the TGF-β/Smad signalling pathway. *Pharm. Biol.* 60 (1), 1407–1416. doi:10.1080/13880209.2022.2097700

Wang, K., Feng, C., Li, C., Yao, J., Xie, X., Gong, L., et al. (2015). Baicalin protects mice from aristolochic acid I-induced kidney injury by induction of CYP1A through the aromatic hydrocarbon receptor. *Int. J. Mol. Sci.* 16 (7), 16454–16468. doi:10.3390/ijms160716454

Wang, T., Lee, H. K., Yue, G. G. L., Chung, A. C. K., Lau, C. B. S., and Cai, Z. (2021a). A novel binary matrix consisting of graphene oxide and caffeic acid for the analysis of scutellarin and its metabolites in mouse kidney by MALDI imaging. *Analyst* 146 (1), 289–295. doi:10.1039/d0an01539c

Wang, W., Sun, W., Cheng, Y., Xu, Z., and Cai, L. (2019). Role of sirtuin-1 in diabetic nephropathy. *J. Mol. Med. Berlin, Ger.* 97 (3), 291–309. doi:10.1007/s00109-019-01743-7

Wang, Y., Jia, Y., Yang, X., Liang, B., Gao, H., and Yang, T. (2018b). A potential role of Baicalin to inhibit apoptosis and protect against acute liver and kidney injury in rat preeclampsia model. *Biomed. and Pharmacother. = Biomedecine and Pharmacother.* 108, 1546–1552. doi:10.1016/j.biopha.2018.09.107

Wang, Y., Liu, T., Cai, Y., Liu, W., and Guo, J. (2023). SIRT6's function in controlling the metabolism of lipids and glucose in diabetic nephropathy. *Front. Endocrinol.* 14, 1244705. doi:10.3389/fendo.2023.1244705

Wang, Z., Wang, L., Luo, J., and Zhang, J. (2021b). Protection against acute renal injury by naturally occurring medicines which act through Nrf2 signaling pathway. *J. food Biochem.* 45 (1), e13556. doi:10.1111/jfbc.13556

Wang, Z., Wu, J., Hu, Z., Luo, C., Wang, P., Zhang, Y., et al. (2020). Dexmedetomidine alleviates lipopolysaccharide-induced acute kidney injury by inhibiting p75NTR-mediated oxidative stress and apoptosis. *Oxidative Med. Cell. Longev.* 2020, 5454210. doi:10.1155/2020/5454210

Wang, Z. L., Wang, S., Kuang, Y., Hu, Z. M., Qiao, X., and Ye, M. (2018a). A comprehensive review on phytochemistry, pharmacology, and flavonoid biosynthesis of Scutellaria baicalensis. *Pharm. Biol.* 56 (1), 465–484. doi:10.1080/13880209.2018.1492620

Wei, L., and Tan, J. (2005). Clinical observation on Breviscapine in treating hypertension patients complicated with micro-albuminuria of renal impairment. *Chin. J. Integr. Med.* 11 (1), 31–33. doi:10.1007/bf02835745

Wei, Y., Pi, C., Yang, G., Xiong, X., Lan, Y., Yang, H., et al. (2016). LC-UV determination of baicalin in rabbit plasma and tissues for application in pharmacokinetics and tissue distribution studies of baicalin after intravenous administration of liposomal and injectable formulations. *Mol. Basel, Switz.* 21 (4), 444. doi:10.3390/molecules21040444

Wen, Y., Wang, Y., Zhao, C., Zhao, B., and Wang, J. (2023). The pharmacological efficacy of baicalin in inflammatory diseases. *Int. J. Mol. Sci.* 24 (11), 9317. doi:10.3390/ijms24119317

Wu, H., Long, X., Yuan, F., Chen, L., Pan, S., Liu, Y., et al. (2014). Combined use of phospholipid complexes and self-emulsifying microemulsions for improving the oral absorption of a BCS class IV compound, baicalin. *Acta Pharm. Sin.* 4 (3), 217–226. doi:10.1016/j.apsb.2014.03.002

Wu, H., Lu, C., Zhou, A., Min, Z., and Zhang, Y. (2009). Enhanced oral bioavailability of puerarin using microemulsion vehicle. *Drug Dev. industrial Pharm.* 35 (2), 138–144. doi:10.1080/03639040801973495

Wu, L., Bi, Y., and Wu, H. (2018). Formulation optimization and the absorption mechanisms of nanoemulsion in improving baicalin oral exposure. *Drug Dev. industrial Pharm.* 44 (2), 266–275. doi:10.1080/03639045.2017.1391831

Wu, W., Wang, X., Yu, X., and Lan, H. Y. (2022). Smad3 signatures in renal inflammation and fibrosis. *Int. J. Biol. Sci.* 18 (7), 2795–2806. doi:10.7150/ijbs.71595

Xianyuan, L., Wei, Z., Yaqian, D., Dan, Z., Xueli, T., Zhanglu, D., et al. (2019). Anti-renal fibrosis effect of asperulosidic acid via TGF-β1/smad2/smad3 and NF-κB signaling pathways in a rat model of unilateral ureteral obstruction. *Phytomedicine Int. J. phytotherapy Phytopharm.* 53, 274–285. doi:10.1016/j.phymed.2018.09.009

Xing, J., Chen, X., Sun, Y., Luan, Y., and Zhong, D. (2005b). Interaction of baicalin and baicalein with antibiotics in the gastrointestinal tract. *J. Pharm. Pharmacol.* 57 (6), 743–750. doi:10.1211/0022357056244

Xing, J., Chen, X., and Zhong, D. (2005a). Absorption and enterohepatic circulation of baicalin in rats. *Life Sci.* 78 (2), 140–146. doi:10.1016/j.lfs.2005.04.072

Xu, D., Wang, B., Chen, P. P., Wang, Y. Z., Miao, N. J., Yin, F., et al. (2019a). c-Myc promotes tubular cell apoptosis in ischemia-reperfusion-induced renal injury by negatively regulating c-FLIP and enhancing FasL/Fas-mediated apoptosis pathway. *Acta Pharmacol. Sin.* 40 (8), 1058–1066. doi:10.1038/s41401-018-0201-9

Xu, Q., Zhou, A., Wu, H., and Bi, Y. (2019b). Development and *in vivo* evaluation of baicalin-loaded W/O nanoemulsion for lymphatic absorption. *Pharm. Dev. Technol.* 24 (9), 1155–1163. doi:10.1080/10837450.2019.1646757

Xu, T., Xu, X., Zhang, L., Zhang, K., Wei, Q., Zhu, L., et al. (2021). Lipidomics reveals serum specific lipid alterations in diabetic nephropathy. *Front. Endocrinol.* 12, 781417. doi:10.3389/fendo.2021.781417

Yang, B., Jain, S., Ashra, S. Y., Furness, P. N., and Nicholson, M. L. (2006). Apoptosis and caspase-3 in long-term renal ischemia/reperfusion injury in rats and divergent effects of immunosuppressants. *Transplantation* 81 (10), 1442–1450. doi:10.1097/01.tp.0000209412.77312.69

Yang, C., Zhao, T., Zhao, Z., Jia, Y., Li, L., Zhang, Y., et al. (2014). Serum-stabilized naked caspase-3 siRNA protects autotransplant kidneys in a porcine model. *Mol. Ther. J. Am. Soc. Gene Ther.* 22 (10), 1817–1828. doi:10.1038/mt.2014.111

Yang, M., Kan, L., Wu, L., Zhu, Y., and Wang, Q. (2019). Effect of baicalin on renal function in patients with diabetic nephropathy and its therapeutic mechanism. *Exp. Ther. Med.* 17 (3), 2071–2076. doi:10.3892/etm.2019.7181

Yao, H., Liu, J., Xu, S., Zhu, Z., and Xu, J. (2017). The structural modification of natural products for novel drug discovery. *Expert Opin. drug Discov.* 12 (2), 121–140. doi:10.1080/17460441.2016.1272757

Yoon, S. B., Lee, Y. J., Park, S. K., Kim, H. C., Bae, H., Kim, H. M., et al. (2009). Anti-inflammatory effects of Scutellaria baicalensis water extract on LPS-activated RAW 264.7 macrophages. *J. Ethnopharmacol.* 125 (2), 286–290. doi:10.1016/j.jep.2009.06.027

Yu, H., Lin, L., Zhang, Z., Zhang, H., and Hu, H. (2020). Targeting NF-κB pathway for the therapy of diseases: mechanism and clinical study. *Signal Transduct. Target. Ther.* 5 (1), 209. doi:10.1038/s41392-020-00312-6

Yu, L., Duan, H., Yu, Y., Zhang, Q., Zhao, J., Zhang, H., et al. (2022). Dose-dependent effects of chronic lead toxicity *in vivo*: focusing on trace elements and gut microbiota. *Chemosphere* 301, 134670. doi:10.1016/j.chemosphere.2022.134670

Yuan, Q., Lv, Y., Ding, H., Ke, Q., Shi, C., Luo, J., et al. (2021). CPT1α maintains phenotype of tubules via mitochondrial respiration during kidney injury and repair. *Cell. death and Dis.* 12 (8), 792. doi:10.1038/s41419-021-04085-w

Yuan, Q., Tang, B., and Zhang, C. (2022). Signaling pathways of chronic kidney diseases, implications for therapeutics. *Signal Transduct. Target. Ther.* 7 (1), 182. doi:10.1038/s41392-022-01036-5

Zeng, W., Qi, W., Mu, J., Wei, Y., Yang, L. L., Zhang, Q., et al. (2019). MG132 protects against renal dysfunction by regulating Akt-mediated inflammation in diabetic nephropathy. *Sci. Rep.* 9 (1), 2049. doi:10.1038/s41598-018-38425-2

Zhang, H., Yang, X., Zhao, L., Jiao, Y., Liu, J., and Zhai, G. (2016). *In vitro* and *in vivo* study of Baicalin-loaded mixed micelles for oral delivery. *Drug Deliv.* 23 (6), 1933–1939. doi:10.3109/10717544.2015.1008705

Zhang, J., Cai, W., Zhou, Y., Liu, Y., Wu, X., Li, Y., et al. (2015). Profiling and identification of the metabolites of baicalin and study on their tissue distribution in rats by ultra-high-performance liquid chromatography with linear ion trap-Orbitrap mass spectrometer. *J. Chromatogr. B, Anal. Technol. Biomed. life Sci.* 985, 91–102. doi:10.1016/j.jchromb.2015.01.018

Zhang, J., Jiang, J., Wang, B., Wang, Y., Qian, Y., Suo, J., et al. (2024). SAP130 released by ferroptosis tubular epithelial cells promotes macrophage polarization via Mincle signaling in sepsis acute kidney injury. *Int. Immunopharmacol.* 129, 111564. doi:10.1016/j.intimp.2024.111564

Zhang, J. Q., Li, Y. Y., Zhang, X. Y., Tian, Z. H., Liu, C., Wang, S. T., et al. (2023). Cellular senescence of renal tubular epithelial cells in renal fibrosis. *Front. Endocrinol.* 14, 1085605. doi:10.3389/fendo.2023.1085605

Zhang, L., Lin, G., Chang, Q., and Zuo, Z. (2005). Role of intestinal first-pass metabolism of baicalein in its absorption process. *Pharm. Res.* 22 (7), 1050–1058. doi:10.1007/s11095-005-5303-7

Zhang, P. L., Rothblum, L. I., Han, W. K., Blasick, T. M., Potdar, S., and Bonventre, J. V. (2008b). Kidney injury molecule-1 expression in transplant biopsies is a sensitive measure of cell injury. *Kidney Int.* 73 (5), 608–614. doi:10.1038/sj.ki.5002697

Zhang, Q., Ye, Q., Huang, X., Xu, A., Liu, Y., Qi, J., et al. (2020d). Revealing active components, action targets and molecular mechanism of Gandi capsule for treating diabetic nephropathy based on network pharmacology strategy. *BMC complementary Med. Ther.* 20 (1), 362. doi:10.1186/s12906-020-03155-4

Zhang, S., Xu, L., Liang, R., Yang, C., and Wang, P. (2020a). Baicalin suppresses renal fibrosis through microRNA-124/TLR4/NF-κB axis in streptozotocin-induced diabetic nephropathy mice and high glucose-treated human proximal tubule epithelial cells. *J. physiology Biochem.* 76 (3), 407–416. doi:10.1007/s13105-020-00747-z

Zhang, X., Zhang, Y., Li, R., Zhu, L., Fu, B., and Yan, T. (2020c). Salidroside ameliorates Parkinson's disease by inhibiting NLRP3-dependent pyroptosis. *Aging* 12 (10), 9405–9426. doi:10.18632/aging.103215

Zhang, X. P., Tian, H., Lai, Y. H., Chen, L., Zhang, L., Cheng, Q. H., et al. (2007). Protective effects and mechanisms of Baicalin and octreotide on renal injury of rats with severe acute pancreatitis. *World J. gastroenterology* 13 (38), 5079–5089. doi:10.3748/wjg.v13.i38.5079

Zhang, X. P., Zhang, L., Yang, P., Zhang, R. P., and Cheng, Q. H. (2008a). Protective effects of baicalin and octreotide on multiple organ injury in severe acute pancreatitis. *Dig. Dis. Sci.* 53 (2), 581–591. doi:10.1007/s10620-007-9868-3

Zhang, X. T., Wang, G., Ye, L. F., Pu, Y., Li, R. T., Liang, J., et al. (2020b). Baicalin reversal of DNA hypermethylation-associated Klotho suppression ameliorates renal injury in type 1 diabetic mouse model. *Tex.* 19 (23), 3329–3347. doi:10.1080/15384101.2020.1843815

Zhang, Y., Yao, H., Li, C., Sun, W., Chen, X., Cao, Y., et al. (2022). Gandi capsule improved podocyte lipid metabolism of diabetic nephropathy mice through SIRT1/AMPK/HNF4A pathway. *Oxidative Med. Cell. Longev.* 2022, 6275505. doi:10.1155/2022/6275505

Zhang, Z., Gao, X., Guo, M., Jiang, H., Cao, Y., and Zhang, N. (2017). The protective effect of baicalin against lead-induced renal oxidative damage in mice. *Biol. trace Elem. Res.* 175 (1), 129–135. doi:10.1007/s12011-016-0731-2

Zhao, L., Wei, Y., Huang, Y., He, B., Zhou, Y., and Fu, J. (2013). Nanoemulsion improves the oral bioavailability of baicalin in rats: *in vitro* and *in vivo* evaluation. *Int. J. nanomedicine* 8, 3769–3779. doi:10.2147/ijn.S51578

Zhao, M., Wang, Y., Li, L., Liu, S., Wang, C., Yuan, Y., et al. (2021). Mitochondrial ROS promote mitochondrial dysfunction and inflammation in ischemic acute kidney injury by disrupting TFAM-mediated mtDNA maintenance. *Theranostics* 11 (4), 1845–1863. doi:10.7150/thno.50905

Zheng, L., Zhang, C., Li, L., Hu, C., Hu, M., Sidikejiang, N., et al. (2017). Baicalin ameliorates renal fibrosis via inhibition of transforming growth factor β1 production and downstream signal transduction. *Mol. Med. Rep.* 15 (4), 1702–1712. doi:10.3892/mmr.2017.6208

Zheng, X. P., Nie, Q., Feng, J., Fan, X. Y., Jin, Y. L., Chen, G., et al. (2020). Kidney-targeted baicalin-lysozyme conjugate ameliorates renal fibrosis in rats with diabetic nephropathy induced by streptozotocin. *BMC Nephrol.* 21 (1), 174. doi:10.1186/s12882-020-01833-6

Zhong, Y., Yang, W., Sun, H., Cheng, R., Meng, F., Deng, C., et al. (2013). Ligand-directed reduction-sensitive shell-sheddable biodegradable micelles actively deliver doxorubicin into the nuclei of target cancer cells. *Biomacromolecules* 14 (10), 3723–3730. doi:10.1021/bm401098w

Zhu, H., Qian, Z., He, F., Liu, M., Pan, L., Zhang, Q., et al. (2013). Novel pharmacokinetic studies of the Chinese formula Huang-Lian-Jie-Du-Tang in MCAO rats. *Phytomedicine Int. J. phytotherapy Phytopharm.* 20 (10), 767–774. doi:10.1016/j.phymed.2012.11.012

Zhu, Y., Fu, Y., and Lin, H. (2016). Baicalin inhibits renal cell apoptosis and protects against acute kidney injury in pediatric sepsis. *Med. Sci. Monit. Int. Med. J. Exp. Clin. Res.* 22, 5109–5115. doi:10.12659/msm.899061

frontiers | Frontiers in Pharmacology

TYPE Systematic Review
PUBLISHED 27 January 2025
DOI 10.3389/fphar.2024.1462965

Check for updates

OPEN ACCESS

EDITED BY
Swayam Prakash Srivastava,
University of Michigan, United States

REVIEWED BY
Aditya Yashwant Sarode,
Columbia University, United States
Davide Costa,
Magna Græcia University, Italy
Edgar Jaimes,
Memorial Sloan Kettering Cancer Center, United States

*CORRESPONDENCE
Guanjie Fan,
fanguanjie@gzucm.edu.cn
Lu Sun,
13826055952@163.com

†These authors share first authorship

RECEIVED 11 July 2024
ACCEPTED 20 December 2024
PUBLISHED 27 January 2025

CITATION
Yuan Y, Chen C, Lin Y, Luo Y, Yang Z, Guo J, Liu Q, Sun L and Fan G (2025) Incidence of hyperkalemia RAASi and SGLT-2i treatment in individuals with diabetic kidney disease: a systematic review and network meta-analysis. *Front. Pharmacol.* 15:1462965. doi: 10.3389/fphar.2024.1462965

COPYRIGHT
© 2025 Yuan, Chen, Lin, Luo, Yang, Guo, Liu, Sun and Fan. 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.

Incidence of hyperkalemia RAASi and SGLT-2i treatment in individuals with diabetic kidney disease: a systematic review and network meta-analysis

Yahui Yuan[1,2,3†], Chun Chen[4†], Yuping Lin[3†], Yehao Luo[1,2,3], Zhaojun Yang[1,2,3], Jingyi Guo[1,2,3], Qiaoyun Liu[1,2,3], Lu Sun[3*] and Guanjie Fan[1,2,3*]

[1]The Second Clinical Medical School, Guangzhou University of Chinese Medicine, Guangzhou, China, [2]Department of Endocrinology, The Second Affiliated Hospital of Guangzhou University of Chinese Medicine, Guangzhou, China, [3]Department of Endocrinology, Guangdong Provincial Hospital of Chinese Medicine, Guangzhou, China, [4]Wangjing Hospital, China Academy of Chinese Medical Sciences, Beijing, China

Background: This study aims to evaluate the incidence of hyperkalemia and serum potassium levels associated with the use of sodium-glucose cotransporter-2 inhibitors (SGLT-2i), renin-angiotensin-aldosterone system inhibitors (RAASi) and concurrent use of these medications in individuals with diabetic kidney disease (DKD).

Methods: A comprehensive systematic search was performed in EMBASE, the Cochrane Central Register of Controlled Trials (CENTRAL), Web of Science, Scopus, and PubMed database, covering studies up to March 2024. Relevant randomized controlled trials (RCT) included adults with DKD who were treated with SGLT-2i and RAASi or their combination, with a minimum follow-up duration of 12 weeks. The primary outcomes assessed were the incidence of hyperkalemia and serum potassium levels were the primary outcomes assessed. The surface under the cumulative ranking curves (SUCRA) was utilized for ranking purposes.

Results: The study included 36 trials, encompassing 45,120 participants, comparing various interventions. SGLT-2i (SUCRA = 88.5%) was found to significantly reduce the risk of hyperkalemia. In contrast, the combination of ACEI/ARB + MRA (SUCRA = 5.7%) increased the risk of hyperkalemia. However, when SGLT-2i was added to the ACEI/ARB + MRA regimen, the incidence of hyperkalemia was found to decrease. Subgroup analyses on MRA showed that ACEI/ARB + spironolactone posed the highest risk of hyperkalemia. ACEI/ARB + SGLT-2i mitigated serum potassium level.

Conclusion: SGLT-2i was effective in reducing the incidence of hyperkalemia incidence, whereas a combination of ACEI/ARB and MRA might elevate the incidence of hyperkalemia in individuals with DKD.

Systematic Review Registration: https://www.crd.york.ac.uk/PROSPERO/#recordDetails, identifier CRD42024552810.

KEYWORDS

diabetic kidney disease, renin-angiotensin-aldosterone system inhibitors, hyperkalemia, SGLT-2i, network meta-analysis

1 Introduction

Diabetic kidney disease (DKD), a major microvascular complications of diabetes mellitus (DM), is characterized by proteinuria and progressive impairment of the renal function caused by diabetes, ultimately leading to end-stage renal disease (ESRD) (McClellan et al., 2011). Approximately 40% of individuals with DM will develop DKD, making it the leading contributor of chronic kidney disease (CKD) globally (Tuttle et al., 2022). As the global prevalence of diabetes is expected to rise by nearly 50% in the subsequent 24 years, from 537 million to 783 million individuals, leading to a corresponding increase in the incidence of DKD (Alicic et al., 2017; Magliano et al., 2021). Effective management of DKD hinges on controlling blood glucose and blood pressure, blocking the renin-angiotensin-aldosterone system (RAAS) with a single agent are cornerstones of treating DKD (de Boer et al., 2020; Lytvyn et al., 2020).

ACEI and ARB are commonly prescribed for DKD patients, due to their proven ability to delay the progression of kidney dysfunction and decrease proteinuria. However, the use of dual RAAS inhibition therapy has been associated with a higher risk of hyperkalemia (Strippoli et al., 2006; Whitlock et al., 2023; Costa et al., 2023). Clinical trials focusing on DKD have indicated that steroid mineralocorticoid receptor antagonists (MRAs) are effective in reducing albuminuria in comparison with placebo, though hyperkalemia remains an important side effect (Barrera-Chimal et al., 2022). Sodium-glucose cotransporter-2 inhibitors (SGLT-2i), a novel class of oral antihyperglycemic medications have been developed to block the majority of glucose reabsorption in the renal proximal tubule, thereby reducing both renal and cardiovascular risks (Cai et al., 2020). Recent research has highlighted the safe use of SGLT-2i in individuals with type 2 diabetes mellitus (T2DM) who are at high cardiovascular risk and have CKD, without an increased risk of hyperkalemia (Albakr et al., 2023).

Hyperkalemia, characterized as an increase serum potassium concentration exceeding 5.0 or 5.5 mmol/L, is a potentially life-threatening electrolyte abnormality. Patients with CKD, DM, heart failure (HF) and those receiving RAASi are particularly prone to developing hyperkalemia (Valdivielso et al., 2021). Individuals with DKD are particularly susceptible to hypoinsulinemia and hyperglycemia, which can further elevate the risk of hyperkalemia (Picard et al., 2020; Montford and Linas, 2017). The effects of hyperkalemia on cardiac function include depolarization, leading to shorter action potentials and an increased risk of cardiovascular morbidity. Additionally, hyperkalemia can manifest as neuromuscular symptoms, metabolic acidosis, and suppression of ammoniagenesis, ultimately raising the likelihood of mortality, progression of CKD, and hospitalization (Palmer et al., 2021). This research aims to evaluate the effect of SGLT-2i, RAASi, and their combination on the incidence of hyperkalemia and serum potassium levels in patients with DKD.

2 Methods

This study has been registered in PROSPERO International Prospective Register of Systematic Reviews (Register number: CRD42024552810). The network meta-analysis (NMA) was meticulously designed, conducted and presented in accordance with quality standards for reporting systematic reviews (Hutton et al., 2015).

2.1 Literature search

EMBASE, the Cochrane Central Register of Controlled Trials (CENTRAL), Web of science, Scopus and PubMed were systematically searched for relevant English-language literature without time restrictions from inception until March 2024. The search term was predefined and detailed in Supplementary Table S1. Moreover, the articles that are included in the reference list can also be searched manually. Data extraction was independently performed by two authors, with any discrepancies resolved through discussion or by consulting a third author to reach a consensus. The extracted information included details such as: the first author, publication time, location of the study conducted, study duration, age, total number of participants included in the study, clinical trial number, diabetes type, and more.

2.2 Eligibility criteria

Eligible trials enrolled adults (≥18 years old) with DKD who were treated with SGLT-2i, angiotensin-converting enzyme inhibitors (ACEI), angiotensin receptor blockers (ARB), renin inhibitor (RI), mineralocorticoid receptor antagonist (MRA) or placebo either as monotherapy or in combination, with a minimum follow-up period of 12 weeks. Our inclusion criteria encompassed randomized controlled trials, *post hoc* analysis, or pooled studies, while conference abstracts and non-English literature were excluded. Additionally, trials with a crossover design were excluded. Each trial had to report at least one outcome related to hyperkalemia or serum potassium levels. Detailed eligibility criteria for inclusion and exclusion in this review are provided in Supplementary Table S2.

2.3 Risk of bias assessment

Assessment of the enrolled trials' methodological quality was conducted with the Cochrane Collaboration Risk of Bias Assessment Tool. Biases such as random-sequence generation, allocation sequence concealment, blinding of participants and clinicians, blinding of outcome assessment, completeness of outcome data, and selective outcome reporting were considered. Studies were labeled as high risk of bias if at least 4 out of 7 items were deemed low risk and no single item was considered high risk. If one item was assessed as high risk, the study was categorized as high risk, while the remaining studies were rated as unclear risk (Higgins et al., 2011; Schwingshackl et al., 2018).

2.4 Data synthesis and analysis

2.4.1 Available data description

The characteristics of the studies and populations in all included trials were thoroughly described, including important variables such

as mean age, sex ratio, and dropout rates. A network diagram was utilized to illustrate the direct comparisons between different interventions for each outcome. The sample sizes for each intervention were represented by the size of the nodes, and the number of included studies was reflected in the width of the lines.

2.4.2 Transitivity assessment

One of the fundamental assumptions in NMA and indirect comparisons is transitivity. The violation of transitivity poses a risk to the validity of key findings in NMA. To assess the transitivity hypothesis, researchers analyzed the distribution of potential effect modifiers in the existing direct comparisons, including baseline serum potassium, baseline serum creatinine, and age.

2.4.3 Inconsistency assessment

The study utilized both local and global approaches to assess consistency in the results. A loop-specific approach and a side-splitting approach were employed to identify significant local inconsistencies (Dias et al., 2010). Additionally, a design-by-treatment interaction model was used to detect inconsistencies stemming from various potential origins across the network at a global level.

2.5 Statistical analysis

An NMA with random effects was conducted to establish the overall effect of one treatment compared to another. The NMA allowed for comparisons of different interventions and established a clinically meaningful ranking. For each outcome (hyperkalemia incidence, serum potassium levels), a league table was utilized to present odds ratios (OR) or mean differences (MDs) along with their corresponding 95% confidence intervals (95% CIs). The surface under the cumulative ranking curve (SUCRA) was used to assess the relative ranking of interventions. The calculations were performed using a frequentist framework in STATA17, and network diagrams were created using the R package "netmeta."

2.6 Subgroup analyses

Subgroup analyses were performed by considering baseline eGFR (<60 mL/min/1.73 m^2 vs. ≥60 mL/min/1.73 m^2), sample size (<100 vs. ≥100), and therapy duration (<48 weeks vs. ≥48 weeks).

2.7 Publication bias

Adjusted funnel plots for comparison were applied to examine the potential existence of small-study effects when 10 or more studies were available (Egger et al., 1997).

2.8 Evidence credibility

The Grading of Recommendations Assessment, Development and Evaluation (GRADE) system was used to assess the credibility of evidence. The CINeMA web tool, available at http://cinema.ispm.ch/, was applied to evaluate the results of the NMA. Within-study risk of bias, reporting bias, indirectness, imprecision, heterogeneity and inconsistency were judged qualitatively (Nikolakopoulou et al., 2020). The level of concerns for each treatment effect of NMA were judged as "no concerns," "some concerns," or "major concerns" for each of the six domains (Papakonstantinou et al., 2020).

3 Results

3.1 Characteristics

Our literature search identified 23,528 records, from which 400 full-text articles undergoing detailed evaluation (Figure 1). Out of these, 364 were excluded, leading to 36 trials meeting the inclusion criteria and providing sufficient information for the current study. The combined population of DKD patients across all relevant articles was 45,120. Among these trials, 26 were multicenter and predominantly conducted in South America, North America, Central America, Europe, Asia, and other regions. The duration of intervention varied from 12 to 216 weeks, with the average age of enrolled patients falling between 34 and 68.7 years old. Supplementary Table S3 were utilized to summarize the characteristics of the studies meeting the inclusion criteria.

3.2 Risk of bias

The risk of bias for each trial is presented in Supplementary Table S4 and Supplementary Figures S1, S2. Main limitations included insufficient reporting on the implementation of blindness among participants, investigators, and outcome assessors in some studies. Among the 36 trials reviewed, 18 trials (50%) were at low risk of randomisation bias, 12 trials (33%) were at low risk of allocation concealment, 24 trials (67%) were at low risk of participant and staff concealment, 17 trials (47%) were at low risk of outcome concealment, 24 trials (67%) were at low risk of incomplete outcome data, 30 trials (83%) were at low risk of selective reporting and 21 studies (58%) were at low risk of other bias. A primary limitation was the insufficient reporting on blinding practices involving participants, investigators, and outcome assessors in some studies.

3.3 Network diagrams

Figure 2 depicted the networks of trials used in the meta-analyses for the risk of hyperkalemia and the level of serum potassium with included studies proportional to the width of lines, and the sample number of each intervention approach proportional to the size of the nodes.

3.4 Transitivity

No evidence of transitivity was observed (Supplementary Figures S3–S5). Thus, we concluded that there was no relevant clinical and methodological heterogeneity across treatment comparisons (intransitivity) in the current review.

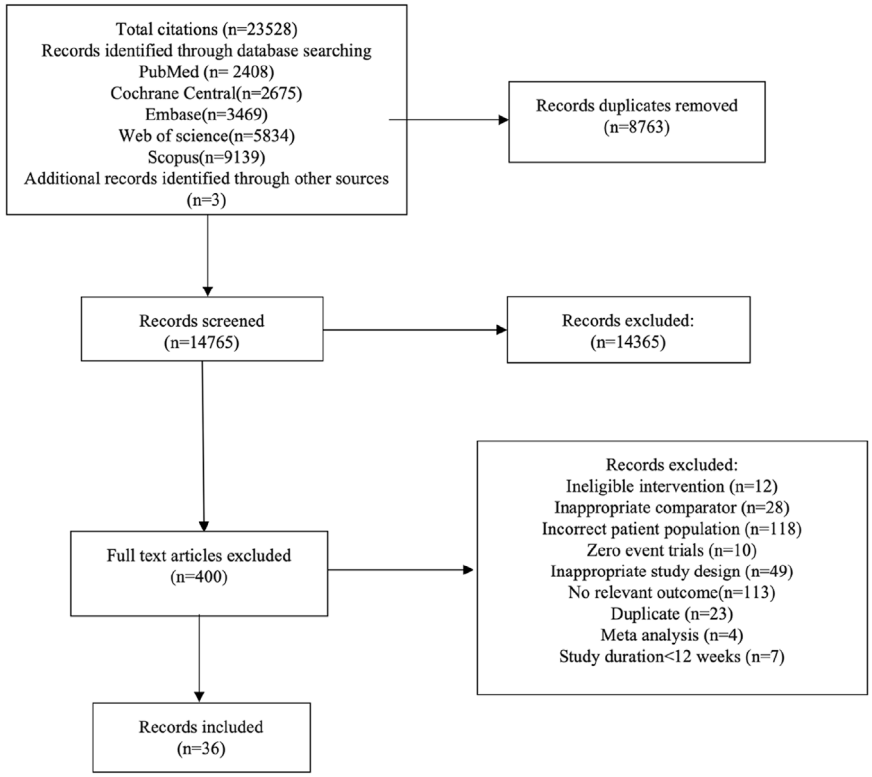


FIGURE 1
Flow-chart of study selection.

3.5 Inconsistency

The forest plot, presented in Supplementary Figures S6, S7, displayed the pooled effect size for interventions across included studies, along with the findings from the test of inconsistency. The overall test of inconsistency for the risk of hyperkalemia and the level of serum potassium did not show statistical significance (P = 0.065 and 0.943, respectively). Evidence of inconsistency was found in the pairwise comparison approach between ACEI + ARB and ACEI/ARB, as well as between ACEI/ARB + MRA along with ACEI/ARB in relation to the risk of hyperkalemia (Supplementary Table S5. The loop-specific method presented some significant inconsistency in the loop formed by ACEI + ARB-ACEI/ARB-ACEI/ARB + MRA for the risk of hyperkalemia (Supplementary Figure S8). No significant differences were observed in serum potassium level between direct and indirect results (Supplementary Table S6; Supplementary Figure S9). Furthermore, a notable inconsistency was found in the design-by-treatment model for the risk of hyperkalemia (P = 0.029), but not for serum potassium level (P = 0.93).

3.6 Contribution matrix

The contribution matrix showed to which extent the direct and indirect comparisons of the various interventions were contributory to the overall effect. The primary contributions to the effects of this study derived from indirect comparisons (Supplementary Table S7).

3.7 Table league

Table 1 showed the results of the NMA for the risk of hyperkalemia and the level of serum potassium. The analysis revealed that ACEI/ARB (OR: 1.95; 95% CI: 1.19–3.18), ACEI/ARB + SGLT-2i (OR: 2.69; 95% CI: 1.44–5.02), placebo (OR: 2.54; 95% CI: 1.04–6.18), and SGLT-2i (OR: 4.12; 95% CI: 1.43–11.87) were more effective than ACEI + ARB in reducing hyperkalemia incidence. Additionally, ACEI/ARB + MRA + SGLT-2i (OR: 3.27; 95% CI: 1.65–6.49), ACEI/ARB + RI (OR: 2.29; 95% CI: 1.15–4.57), ACEI/ARB + SGLT-2i (OR: 4.21; 95% CI: 2.34–7.58), placebo (OR: 3.97; 95% CI: 1.37–11.51) and SGLT-2i (OR: 6.44; 95% CI: 1.91–21.75) were found to be more effective in reducing hyperkalemia incidence compared to ACEI/ARB + MRA. Notably, SGLT-2i demonstrated greater efficacy than ARB (OR: 3.72; 95% CI: 1.70–8.13) and ACEI (OR: 2.77; 95% CI: 1.02–7.51) in reducing the incidence of hyperkalemia.

In terms of lowering serum potassium levels, ACEI/ARB + SGLT-2i (MD: −0.19 mmol/L; 95% CI: −0.36 to −0.03), ACEI/ARB (MD: 0.22 mmol/L; 95% CI: 0.07–0.37), ARB (MD: −0.22 mmol/L; 95% CI: −0.43 to −0.02), and RI (MD: −0.33 mmol/L; 95% CI: −0.58 to −0.08) were more effective compared to ACEI/ARB + MRA + SGLT-2i. Similarly, ACEI/ARB + SGLT-2i (MD: −0.18 mmol/L; 95% CI: −0.30 to −0.06), ACEI/ARB (MD: 0.20 mmol/L; 95% CI: 0.12–0.28), ACEI (MD: 0.18 mmol/L; 95% CI: 0.04–0.33), ARB (MD: −0.21 mmol/L; 95% CI: −0.34 to −0.07), and RI (MD: −0.32 mmol/L; 95% CI: −0.51 to −0.12) achieved lower potassium levels compared to

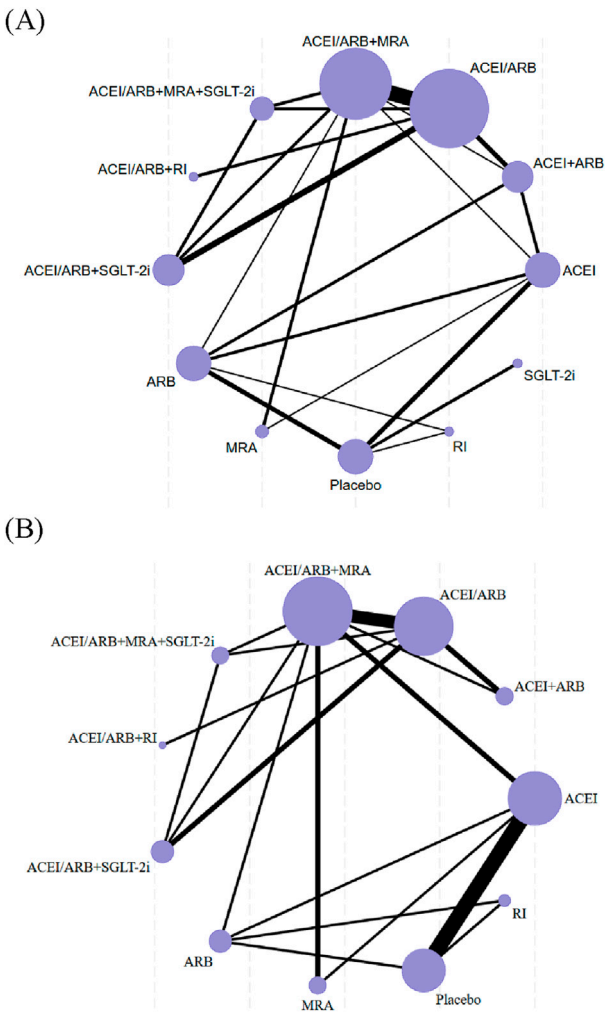


FIGURE 2
Network diagrams for hyperkalemia **(A)** and serum potassium **(B)**. The size of the nodes is proportional to the total number of participants, and the thickness of the lines is proportional to the number of studies evaluating each direct comparison.

ACEI/ARB + MRA. Furthermore, ACEI or ARB alone (MD: −0.19 mmol/L; 95% CI: −0.33 to −0.05) were more effective in reducing serum potassium levels than the combination of ACEI + ARB.

3.8 SUCRA

Figures 3, 4 showed the SUCRA values for hyperkalemia incidence and serum potassium levels. In terms of the risk of hyperkalemia, SGLT-2i (88.5%), ACEI/ARB + SGLT-2i (76.6%), and RI (67.6%) were identified as the top three most beneficial interventions. Regarding serum potassium levels, the placebo had the highest probability of being the most effective (91.4%), followed by RI (87.7%), and then ARB (62.9%).

3.9 Subgroup analyses

We conducted subgroup analyses to study the effect of eGFR, study duration, sample size, and mean age. The results of these subgroup analyses were consistent with the main analyses (Supplementary Tables S8–S10). Furthermore, a subgroup analysis on MRA was performed to evaluate hyperkalemia incidence, presented in Supplementary Table S11. The findings indicated that when used in combination with ACEI or ARB, like spironolactone (OR: 0.06; 95% CI: 0.02–0.23), esaxerenone (OR: 0.23; 95% CI: 0.12–0.42), and finerenone (OR: 0.45; 95% CI: 0.40–0.51), there was an increased frequency of hyperkalemia compared to ACEI or ARB alone. Spironolactone showed the least effectiveness in reducing the incidence of hyperkalemia. Moreover, the concurrent use of finerenone and ACEI or ARB was found to significantly decrease the risk of hyperkalemia when compared to spironolactone with ACEI/ARB (OR 0.14, 95% CI: 0.04–0.52).

3.10 Publication bias

There was no asymmetry in the funnel plots for either hyperkalemia or serum potassium levels (Supplementary Figures S10, S11).

3.11 Confidence of evidence

The confidence of evidence was low or very low for most of the comparisons regarding the risk of hyperkalemia (Supplementary Table S12). The confidence of evidence was moderate or very low regarding the level of serum potassium level (Supplementary Table S13).

4 Discussion

4.1 Principal findings

The NMA was used to investigate the effect of therapeutic drugs (ACEI, ARB, ACEI/ARB, ACEI + ARB, SGLT-2i, RI, MRA, Placebo, ACEI/ARB + SGLT-2i, ACEI/ARB + RI, ACEI/ARB + MRA, ACEI/ARB + MRA + SGLT-2i) on serum potassium levels in individuals with DKD. Results from this study demonstrated that ACEI/ARB combined with MRA was the least effective in reducing the incidence of hyperkalemia, whereas SGLT-2i emerged as the most effective drug. Interestingly, SGLT-2i even showed potential in partially counteracting the elevated risk associated with MRA combination therapy. Similarly, combined use of ACEI/ARB along with MRA resulted in a significant rise in serum potassium levels. Our findings offer valuable insights into the intricate interplay between drug classes and their impact on electrolyte homeostasis. Nevertheless, the confidence of the evidence ranged from very low to moderate for many of the comparisons, highlighting the necessity for additional high-quality studies to strengthen its credibility.

TABLE 1 League table showing the results of the network meta-analysis comparing the effects (MD or OR) of all interventions and 95% CI on hyperkalemia (upper-right) and serum potassium (bottom-left) in individuals with DKD.

SGLT-2i	1.20 (0.07, 21.57)	1.62 (0.92, 2.87)	2.84 (0.30, 26.75)	3.72 (1.70, 8.13)	1.53 (0.47, 5.03)	2.81 (0.83, 9.55)	1.97 (0.51, 7.56)	6.44 (1.91, 21.75)	2.12 (0.68, 6.60)	4.12 (1.43, 11.87)	2.77 (1.02, 7.51)
——	RI	1.35 (0.08, 23.04)	2.37 (0.07, 81.31)	3.10 (0.18, 52.74)	1.28 (0.06, 25.17)	2.35 (0.12, 46.9)	1.64 (0.08, 34.45)	5.38 (0.27, 106.62)	1.77 (0.09, 34.05)	3.44 (0.18, 64.39)	2.31 (0.12, 42.88)
——	0.01 (−0.16, 0.17)	Placebo	1.75 (0.20, 15.25)	**2.29 (1.34, 3.92)**	0.94 (0.33, 2.68)	1.73 (0.59, 5.12)	1.21 (0.36, 4.08)	**3.97 (1.37, 11.51)**	1.30 (0.49, 3.48)	**2.54 (1.04, 6.18)**	1.71 (0.75, 3.87)
——	−0.16 (−0.41, 0.09)	−0.17 (−0.38, 0.04)	MRA	1.31 (0.16, 10.97)	0.54 (0.07, 4.13)	0.99 (0.13, 7.82)	0.69 (0.09, 5.55)	2.27 (0.31, 16.33)	0.74 (0.10, 5.48)	1.45 (0.19, 11.02)	0.97 (0.12, 7.96)
——	−0.11 (−0.28, 0.06)	−0.12 (−0.27, 0.03)	0.05 (−0.16, 0.26)	ARB	0.41 (0.16, 1.07)	0.76 (0.28, 2.05)	0.53 (0.17, 1.64)	1.73 (0.66, 4.58)	0.57 (0.24, 1.38)	1.11 (0.51, 2.42)	0.75 (0.34, 1.64)
——	−0.14 (−0.37, 0.09)	−0.15 (−0.35, 0.05)	0.02 (−0.19, 0.24)	−0.03 (−0.21, 0.15)	ACEI/ ARB+SGLT-2i	1.84 (0.99, 3.40)	1.29 (0.57, 2.88)	**4.21 (2.34, 7.58)**	1.38 (0.93, 2.06)	**2.69 (1.44, 5.02)**	1.81 (0.68, 4.80)
——	−0.21 (−0.55, 0.14)	−0.21 (−0.54, 0.11)	−0.04 (−0.38, 0.29)	−0.10 (−0.41, 0.22)	−0.07 (−0.36, 0.23)	ACEI/ARB+RI	0.70 (0.29, 1.70)	**2.29 (1.15, 4.57)**	0.75 (0.46, 1.24)	1.46 (0.73, 2.92)	0.98 (0.36, 2.73)
——	**−0.33 (−0.58, −0.08)**	**−0.34 (−0.56, −0.12)**	−0.17 (−0.40, 0.06)	**−0.22 (−0.43, −0.02)**	**−0.19 (−0.36, −0.03)**	−0.13 (−0.44, 0.18)	ACEI/ ARB+MRA+SGLT-2i	**3.27 (1.65, 6.49)**	1.08 (0.53, 2.17)	2.09 (0.89, 4.92)	1.41 (0.44, 4.46)
——	**−0.32 (−0.51, −0.12)**	**−0.33 (−0.48, −0.17)**	−0.16 (−0.33, 0.02)	**−0.21 (−0.34, −0.07)**	**−0.18 (−0.30, −0.06)**	−0.11 (−0.40, 0.17)	0.02 (−0.14, 0.17)	ACEI/ ARB+MRA	0.33 (0.22, 0.49)	0.64 (0.34, 1.20)	0.43 (0.16, 1.16)
——	−0.12 (−0.33, 0.09)	−0.12 (−0.30, 0.05)	0.05 (−0.15, 0.24)	−0.01 (−0.16, 0.15)	0.02 (−0.08, 0.13)	0.09 (−0.18, 0.36)	**0.22 (0.07, 0.37)**	**0.20 (0.12, 0.28)**	ACEI/ARB	**1.95 (1.19, 3.18)**	1.31 (0.53, 3.25)
——	**−0.30 (−0.55, −0.06)**	**−0.31 (−0.53, −0.09)**	−0.14 (−0.38, 0.09)	−0.20 (−0.40, 0.01)	−0.16 (−0.34, 0.01)	−0.10 (−0.41, 0.21)	0.03 (−0.18, 0.23)	0.01 (−0.14, 0.17)	**−0.19 (−0.33, −0.05)**	ACEI+ARB	0.67 (0.30, 1.51)
——	−0.13 (−0.31, 0.04)	**−0.14 (-0.23, −0.05)**	0.03 (−0.18, 0.23)	−0.03 (−0.17, 0.12)	0.01 (−0.18, 0.20)	0.07 (−0.25, 0.39)	0.20 (−0.01, 0.41)	**0.18 (0.04, 0.33)**	−0.02 (−0.18, 0.15)	0.17 (−0.04, 0.38)	ACEI

The values upper-right the therapeutic drugs correspond to the OR and 95% CI in the incidence of hyperkalemia between the row and columns (e.g., the OR in the incidence of hyperkalemia between ACEI/ARB + MRA and ACEI/ARB + MRA + SGLT-2i is 3.27). The values bottom-left the therapeutic drugs correspond to the MD and 95% CI in serum potassium levels (mmol/L) between the column and the row (e.g., the MD serum potassium levels between ACEI/ARB + MRA and ACEI/ARB is 0.2 mmol/L). Bold values indicate comparisons with significant differences.

10.3389/fphar.2024.1462965

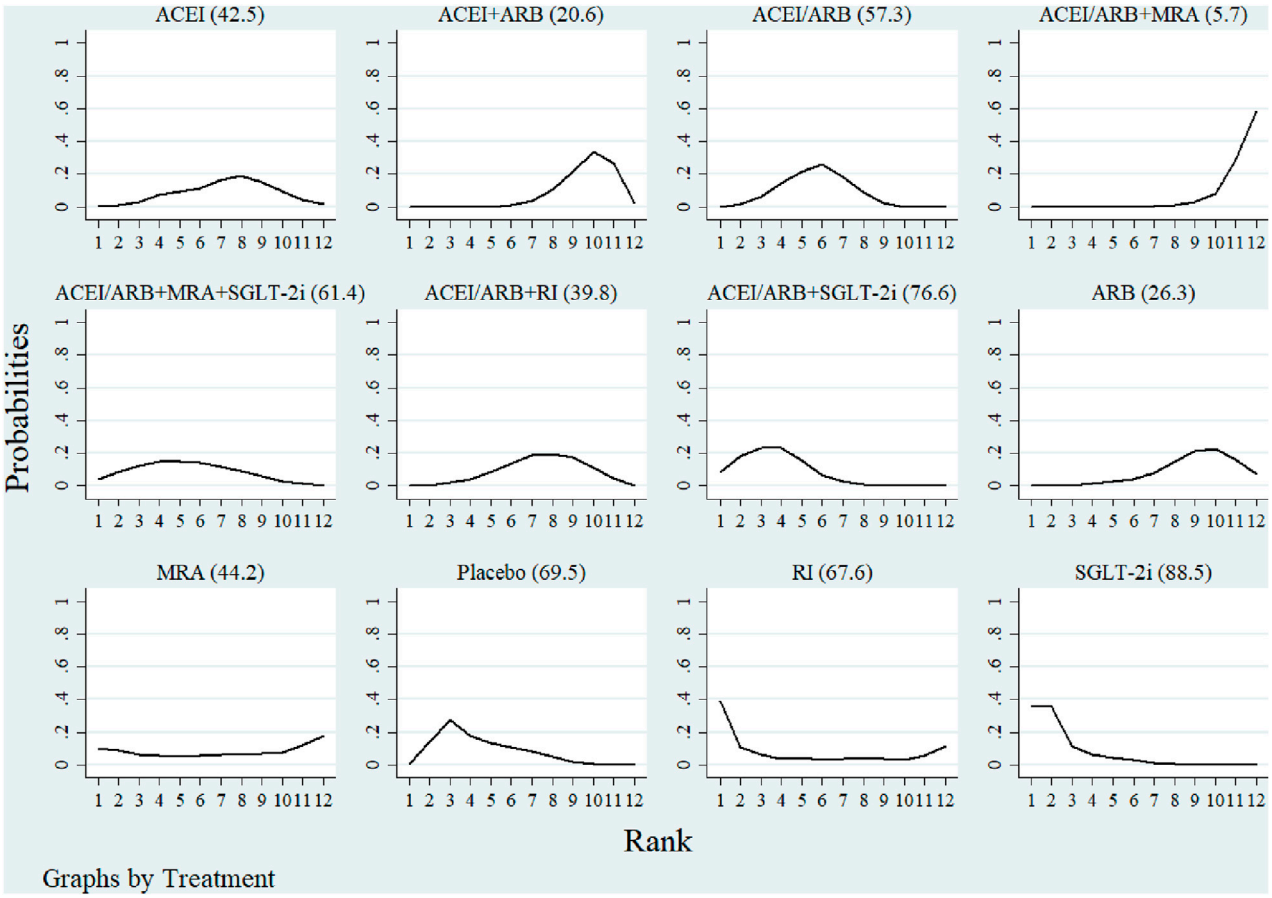


FIGURE 3
Ranking of hyperkalemia by SUCRA. The larger the SUCRA value, the better the result.

4.2 Comparison with previous studies and reasons for difference

Of note, combined use of ACEI and ARB elevated the incidence of hyperkalemia and the level serum potassium in patients with DKD when compared to using ACEI or ARB alone. Likewise, the previous studies found ACEI plus ARB dual therapy may result in a greater likelihood of hyperkalemia compared to using either medication alone (Whitlock et al., 2023; Nishida et al., 2012). The important mechanisms leading to hyperkalemia while on ACEI or ARB include decreased aldosterone levels, reduced sodium delivery to the distal nephron, impaired collecting duct function, and excessive potassium intake (Raebel, 2012). Therefore, it is not recommended to use ACEI and ARB together.

When RI aliskiren is combined with an ACEI or ARB, there is a notable rise in the likelihood of hyperkalemia (Parving et al., 2012). Yet, our research discovered that ACEI/ARB plus RI had a lower risk of hyperkalemia when compared to combining ACEI/ARB with MRA. This aligns with the finding from a study that observed no elevated risk of hospitalization due to hyperkalemia among elderly patients (aged 66 and older) receiving treatment with either ACEi or ARB, when aliskiren was used (Gilbert et al., 2013). In addition, small fluctuations in serum potassium levels were typically minor and not likely to have a meaningful impact on patients with heart failure or CKD (Weir and Rolfe, 2010). Conversely, significant decreases in serum potassium levels were observed with RI alone in this study. The different effects of RI and MRA on hyperkalemia could be attributed to different mechanisms of action. RI disrupts angiotensin II-induced stimulation of aldosterone secretion, leading to an increase in serum potassium levels, while MRA blocks the interaction between aldosterone and its receptor (Weir and Rolfe, 2010). Moreover, it is important to interpret these results cautiously, as only one article was available for RI alone. Therefore, further research is needed to confirm these findings and to fully understand the mechanisms underlying the effects of RI and MRA on serum potassium levels and hyperkalemia risk in patients with DKD.

The results of this study indicated that SGLT-2i alone had the lowest risk of hyperkalemia in patients with DKD, which is consistent with previous studies (Luo et al., 2023; Yang et al., 2022). The combination of ACEI/ARB and SGLT-2i was found to be linked to a reduced risk of hyperkalemia in comparison to ACEI plus ARB or ACEI/ARB plus MRA and did not increase the incidence of hyperkalemia compared to placebo, in line with previous research (Kaze et al., 2022; Pozzi et al., 2024). Previous research has shown that MRA added to SGLT-2i reduced the risk of hyperkalemia in patients with heart failure and reduced ejection fraction compared to MRA alone (Ahmed et al., 2023; Kristensen et al., 2020). The risk of hyperkalemia was significantly lower with the combination of ACEI/ARB and SGLT-2i plus MRA compared to ACEI/ARB plus MRA,

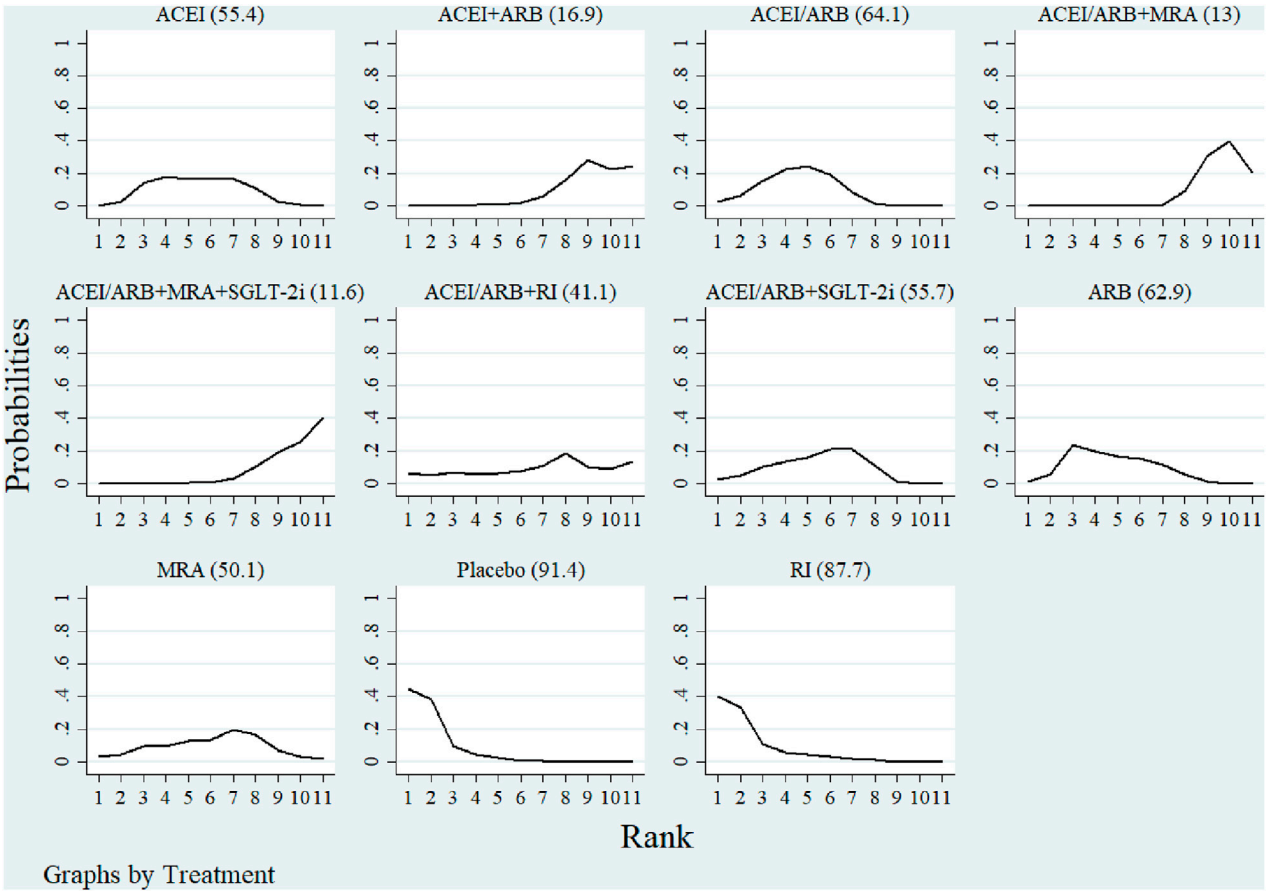


FIGURE 4
Ranking of serum potassium by SUCRA. The larger the SUCRA value, the better the result.

suggesting a reversal of hyperkalemia occurrence in DKD patients by SGLT-2i, which is supported by a meta-analysis (Luo et al., 2023). However, SGLT-2i did not reverse absolute changes in serum potassium induced by the concurrent use of ACEI/ARB plus MRA. Possible reasons for this inconsistency could be related to differences in sample sizes (44249 for hyperkalemia and 17877 for serum potassium) and heterogeneity between studies. Therefore, a more thorough evaluation of individual risk factors and appropriate management strategies may be necessary in clinical practice. Additional investigation is necessary to ascertain if SGLT-2i can counteract the effects of MRA on serum potassium levels. SGLT-2i regulate potassium levels in a unique manner when compared to ACEI, ARB, or MRA. These mechanisms involve increased negative charge in the renal tubules (De La Flor et al., 2023; Neuen et al., 2022), a slight elevation in aldosterone levels (Lytvyn et al., 2022), and long-term sustained use of SGLT-2i for kidney function preservation, which could explain their counterbalancing effect on the risk of drug-induced hyperkalemia. These findings underscore the potential of SGLT-2i as a promising therapeutic option for DKD patients, not only for their well-established renal and cardiovascular benefits but also for their favorable effect on potassium homeostasis.

Our research revealed that the concomitant use of ACEI/ARB along with MRA posed the highest risk of hyperkalemic adverse events in patients with DKD. MRA alone did not increase the risk of hyperkalemia, but when combined with ACEI/ARB, it significantly elevated the risk, consistent with previous research (Luo et al., 2023). The similar results were observed in the absolute changes in serum potassium. The significant elevation in the risk of hyperkalemia observed with ACEI/ARB plus MRA highlights the need for careful monitoring and individualized dosing strategies when employing this combination therapy. Subgroup analyses on MRA indicated that the concurrent use of ACEI/ARB plus spironolactone was the most pronounced in increasing the risk of hyperkalemia. A previous study has shown that the risk of hyperkalemia was elevated by ACEI/ARB together with spironolactone, necessitating careful monitoring of patients' serum potassium levels (Liu et al., 2018). Novel non-steroidal MRAs were associated with a lower risk of hyperkalemia compared to steroidal MRAs due to various factors such as tissue distribution, receptor inactivation, drug half-life and other factors (Barrera-Chimal et al., 2022). The previous study found that combination therapy with SGLT-2i could lower the hyperkalemia risk associated with finerenone (Agarwal et al., 2022; Shaikh et al., 2022). The SGLT-2i could counteract the hyperkalemia induced by ACEI/ARB plus finerenone or esaxerenone in this study. Therefore, clinicians should consider these findings when selecting the appropriate therapy for individual patients, balancing the potential benefits and risks of each treatment option.

Our study systematically assessed the impact of SGLT-2i and RAAS inhibitors on the incidence of hyperkalemia in patients with

DKD, providing a detailed comparison of different drug combinations. This meta-analysis clearly reveals the effectiveness of SGLT-2i in reducing the risk of hyperkalemia, contrasting sharply with the increased risk of hyperkalemia associated with traditional RAAS inhibitor combinations. Although recent research also addresses the use of these medications, our study offers a more comprehensive and precise ranking of drug effects by including multiple randomized controlled trials and employing a network meta-analysis approach. Furthermore, our study particularly highlights the significant increase in the risk of hyperkalemia when using the ACEI/ARB + MRA combination, especially when combined with SGLT-2i, which was found to have a protective effect against hyperkalemia. This finding provides crucial evidence for the selection of drug combinations in clinical practice and offers a new perspective for optimizing treatment strategies for patients with DKD.

4.3 Strengths and limitations of the study

NMA methods, which are a combination of direct and indirect comparison simultaneously, were used in this systematic review. Another strength included identifying inconsistencies and assessing the confidence of evidence. Moreover, this study demonstrated that SGLT-2i presented a reduced risk of hyperkalemia and partially counteracted the hyperkalemia induced by the concomitant use of ACEI/ARB along with MRA in individuals with DKD. These findings are valuable for evidence-based decision-making in managing individuals with DKD.

The primary limitation of this review was the relatively small number and quality of included studies, with only 36 trials and 10 assessed at low risk of bias. Another significant limitation was the varying definitions of hyperkalemia in the original literature, with some studies using thresholds of over 5.5 mmol/L and others over 6 mmol/L. In addition, while our results contribute significantly to the understanding of drug effects on potassium levels in DKD, the statistical inconsistency in hyperkalemia, which also presented in the GRADE rating, reducing the confidence in the effect estimates and rankings. Consequently, our findings should be interpreted with caution and serve as a catalyst for further high-quality research in this area.

Our findings suggest that SGLT-2i is effective in reducing the incidence of hyperkalemia in individuals with DKD, whereas combinations of ACEI/ARB and MRA may elevate this risk. However, these pharmacological effects must be considered alongside the social determinants of health and social capital that influence disease management (Costa et al., 2022). Therefore, future research should prioritize the integration of social factors into clinical trials and observational studies to provide a more holistic understanding of DKD management.

4.4 Conclusion

SGLT-2i was found to be the most effective therapeutic drugs for lowering the risk of hyperkalemia in individuals with DKD, while MRA has been linked to a heightened risk of hyperkalemia. The potential of SGLT-2i to reverse the elevation of serum potassium induced by ACEI/ARB combined with MRA is still under investigation. The study found no significant evidence of transitivity or inconsistency in the clinical and methodological heterogeneity across the treatment comparisons, suggesting that the observed effects are robust. However, the confidence in the evidence for hyperkalemia risk was generally low or very low, and moderate to very low for serum potassium levels, indicating that further research is needed to strengthen these findings.

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

YY: Methodology, Software, Supervision, Writing–original draft. CC: Methodology, Software, Writing–review and editing. YpL: Methodology, Software, Writing–review and editing. YhL: Formal analysis, Writing–review and editing. ZY: Formal analysis, Writing–review and editing. JG: Formal analysis, Writing–review and editing. QL: Formal analysis, Writing–review and editing. LS: Methodology, Software, Writing–review and editing. GF: Conceptualization, Writing–review and editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This study was supported by the Guangdong Science and Technology Department (YN2021DB02) and the Special Research Fund for Clinical Studies of Innovative Drugs after Market Launch (WKZX2023CX150007).

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.

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.2024.1462965/full#supplementary-material

References

Agarwal, R., Joseph, A., Anker, S. D., Filippatos, G., Rossing, P., Ruilope, L. M., et al. (2022). Hyperkalemia risk with finerenone: results from the FIDELIO-DKD trial. *J. Am. Soc. Nephrol.* 33 (1), 225–237. doi:10.1681/ASN.2021070942

Ahmed, A., Ahmed, W., Arshad, M. S., Suri, A., Amin, E., Shahid, I., et al. (2023). Meta-analysis evaluating risk of hyperkalemia stratified by baseline MRA usage in patients with heart failure receiving SGLT2 inhibitors. *Cardiovasc Drugs Ther.* 38, 1055–1058. doi:10.1007/s10557-023-07446-z

Albakr, R. B., Sridhar, V. S., and Cherney, D. Z. I. (2023). Novel therapies in diabetic kidney disease and risk of hyperkalemia: a review of the evidence from clinical trials. *Am. J. Kidney Dis.* 82 (6), 737–742. doi:10.1053/j.ajkd.2023.04.015

Alicic, R. Z., Rooney, M. T., and Tuttle, K. R. (2017). Diabetic kidney disease: challenges, progress, and possibilities. *Clin. J. Am. Soc. Nephrol.* 12 (12), 2032–2045. doi:10.2215/CJN.11491116

Barrera-Chimal, J., Lima-Posada, I., Bakris, G. L., and Jaisser, F. (2022). Mineralocorticoid receptor antagonists in diabetic kidney disease - mechanistic and therapeutic effects. *Nat. Rev. Nephrol.* 18 (1), 56–70. doi:10.1038/s41581-021-00490-8

Cai, Y., Liu, X., and Xu, G. (2020). Combination therapy with SGLT2 inhibitors for diabetic kidney disease. *Biomed. Pharmacother.* 127, 110192. doi:10.1016/j.biopha.2020.110192

Costa, D., Ielapi, N., Caprino, F., Giannotta, N., Sisinni, A., Abramo, A., et al. (2022). Social aspects of diabetic foot: a scoping review. *Soc. Sci.* 11, 149. doi:10.3390/socsci11040149

Costa, D., Patella, G., Provenzano, M., Ielapi, N., Faga, T., Zicarelli, M., et al. (2023). Hyperkalemia in CKD: an overview of available therapeutic strategies. *Front. Med. (Lausanne)* 10, 1178140. doi:10.3389/fmed.2023.1178140

de Boer, I. H., Caramori, M. L., Chan, J. C. N., Heerspink, H. J. L., Hurst, C., Khunti, K., et al. (2020). Executive summary of the 2020 KDIGO Diabetes Management in CKD Guideline: evidence-based advances in monitoring and treatment. *Kidney Int.* 98 (4), 839–848. doi:10.1016/j.kint.2020.06.024

De La Flor, J. C., Villa, D., Cruzado, L., Apaza, J., Valga, F., Zamora, R., et al. (2023). Efficacy and safety of the use of SGLT2 inhibitors in patients on incremental hemodialysis: maximizing residual renal function, is there a role for SGLT2 inhibitors? *Biomedicines* 11 (7), 1908. doi:10.3390/biomedicines11071908

Dias, S., Welton, N. J., Caldwell, D. M., and Ades, A. E. (2010). Checking consistency in mixed treatment comparison meta-analysis. *Stat. Med.* 29 (7-8), 932–944. doi:10.1002/sim.3767

Egger, M., Davey Smith, G., Schneider, M., and Minder, C. (1997). Bias in meta-analysis detected by a simple, graphical test. *BMJ* 315 (7109), 629–634. doi:10.1136/bmj.315.7109.629

Gilbert, C. J., Gomes, T., Mamdani, M. M., Hellings, C., Yao, Z., Garg, A. X., et al. (2013). No increase in adverse events during aliskiren use among ontario patients receiving angiotensin-converting enzyme inhibitors or angiotensin-receptor blockers. *Can. J. Cardiol.* 29 (5), 586–591. doi:10.1016/j.cjca.2013.02.015

Higgins, J. P., Altman, D. G., Gøtzsche, P. C., Jüni, P., Moher, D., Oxman, A. D., et al. (2011). The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. *Bmj* 343, d5928. doi:10.1136/bmj.d5928

Hutton, B., Salanti, G., Caldwell, D. M., Chaimani, A., Schmid, C. H., Cameron, C., et al. (2015). The PRISMA extension statement for reporting of systematic reviews incorporating network meta-analyses of health care interventions: checklist and explanations. *Ann. Intern Med.* 162 (11), 777–784. doi:10.7326/M14-2385

Kaze, A. D., Zhuo, M., Kim, S. C., Patorno, E., and Paik, J. M. (2022). Association of SGLT2 inhibitors with cardiovascular, kidney, and safety outcomes among patients with diabetic kidney disease: a meta-analysis. *Cardiovasc Diabetol.* 21 (1), 47. doi:10.1186/s12933-022-01476-x

Kristensen, S. L., Docherty, K., Jhund, P., Bengtsson, O., Demets, D., Inzucchi, S., et al. (2020). Dapagliflozin reduces the risk of hyperkalaemia in patients with heart failure and reduced ejection fraction: a secondary analysis DAPA-HF. *Eur. Heart J.* 41 (Suppl. ment_2), ehaa946.0939. doi:10.1093/ehjci/ehaa946.0939

Liu, Y., Dai, S., Liu, L., Liao, H., and Xiao, C. (2018). Spironolactone is superior to hydrochlorothiazide for blood pressure control and arterial stiffness improvement: a prospective study. *Medicine (Baltimore)*, 97(16):e0500. doi:10.1097/MD.0000000000010500

Luo, X., Xu, J., Zhou, S., Xue, C., Chen, Z., and Mao, Z. (2023). Influence of SGLT2i and RAASi and their combination on risk of hyperkalemia in DKD: a network meta-analysis. *Clin. J. Am. Soc. Nephrol.* 18 (8), 1019–1030. doi:10.2215/CJN.0000000000000205

Lytvyn, Y., Bjornstad, P., van Raalte, D. H., Heerspink, H. L., and Cherney, D. Z. I. (2020). The new biology of diabetic kidney disease-mechanisms and therapeutic implications. *Endocr. Rev.* 41 (2), 202–231. doi:10.1210/endrev/bnz010

Lytvyn, Y., Kimura, K., Peter, N., Lai, V., Tse, J., Cham, L., et al. (2022). Renal and vascular effects of combined SGLT2 and angiotensin-converting enzyme inhibition. *Circulation* 146 (6), 450–462. doi:10.1161/CIRCULATIONAHA.122.059150

Magliano, D. J., Boyko, E. J., and I.D.F.D.A.t.e.s. Committee (2021). "IDF diabetes atlas," in *Idf diabetes atlas* (Brussels: International Diabetes Federation © International Diabetes Federation).

McClellan, W. M., Warnock, D. G., Judd, S., Muntner, P., Kewalramani, R., Cushman, M., et al. (2011). Albuminuria and racial disparities in the risk for ESRD. *J. Am. Soc. Nephrol.* 22 (9), 1721–1728. doi:10.1681/ASN.2010101085

Montford, J. R., and Linas, S. (2017). How dangerous is hyperkalemia? *J. Am. Soc. Nephrol.* 28 (11), 3155–3165. doi:10.1681/ASN.2016121344

Neuen, B. L., Oshima, M., Agarwal, R., Arnott, C., Cherney, D. Z., Edwards, R., et al. (2022). Sodium-glucose cotransporter 2 inhibitors and risk of hyperkalemia in People with type 2 diabetes: a meta-analysis of individual participant data from randomized, controlled trials. *Circulation* 145 (19), 1460–1470. doi:10.1161/CIRCULATIONAHA.121.057736

Nikolakopoulou, A., Higgins, J. P. T., Papakonstantinou, T., Chaimani, A., Del Giovane, C., Egger, M., et al. (2020). CINeMA: an approach for assessing confidence in the results of a network meta-analysis. *PLoS Med.* 17 (4), e1003082. doi:10.1371/journal.pmed.1003082

Nishida, Y., Takahashi, Y., Nakayama, T., and Asai, S. (2012). Comparative effect of angiotensin II type I receptor blockers and calcium channel blockers on laboratory parameters in hypertensive patients with type 2 diabetes. *Cardiovasc Diabetol.* 11, 53. doi:10.1186/1475-2840-11-53

Palmer, B. F., Carrero, J. J., Clegg, D. J., Colbert, G. B., Emmett, M., Fishbane, S., et al. (2021). Clinical management of hyperkalemia. *Mayo Clin. Proc.* 96 (3), 744–762. doi:10.1016/j.mayocp.2020.06.014

Papakonstantinou, T., Nikolakopoulou, A., Higgins, J. P. T., Egger, M., and Salanti, G. (2020). CINeMA: Software for semiautomated assessment of the confidence in the results of network meta-analysis. *Campbell Syst. Rev.* 16 (1), e1080. doi:10.1002/cl2.1080

Parving, H. H., Brenner, B. M., McMurray, J. J. V., de Zeeuw, D., Haffner, S. M., Solomon, S. D., et al. (2012). Cardiorenal end points in a trial of aliskiren for type 2 diabetes. *N. Engl. J. Med.* 367 (23), 2204–2213. doi:10.1056/NEJMoa1208799

Picard, K., Barreto Silva, M. I., Mager, D., and Richard, C. (2020). Dietary potassium intake and risk of chronic kidney disease progression in predialysis patients with chronic kidney disease: a systematic review. *Adv. Nutr.* 11 (4), 1002–1015. doi:10.1093/advances/nmaa027

Pozzi, A., Cirelli, C., Merlo, A., Rea, F., Scangiuzzi, C., Tavano, E., et al. (2024). Adverse effects of sodium-glucose cotransporter-2 inhibitors in patients with heart failure: a systematic review and meta-analysis. *Heart Fail Rev.* 29 (1), 207–217. doi:10.1007/s10741-023-10363-w

Raebel, M. A. (2012). Hyperkalemia associated with use of angiotensin-converting enzyme inhibitors and angiotensin receptor blockers. *Cardiovasc Ther.* 30 (3), e156–e166. doi:10.1111/j.1755-5922.2010.00258.x

Schwingshackl, L., Chaimani, A., Hoffmann, G., Schwedhelm, C., and Boeing, H. (2018). A network meta-analysis on the comparative efficacy of different dietary approaches on glycaemic control in patients with type 2 diabetes mellitus. *Eur. J. Epidemiol.* 33 (2), 157–170. doi:10.1007/s10654-017-0352-x

Shaikh, A., Ray, J., and Campbell, K. N. (2022). Role of finerenone in the treatment of diabetic kidney disease: patient selection and clinical perspectives. *Ther. Clin. Risk Manag.* 18, 753–760. doi:10.2147/TCRM.S325916

Strippoli, G. F., Bonifati, C., Craig, M., Navaneethan, S. D., and Craig, J. C. (2006). Angiotensin converting enzyme inhibitors and angiotensin II receptor antagonists for preventing the progression of diabetic kidney disease. *Cochrane Database Syst. Rev.* 2006 (4), Cd006257. doi:10.1002/14651858.CD006257

Tuttle, K. R., Agarwal, R., Alpers, C. E., Bakris, G. L., Brosius, F. C., Kolkhof, P., et al. (2022). Molecular mechanisms and therapeutic targets for diabetic kidney disease. *Kidney Int.* 102 (2), 248–260. doi:10.1016/j.kint.2022.05.012

Valdivielso, J. M., Balafa, O., Ekart, R., Ferro, C. J., Mallamaci, F., Mark, P. B., et al. (2021). Hyperkalemia in chronic kidney disease in the new era of kidney protection therapies. *Drugs* 81 (13), 1467–1489. doi:10.1007/s40265-021-01555-5

Weir, M. R., and Rolfe, M. (2010). Potassium homeostasis and renin-angiotensin-aldosterone system inhibitors. *Clin. J. Am. Soc. Nephrol.* 5 (3), 531–548. doi:10.2215/CJN.07821109

Whitlock, R., Leon, S. J., Manacsa, H., Askin, N., Rigatto, C., Fatoba, S. T., et al. (2023). The association between dual RAAS inhibition and risk of acute kidney injury and hyperkalemia in patients with diabetic kidney disease: a systematic review and meta-analysis. *Nephrol. Dial. Transpl.* 38 (11), 2503–2516. doi:10.1093/ndt/gfad101

Yang, S., Zhao, L., Mi, Y., and He, W. (2022). Effects of sodium-glucose cotransporter-2 inhibitors and aldosterone antagonists, in addition to renin-angiotensin system antagonists, on major adverse kidney outcomes in patients with type 2 diabetes and chronic kidney disease: a systematic review and network meta-analysis. *Diabetes Obes. Metab.* 24 (11), 2159–2168. doi:10.1111/dom.14801

frontiers | Frontiers in Pharmacology

TYPE Systematic Review
PUBLISHED 27 March 2025
DOI 10.3389/fphar.2025.1517272

Check for updates

OPEN ACCESS

EDITED BY
Swayam Prakash Srivastava,
University of Michigan, United States

REVIEWED BY
Xiuge Wang,
The Affiliated Hospital of Changchun University of Traditional Chinese Medicine, China
Xieyu Zhang,
China Academy of Chinese Medical Sciences, China

*CORRESPONDENCE
Yanbing Gong,
gyb_1226@163.com

RECEIVED 25 October 2024
ACCEPTED 07 March 2025
PUBLISHED 27 March 2025

CITATION
Guo J, Wei M, Zhang W, Jiang Y, Li A, Wang C, Yin D, Sun A and Gong Y (2025) Clinical efficacy and safety of sodium-glucose cotransporter protein-2 (SGLT-2) inhibitor, glucagon-like peptide-1 (GLP-1) receptor agonist, and Finerenone in type 2 diabetes mellitus with non-dialysis chronic kidney disease: a network meta-analysis of randomized clinical trials. *Front. Pharmacol.* 16:1517272. doi: 10.3389/fphar.2025.1517272

COPYRIGHT
© 2025 Guo, Wei, Zhang, Jiang, Li, Wang, Yin, Sun and Gong. 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.

Clinical efficacy and safety of sodium-glucose cotransporter protein-2 (SGLT-2) inhibitor, glucagon-like peptide-1 (GLP-1) receptor agonist, and Finerenone in type 2 diabetes mellitus with non-dialysis chronic kidney disease: a network meta-analysis of randomized clinical trials

Jingyi Guo[1], Maoying Wei[1], Wenhua Zhang[1], Yijia Jiang[1], Aijing Li[1], Churan Wang[1], Dan Yin[1], Anning Sun[1] and Yanbing Gong[2]*

[1]Dongzhimen Hospital, Beijing University of Chinese Medicine, Beijing, China, [2]Beijing University of Chinese Medicine, Beijing, China

Objective: To investigate the safety and clinical efficacy of sodium-glucose cotransporter protein-2 (SGLT-2) inhibitors, glucagon-like peptide-1 (GLP-1) receptor agonists and Finerenone in treating patients with type 2 diabetes mellitus (T2DM) combined with non-dialysis chronic kidney disease (CKD).

Methods: Cochrane Library, PubMed, EMBASE, Web of Science, CNKI, CQVIP database, and WanFang from their inception up to November 2023 were searched to compare the efficacy and safety of SGLT-2 inhibitors, GLP-1 RA receptor agonists and Finerenone in the treatment of T2DM patients with non-dialysis CKD. To assess the methodological quality and risk of bias in the included studies, we utilized the Cochrane Risk of Bias Assessment tool (RoB 2.0). The confidence of evidence was examined using Confidence in Network Meta-Analysis (CINeMA). Traditional meta-analysis of variables was conducted using Stata 17.0 software with a random-effects model. We assessed publication bias using funnel plots and explored potential sources of heterogeneity through subgroup analysis.

Results: A total of 39 studies (99,599 patients) were included. Compared to Placebo (PBO), SGLT-2 inhibitors demonstrated superior efficacy in reducing glycosylated hemoglobin (HbA1c) (MD = −0.33; 95%CI: from −0.52 to −0.15), systolic blood pressure (SBP) (MD from −5.52 to −1.50; 95%CI from −8.80 to −0.23), body weight (MD from −3.81 to −1.29; 95%CI from −6.34 to −0.84) and diastolic blood pressure (DBP) (MD = −1.86; 95%CI: −3.18, −40.54). The efficacy of Liraglutide in reducing Low-Density Lipoprotein Cholesterol (LDL-C) surpassed that of other agents (MD from −1.58 to −1.41; 95% CI from −2.05 to −0.81). Finerenone significantly reduced SBP (MD = −1.65; 95%

CI: −2.48, −0.81) compared to PBO. According to the SUCRA based relative ranking of treatments, Empagliflozin was the most effective in reducing HbA1c and DBP. Semaglutide was the least harmful to estimated glomerular filtration rate. Liraglutide was the most effective in reducing LDL-C. Bexagliflozin, Canagliflozin were the most effective in reducing SBP and body weight. Finerenone had the lowest incidence of urinary tract infection, Hypoglycemia was the lowest in the Luseogliflozin group. Ertugliflozin was the least likely to cause acute kidney injury. Canagliflozin had the lowest probability of any adverse event.

Conclusion: The safety of these drugs has been confirmed, except for some special drugs. SGLT-2 inhibitors had a preferential glucose-lowering and weight-loss function, GLP-1 receptor agonists had a preferential lowering of LDL-C and blood glucose, and Finereone significantly reduced SBP compared with PBO. Systematic Review Registration: PROSPERO, CRD42024571544.

KEYWORDS

SGLT-2 inhibitors, GLP-1 receptor agonists, Finerenone, type 2 diabetes mellitus, chronic kidney disease, network meta-analysis

1 Introduction

Diabetes is a global public health concern, and with the recent surge in diabetes patients, it is projected to impact 784 million individuals by 2045, posing a significant threat to human wellbeing. Diabetic kidney disease is a prominent microvascular complication of diabetes mellitus, with an estimated 40% of individuals with diabetes projected to develop chronic kidney disease (CKD) during their lifetime, potentially necessitating the need for renal replacement therapy (Afkarian et al., 2016; Scilletta et al., 2023; Zoja et al., 2020). Hence, it is imperative to prevent further progression of kidney disease in the management of type 2 diabetes mellitus (T2DM) patients with CKD.

The SGLT-2 inhibitors represent a novel class of oral hypoglycemic medications. GLP-1 receptor agonists reduce Glycosylated hemoglobin (HbA1c) by stimulating insulin secretion and reducing glucagon secretion, while also decreasing appetite through delayed gastric emptying (Filippatos et al., 2013). Several randomized controlled trials (RCTs) have demonstrated the effectiveness and safety of these two drugs (Perkovic et al., 2019; Mann et al., 2020). Finerenone, a nonsteroidal selective mineralocorticoid receptor antagonist, has shown in large RCTs to slow down CKD progression and improve cardiovascular outcomes (Pitt et al., 2021; Filippatos et al., 2021; Bakris G. L. et al., 2020). It was approved by the Food and Drug Administration (FDA) in July 2021 for treating T2DM in CKD patients. Although GLP-1 receptor agonists have been found to lower blood pressure and body weight while improving cardiovascular outcomes, there is still no clear conclusion when compared to SGLT-2 inhibitors and Finerenone for treating T2DM in non-dialysis CKD (Sun et al., 2015; Shah et al., 2014).

There is currently a lack of comprehensive evaluation of the efficacy and safety of several drugs for treating T2DM combined with CKD. Network meta-analysis (NMA) combines direct and indirect evidence to compare multiple treatments and assess their interrelationship. Our study focused on non-dialysis CKD patients (eGFR >15 mL/min/1.73 m^2) as these drugs are not recommended for patients with low eGFR (Chinese Diabetes Society, 2025; American Diabetes Association Professional Practice Committee, 2023). Therefore, we conducted an NMA of RCTs to assess the clinical efficacy and safety of SGLT-2 inhibitors, GLP-1 receptor agonists, and Finereone in non-dialysis CKD patients with T2DM.

2 Methods

The reporting of this NMA follows the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) reporting guideline, and the PRISMA extension statement for Reporting of Systematic Reviews Incorporating Network Meta-analysis of healthcare interventions (PRISMA-NMA) (Moher et al., 2009; Hutton et al., 2015). The study is registered with PROSPERO, number CRD42024571544.

2.1 Literature review

Two investigators (J. Guo and Y. Jiang) independently searched and identified relevant studies from various databases, including Cochrane Library, PubMed, EMBASE, Web of Science, CNKI, CQVIP database, and WanFang data, from inception to November 2023. To ensure comprehensive retrieval, a combination of subject words and free words was used. Key search terms included “SGLT-2 inhibitor”, “GLP-1 receptor agonist”, “Finerenone”, “Type 2 diabetes mellitus”, “chronic kidney disease”, *etc.* The detailed search strategies for each database are described in Supplementary Appendix 2.

2.2 Study selection

This trial included double-blind RCTs comparing SGLT-2 inhibitor, GLP-1 receptor agonist, and Finerenone or directly with placebo in adults with T2DM and non-dialysis CKD. Studies using other control drugs, studies with repeated publications and incomplete data, studies with eGFR<15 mL/min/1.73 m^2, studies published in languages other than Chinese

or English, and studies using the drug within 3 months before screening were excluded.

2.3 Outcomes

The clinical outcomes assessed changes in Glycosylated hemoglobin (HbA1c), estimate glomerular filtration rate (eGFR), low-density lipoprotein cholesterol (LDL-C), systolic blood pressure (SBP), diastolic blood pressure (DBP), and body weight from baseline. Safety endpoints included any adverse events (any AE), urinary tract infections (UTI), Hypoglycemia and Acute Kidney Injury (AKI).

2.4 Data extraction

The search results were screened by two blinded independent researchers (J. Guo and Y. Jiang) according to the inclusion and exclusion criteria, and the abstracts of the remaining literatures were reviewed using EndNote20. Two researchers used standard data extraction tables for information extraction, judgment, and literature extraction information including: study author, publication year, intervention measures, outcomes, etc. In case of disagreement, a third researcher (W. Zhang) assisted in making a judgment.

2.5 Risk of bias assessment

The risk of bias assessment was conducted by 2 researchers (J. Guo and Y. Jiang) using the Cochrane Risk of Bias Assessment tool (RoB 2.0) (Sterne et al., 2019). Each study was classified as having low risk, some concerns or high risk of bias.

2.6 Statistical analysis

For outcome indicators, odds ratio (OR) was used for bicategorical variables, mean difference (MD) was used for continuous variables, and 95% confidence interval (95% CI) was used to represent statistical results. The findings were considered statistically significant if the 95%CI did not include the null value (0 for MD and 1 for OR). For each result were calculated using a random effects model. Bilateral P-values <0.05 were considered statistically significant. We evaluated the between-study heterogeneity using the I^2 statistic and its associated p-values. Specifically, I^2 values of 25%, 50%, and 75% were indicative of low, moderate, and high levels of statistical heterogeneity, respectively. Subsequently, subgroup analyses were conducted to investigate potential sources of this heterogeneity. STATA 17.0 was used for statistical analysis, evidence network and surface under the cumulative raking curve (SUCRA). We evaluated publication bias of articles using funnel plots and Egger's test. We examined the confidence of evidence using the CINeMA (Salanti et al., 2014) web application, which grades the confidence of the results as high, moderate, low, and very low.

3 Result

3.1 Characteristics of the included studies

A total of 4,929 articles were retrieved. After removing duplicates, 3,182 remained. Following the title and abstract review, 1719 were selected for full text reading. Ultimately, 39 studies involving 99,599 patients were included: 23 used SGLT-2 inhibitor, 4 used GLP-1 receptor agonist and 12 used Finerenone (Figure 1). Baseline characteristics were comparable between groups. The characteristics of the included studies are shown in Table 1. Figure 2, and Supplementary Appendix 3 shows the network. (Barnett et al., 2014; Haneda et al., 2016; Curovic et al., 2022; Yale et al., 2013; Chen, 2016; Yamout et al., 2014; Pollock et al., 2019; Cherney et al., 2016; Kohan et al., 2014; Wada et al., 2022a; Wada et al., 2022b; Takashima et al., 2018; Dagogo-Jack et al., 2021; Fioretto et al., 2018; Cherney et al., 2021; Allegretti et al., 2019; Cherney et al., 2023; Sivalingam et al., 2024; George et al., 2018; Gao, 2022; Wanner et al., 2018; Sarafidis et al., 2023; Cristian et al., 2020; Bakris et al., 2020; Bakris et al., 2015; Koya et al., 2023; Agarwal et al., 2022a; Agarwal et al., 2023; Mahaffey et al., 2019; Bhatt et al., 2021; Agarwal, et al., 2022b; Zhang et al., 2023; Rosas et al., 2023; Perakakis et al., 2024; Tuttle et al., 2022; Pitt et al., 2021; Bakris et al., 2020; Filippatos et al., 2021; Perkovic et al., 2019).

3.2 Risk of bias

The RoB 2.0 was employed to evaluate the risk of bias in the 39 included studies, among which one study was classified as "high risk", eight studies were categorized as having "some concerns", and the remaining thirty studies were deemed to have a "low risk". The distribution of each category is presented in Figure 3, while the quality assessment for each individual study can be found in Supplementary Appendix 4.

3.3 Evaluation of statistical inconsistency

The consistency test results show that the network has good consistency ($P > 0.05$), and the loop-specific method did not suggest any inconsistency between closed loops. In addition, the node-segmentation approach did not suggest statistical inconsistency for any outcome (Supplementary Appendix 5).

3.4 Outcomes

3.4.1 Glycosylated hemoglobin (HbA1c)

A total of 24 trials (37,252 patients) evaluated HbA1c (Yale et al., 2013; Chen, 2016; Gao, 2022; Barnett et al., 2014; Yamout et al., 2014; Cherney et al., 2016; Cherney et al., 2023; Curovic et al., 2022; Takashima et al., 2018; Sivalingam et al., 2024; Pollock et al., 2019; Fioretto et al., 2018; Kohan et al., 2014; Allegretti et al., 2019; Haneda et al., 2016; Wada et al., 2022a; George et al., 2018; Dagogo-Jack et al., 2021; Cherney et al., 2021; Pitt et al., 2021; Bakris George et al., 2020; Wanner et al., 2018; Perkovic et al., 2019; Wada et al., 2022b).

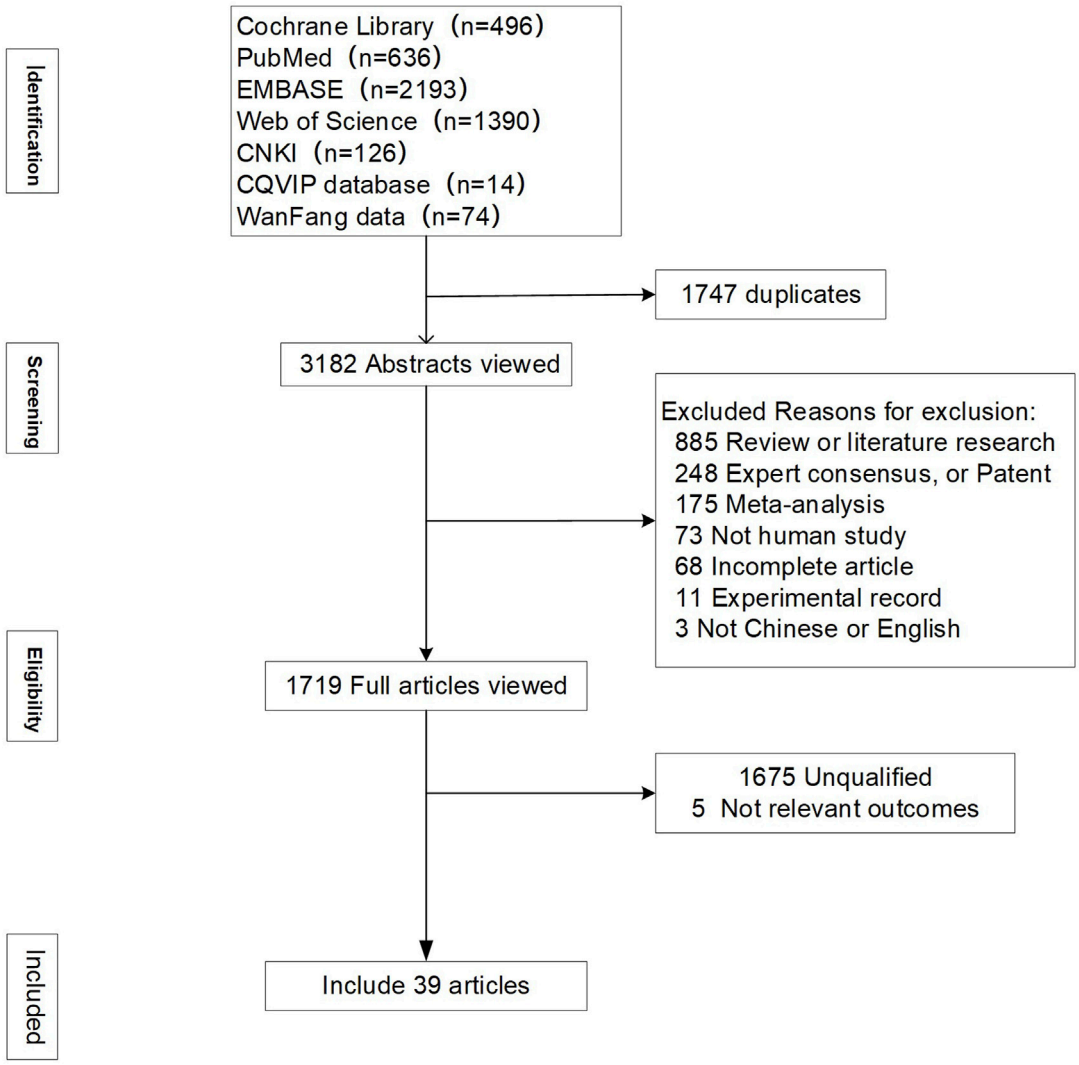


FIGURE 1
Process for identifying studies eligible for the meta-analysis.

Our NMA showed that Empagliflozin (MD = −0.33; 95%CI: −0.45, −0.22) and Canagliflozin (MD = −0.33; 95%CI: −0.52, −0.15) significantly reduced HbA1c compared to PBO group. The results of pairwise comparison showed that Empagliflozin (MD = −0.38; 95%CI: −0.62, −0.14) and Canagliflozin (MD = −0.38; 95%CI: −0.65, −0.10) were better than Finerenone (Supplementary Appendix 6a). Results from SUCRA showed that Empagliflozin was the most effective medicine for lowering HbA1c (SUCRA 73%), followed by Semaglutide (SUCRA 72%) and Canagliflozin (SUCRA 71.8%). Ertuglilozin was ranked 8th (SUCRA 43.4%) and placebo 10th (SUCRA 14.0%) (Figure 4; Supplementary Appendix 7b).

3.4.2 Estimate glomerular filtration rate (eGFR)

27 articles involving 32,360 participants assessed eGFR changes (Yale et al., 2013; Chen, 2016; Gao, 2022; Barnett et al., 2014; Yamout et al., 2014; Cherney et al., 2016; Cherney et al., 2023; Curovic et al., 2022; Takashima et al., 2018; Sivalingam et al., 2024; Pollock et al., 2019; Cristian et al., 2020; Fioretto et al., 2018; Kohan et al., 2014; Allegretti et al., 2019; Haneda et al., 2016; Sarafidis et al., 2023; Wada et al., 2022a; Bakris G. L. et al., 2020; George et al., 2018; Dagogo-Jack et al., 2021; Cherney et al., 2021; Bakris et al., 2020; Filippatos et al., 2021; Bakris et al., 2015; Perkovic et al., 2019; Wada et al., 2022a). There was no significant difference in pairwise comparison between drugs compared with PBO group (Supplementary Appendix 6b in the Supplement). Ranked according to the efficacy of SUCRA against all medicines, Semaglutide had the least damage to kidney function (SUCRA 79.5%), followed by Empagliflozin (SUCRA 79.3%). Dapagliflozin, Luseogliflozin, Bexagliflozin and Ertugliflozin (SUCRA from 24.4% to 27.2%) had the greatest impact on renal function (Figure 4; Supplementary Appendix 8b).

3.4.3 Low-density lipoprotein cholesterol (LDL-C)

Eight studies involving 4,876 participants assessed LDL-C changes (Yale et al., 2013; Chen, 2016; Barnett et al., 2014; Yamout et al., 2014; Curovic et al., 2022; Pollock et al., 2019; Haneda et al., 2016; Wanner et al., 2018). Liraglutide was superior to Canagliflozin (MD = −1.45; 95%CI: −1.87, −1.04),

TABLE 1 Basic characteristics of the included literature.

Study ID	Stage of CKD	Sample size			Age, mean, y (SD)			Treatment			Outcomes
		PBO group	Treatment group 1	Treatment group 2	PBO group	Treatment group 1	Treatment group 2	PBO group	Treatment group 1	Treatment group 2	
Yale et al. (2013)	CKD III	90	90	89	68.2 (8.4)	69.5 (8.2)	67.9 (8.2)	PBO	Cana	Cana	①②③④⑤⑥⑦⑩
Chen (2016)	CKD II CKD III	32	32					PBO	Lira		①②③④⑤⑧
Gao (2022)	Mild/ moderate CKD	26	26		60.25 (1.52)	60.32 (1.66)		PBO	Lira		①②⑧⑩
Barnett et al. (2014)	CKD II	95	98	97	62.6 (8.1)	63.2 (8.5)	62.0 (8.4)	PBO	Empa	Empa	①②③④⑤⑥⑦⑧⑩
	CKD III	187	187		65.1 (8.2)	64.6 (8.9)	64.6 (8.9)	PBO	Empa		①②③④⑤⑥⑦⑧⑩
	CKD IV	37	37		62.9 (11.9)	65.4 (10.2)	65.4 (10.2)	PBO	Empa		①②③④⑤⑥⑦⑧⑩
Rosas et al. (2023)	CKD	1,034	1,065		64.2 (9.7)			PBO	Fine		⑩
Yamout et al. (2014)	CKD IIIA	266	216	239	66.3 (7.5)	66.2 (8.0)	66.3 (6.9)	PBO	Cana	Cana	①②③④⑥⑦⑩
	CKD IIIB	116	122	126	68.4 (7.6)	69.5 (7.9)	68.1 (8.1)	PBO	Cana	Cana	①②③④⑥⑦⑩
Cherney et al. (2016)	CKD	248	388		60.4 (10.2)	58.4 (10.5)		PBO	Empa		①②④⑤⑥⑦⑧⑩
	CKD	87	128		60.5 (10.3)	59.3 (9.9)		PBO	Empa		①②④⑤⑥⑦⑧⑩
Cherney et al. (2023)	CKD III	260	263	264	69.3 (8.1)	69.6 (7.5)	69.5 (8.2)	PBO	Sota	Sota	①②④⑥⑦⑧⑩
Curovic et al. (2022)	CKD II CKD IIIA	15	17		62.3 (7.8)	63.7 (9.0)		PBO	Dapa		①②③④⑤⑥
Takashima et al. (2018)	CKD II CKD IIIA	20	20		65.4 (10.4)	64.7 (9.8)		PBO	Dapa		①②④⑤
Sivalingam et al. (2024)	CKD	30	30		69.4 (9.1)	70.5 (6.8)		PBO	Sema		①②⑥⑦⑧
Pollock et al. (2019)	CKD II CKD III	148	145		64.7 (8.5)	64.7 (8.6)		PBO	Dapa		①②③④⑦⑧⑩
Koya et al. (2023)	CKD	2,831	2,827		63 (10)			PBO	Fine		④⑥⑧⑨⑩
Cristian et al. (2020)	CKD II	1,043	772		56.9 (9.5)	56.9 (9.3)		PBO	Exen		②⑦⑨⑩
	CKD III	207	182		62.2 (9.0)	62.5 (9.0)		PBO	Exen		②⑧⑨⑩
Fioretto et al. (2018)	CKD IIIA	161	160		66.2	65.3		PBO	Dapa		①②④⑥⑦⑧⑩
Kohan et al. (2014)	CKD III	84	83	85	67 (8.6)	66 (8.9)	68 (7.7)	PBO	Dapa	Dapa	①②④⑤⑥⑦⑧⑩
Allegretti et al. (2019)	CKD III	155	157		69.9 (8.29)	69.3 (8.36)		PBO	Bexa		①②④⑥⑦⑧⑨

(Continued on following page)

10.3389/fphar.2025.1517272

TABLE 1 (*Continued*) Basic characteristics of the included literature.

Study ID	Stage of CKD	Sample size			Age, mean, y (SD)			Treatment			Outcomes
		PBO group	Treatment group 1	Treatment group 2	PBO group	Treatment group 1	Treatment group 2	PBO group	Treatment group 1	Treatment group 2	
Haneda et al. (2016)	CKD	50	95		68.4 (8.9)	67.9 (8.9)		PBO	Luse		①②③④⑤⑥⑦⑧⑩
Tuttle et al. (2022)	CKD IIIA	519	1,003		67.1 (8.1)	67.1 (7.5)		PBO	Empa		⑦⑧
	CKD IIIB	277	445		67.9 (8.2)	67.7 (8.7)		PBO	Empa		⑦⑧
	CKD IV	52	71		63.7 (10.7)	68.8 (9.1)		PBO	Empa		⑦⑧
Sarafidis et al. (2023)	CKD IV	450	440		67 (9)	67 (9)		PBO	Fine		②④⑨⑩
Mahaffey et al. (2019)	CKD	1,092	1,089		61.7 (9.4)	61.1 (9.7)		PBO	Cana		⑦⑧⑨⑩
	CKD	1,107	1,113		64.6 (8.9)	64.6 (8.2)		PBO	Cana		⑦⑧⑨⑩
Wada et al. (2022a)	CKD	154	154		62.4 (11.1)	62.5 (10.5)		PBO	Cana		①②④⑤⑦⑧⑩
Bakris et al. (2020a)	CKD IV	90	84		66 (9)	64 (10)		PBO	Cana		②⑨⑩
George et al. (2018)	CKD III	154	158	155	67.5 (8.9)	66.7 (8.3)	67.5 (8.5)	PBO	Ertu	Ertu	①②⑦⑩
Dagogo-Jack et al. (2021)	CKD III	598	618	560	68.0 (7.5)	68.3 (7.7)	68.2 (7.5)	PBO	Ertu	Ertu	①②④⑥⑦⑧⑨⑩
Cherney et al. (2021)	CKD IV	93	92	92	68.0 (8.3)	66.8 (10)	67.3 (9.6)	PBO	Sota	Sota	①②④⑥⑦⑧⑩
Bhatt et al. (2021)	CKD	5,292	5,292					PBO	Sota		⑦⑧⑩
Pitt et al. (2021)	CKD	3,666	3,686		64.1 (10)	64.1 (9.7)		PBO	Fine		①④⑥⑦⑧⑨⑩
Bakris et al. (2020b)	CKD	2,841	2,833		65.7 (9.2)	65.4 (8.9)		PBO	Fine		①②④⑥⑦⑧⑨⑩
Filippatos et al. (2021)	CKD	1,302	1,303		67.1 (8.4)	66 (8.2)		PBO	Fine		②④⑦⑧⑨⑩
	CKD	1,539	1,530		64.5 (9.6)	64.4 (9.4)		PBO	Fine		②④⑦⑧⑨⑩
Agarwal et al. (2023)	CKD II CKD III	27	92		60.8 (8.4)			PBO	Fine		④⑩
Bakris et al., (2015)	CKD II CKD III	94	727		63.26 (8.68)			PBO	Fine		②⑩
Wanner et al. (2018)	CKD I ~ IV	752	1,498		66 (8.5)	66.2 (8)		PBO	Empa		①③④⑥
Perkovic et al. (2019)	CKD II CKD III	2,199	2,202		63.2 (9.2)	62.9 (9.2)		PBO	Cana		①②④⑤⑦⑧⑨⑩
Agarwal et al. (2022a)	CKD	2,328	2,291		65.4 (9.3)	65.2 (9.0)		PBO	Fine		⑨⑩

(Continued on following page)

10.3389/fphar.2025.1517272

TABLE 1 (*Continued*) Basic characteristics of the included literature.

Study ID	Stage of CKD	Sample size			Age, mean, y (SD)			Treatment			Outcomes
		PBO group	Treatment group 1	Treatment group 2	PBO group	Treatment group 1	Treatment group 2	PBO group	Treatment group 1	Treatment group 2	
Wada et al. (2022b)	CKD II CKD III	303	301		60.9 (9.1)	60.6 (9.1)		PBO	Cana		①②④⑤⑦⑨⑩
		1,894	1,899		63.5 (9.2)	63.2 (9.1)		PBO	Cana		①②④⑤⑦⑨⑩
Zhang et al. (2023)	CKD	184	188		60.68 (10.13)	59.85 (10.16)		PBO	Fine		⑨⑩
Agarwal et al. (2022b)	CKD	6,507	6,519		64.8 (9.7)	64.7 (9.4)		PBO	Fine		④⑦⑧⑨⑩
Perakakis et al. (2024)	CKD	5,297	5,340		64.31 (9.56)	64.29 (9.27)		PBO	Fine		⑩
		1,025	1,067		61.71 (10.08)	61.94 (9.76)		PBO	Fine		⑩
		139	152		72.40 (8.02)	70.89 (7.06)		PBO	Fine		⑩

Abbreviations: ① Change in HbA1c from baseline; ② Change in eGFR from baseline; ③ Change in LDL-C from baseline; ④Change in SBP from baseline; ⑤ Change in DBP from baseline; ⑥Change in body weight from baseline; ⑦UTI; ⑧Hypoglycemia; ⑨AKI; ⑩any AE. NI, No Information. PBO: PBO group. CKD: chronic kidney disease. Cana: Canagliflozin. Lira: Liraglutide. Empa: Empagliflozin. Fine: Finerenone. Sota: Sotagliflozin. Dapa: Dapagliflozin. Sema: Semaglutide. Exen: Exenatide Beza: Bexagliflozin. Luse: Luseoglifozin. Ertu: Ertugliflozin.

Luseoglifozin (MD = −1.41; 95%CI: −2.01, −0.81), PBO (MD = −1.50; 95%CI: −1.89, −1.11), Dapagliflozin (MD = −1.58; 95%CI: −2.05, −1.10), and Empagliflozin (MD = −1.56; 95%CI: −1.96, −1.15) (Supplementary Appendix 6c) and was also the best LDL-C-lowering agent (SUCRA 100%) (Figure 4; Supplementary Appendix 9b).

3.4.4 Blood pressure (BP)

26 articles (56,627 participants) reported the changes in SBP (Yale et al., 2013; Chen, 2016; Barnett et al., 2014; Yamout et al., 2014; Cherney et al., 2016; Cherney et al., 2023; Curovic et al., 2022; Takashima et al., 2018; Filippatos et al., 2021; Agarwal et al., 2023; Wanner et al., 2018; Perkovic et al., 2019; Wada et al., 2022b; Agarwal et al., 2022a; Pollock et al., 2019; Koya et al., 2023; Fioretto et al., 2018; Kohan et al., 2014; Allegretti et al., 2019; Haneda et al., 2016; Sarafidis et al., 2023; Wada et al., 2022b; Dagogo-Jack et al., 2021; Cherney et al., 2021; Pitt et al., 2021; Bakris et al., 2020). Compared to the PBO group, Bexagliflozin (MD = −5.52; 95%CI: −8.80, −2.24), Empagliflozin (MD = −4.33; 95%CI: −5.13, −3.53), Dapagliflozin (MD = −3.79; 95%CI: −5.91, −1.66), Canagliflozin (MD = −3.16; 95%CI: −4.56, −1.75), Finerenone (MD = −1.65; 95%CI: −2.48, −0.81) and Ertugliflozin (MD = −1.50; 95%CI: −2.78, −0.23) significantly reduced SBP. Bexagliflozin and Empagliflozin were significantly superior to Finerenone (MD = −3.87; 95%CI: −7.26, −0.49) (MD = −2.69; 95%CI: −3.78, −1.59), Ertugliflozin (MD = −4.01, 95%CI: −7.53, −0.50) (MD = −2.83; 95%CI: −4.33, −1.32) and Sotagliflozin (MD = −4.98; 95%CI: −9.10, −0.87) (MD = −3.80; 95%CI: −6.41, −1.19) (Supplementary Appendix 6d). We analysed 11 articles with 11,497 participants about DBP (Yale et al., 2013; Chen, 2016; Barnett et al., 2014; Cherney et al., 2016; Curovic et al., 2022; Takashima et al., 2018; Kohan et al., 2014; Haneda et al., 2016; Wada et al., 2022a; Perkovic et al., 2019; Wada et al., 2022b). Compared to PBO group, Empagliflozin (MD = −1.86; 95%CI: −3.18, −40.54) significantly reduced DBP (Supplementary Appendix 6e). The SUCRA results showed that Bexagliflozin (SUCRA 89.6%) and Empagliflozin (SUCRA 82.2%) ranked first and second in reducing SBP. Empagliflozin had the best effect on DBP reduction (SUCRA 72.6%). PBO was last in reducing both SBP and DBP (SUCRA 12.1% and 21.4%) (Figure 4; Supplementary Appendix 10b, 11b).

3.4.5 Body weight

A total of 17 trials (27,839 participants) evaluated body weight (Wada et al., 2022a; Barnett et al., 2014; Yamout et al., 2014; Cherney et al., 2016; Cherney et al., 2023; Curovic et al., 2022; Sivalingam et al., 2024; Koya et al., 2023; Fioretto et al., 2018; Kohan et al., 2014; Allegretti et al., 2019; Haneda et al., 2016; Dagogo-Jack et al., 2021; Cherney et al., 2021; Pitt et al., 2021; Bakris et al., 2020; Wanner et al., 2018). Compared to PBO group, Canagliflozin (MD = −3.81, 95%CI: −6.34, −1.27), Ertugliflozin (MD = −2.36, 95%CI: −3.87, −0.84) and Empagliflozin (MD = −1.29, 95%CI: −1.42, −1.16) significantly reduced body weight. The therapeutic effect of Canagliflozin was significantly better than that of Sotagliflozin (MD = −3.55, 95%CI: −6.75, −0.34), Finerenone (MD = −3.94, 95%CI: −6.73, −1.16), and Dapagliflozin (MD = −6.44, 95%CI: −10.52, −2.37). The therapeutic efficacy of Ertugliflozin (MD = −2.50; 95%CI: −4.40, −0.59) (MD = −4.99; 95% CI: −8.53, −1.46) and Empagliflozin (MD = −1.43; 95%CI: −2.58, −0.28)

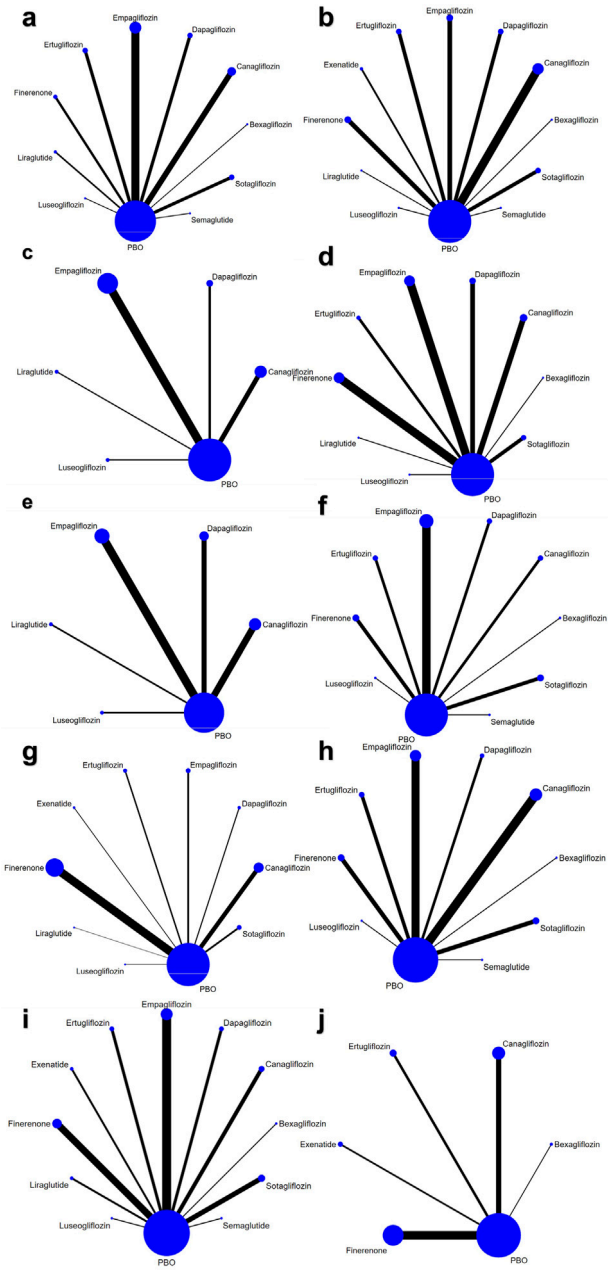


FIGURE 2
Network of eligible treatment comparisons for **(a)** HbA1c, **(b)** eGFR **(c)** LDL-C, **(d)** SBP, **(e)** DBP, **(f)** Body Weight, **(g)** any AE, **(h)** UTI, **(i)** Hypoglycemia, **(j)** AKI.

(MD = −3.93; 95%CI: −7.12, −0.74) was significantly superior to that of Finerenone and Dapagliflozin (Supplementary Appendix 6f). When analyzed in combination with SUCRA, Canagliflozin (SUCRA 90.2%) had the best treatment effect. The least effective for weight loss was Dapagliflozin (SUCRA 4.8%) (Figure 4; Supplementary Appendix 12b).

3.4.6 Adverse events

We analyzed any AE, UTI, Hypoglycemia and AKI.

A total of 31 articles involving 92,867 subjects were included to evaluate the occurrence of any AE (Yale et al., 2013; Gao, 2022; Barnett et al., 2014; Rosas et al., 2023; Yamout et al., 2014; Cherney et al., 2016; Pollock et al., 2019; Koya et al., 2023; Cristian et al., 2020; Fioretto et al., 2018; Kohan et al., 2014; Haneda et al., 2016; Sarafidis et al., 2023; Mahaffey et al., 2019; Wada et al., 2022a; Bakris et al., 2020; George et al., 2018; Dagogo-Jack et al., 2021; Cherney et al., 2021; Bhatt et al., 2021; Pitt et al., 2021; Bakris et al., 2020; Filippatos et al., 2021; Agarwal et al., 2023; Bakris et al., 2015; Perkovic et al., 2019; Agarwal, et al., 2022b; Wada et al., 2022a; Zhang et al., 2023; Agarwal et al., 2022a; Perakakis et al., 2024). Compared to PBO group, Exenatide (OR = 0.79; 95%CI: 0.66, 0.95) showed greater risk. Canagliflozin was safer than PBO group, Sotagliflozin, Finerenone and Exenatide (OR from 1.16 to 1.51; 95%CI from 1.04 to 1.83). Compared with Exenatide, Sotagliflozin, Finerenone and Empagliflozin showed better safety (OR from 0.77 to 1.54; 95%CI from 0.63 to 2.09) (Supplementary Appendix 6g). The SUCRA analysis indicated that Canagliflozin was probably the drug with the best safety profile (SUCRA 84.2%), followed by Empagliflozin (SUCRA 83.3%). Exenatide had the worst safety profile (SUCRA 11.6%) (Figure 4; Supplementary Appendix 13b).

A total of 25 trials (67,632 participants) evaluated UTI (Yale et al., 2013; Barnett et al., 2014; Yamout et al., 2014; Cherney et al., 2016; Cherney et al., 2023; Sivalingam et al., 2024; Pollock et al., 2019; Cristian et al., 2020; Fioretto et al., 2018; Kohan et al., 2014; Allegretti et al., 2019; Haneda et al., 2016; Tuttle et al., 2022; Mahaffey et al., 2019; Wada et al., 2022b; George et al., 2018; Dagogo-Jack et al., 2021; Bhatt et al., 2021; Pitt et al., 2021; Bakris et al., 2020; Filippatos et al., 2021; Perkovic et al., 2019; Wada et al., 2022a; Agarwal et al., 2022b; Cherney et al., 2021) (Supplementary Appendix 6h in the Supplement). In terms of the occurrence of UTI, we found that Canagliflozin seemed to exhibit a worse safety profile compared with PBO group (OR = 0.89; 95%CI: 0.80, 0.99). There were no significant differences between other drugs in pairwise comparisons. Finerenone was the safest in the occurrence of UTI (SUCRA 74.8%), followed by Dapagliflozin and Empagliflozin (SUCRA 70.3% and 69.0%). We also found that Semaglutide (SUCRA 19.7%) was probably the most likely treatment to cause UTI to occur (Figure 4; Supplementary Appendix 14b).

The occurrence of Hypoglycemia was assessed in 24 studies involving 65,498 participants (Chen, 2016; Gao, 2022; Barnett et al., 2014; Cherney et al., 2016; Cherney et al., 2023; Sivalingam et al., 2024; Pollock et al., 2019; Koya et al., 2023; Cristian et al., 2020; Fioretto et al., 2018; Kohan et al., 2014; Allegretti et al., 2019; Haneda et al., 2016; Tuttle et al., 2022; Mahaffey et al., 2019; Wada et al., 2022b; Dagogo-Jack et al., 2021; Cherney et al., 2021; Bhatt et al., 2021; Pitt et al., 2021; Bakris et al., 2020; Filippatos et al., 2021; Perkovic et al., 2019; Agarwal et al., 2022a). (Supplementary Appendix 6i). Finerenone (OR = 1.18; 95%CI: 1.07, 1.31), Empagliflozin (OR = 1.13; 95%CI: 1.01, 1.27) were better than PBO group in reducing the incidence of Hypoglycemia. There were no significant difference between other drugs. In our analysis, Finerenone (SUCRA 65.5%) had a better safety than Empagliflozin (SUCRA 55.8%). Liraglutide (SUCRA 17.2%) may be a risk factor for Hypoglycemia. The safety of Luseogliflozin (SUCRA 73.7%) was probably optimal (Figure 4; Supplementary Appendix 15b).

60,930 participants involved in 15 articles were assessed for the occurrence of AKI (Koya et al., 2023; Cristian et al., 2020; Allegretti et al., 2019; Sarafidis et al., 2023; Mahaffey et al., 2019; Bakris et al.,

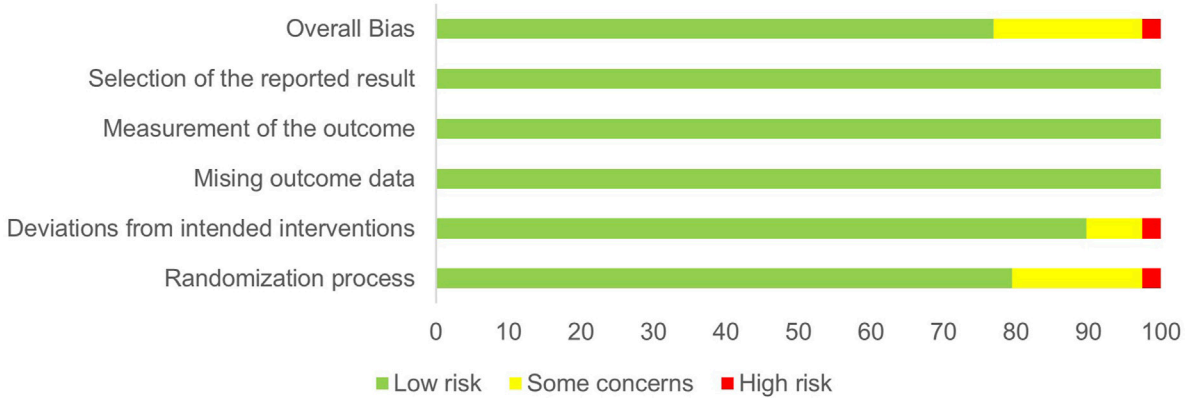


FIGURE 3
Risks of bias.

2020; Dagogo-Jack et al., 2021; Pitt et al., 2021; Bakris et al., 2020; Filippatos et al., 2021; Perkovic et al., 2019; Agarwal et al., 2022b; Wada et al., 2022a; Zhang et al., 2023; Agarwal et al., 2022a). There were no significant difference in pairwise comparison between drugs compared with PBO group (Supplementary Appendix 6j in the Supplement). Combined with SUCRA analysis, Ertugliflozin was the medicines with the fewest AKI (SUCRA 72.5%), significantly better than Canagliflozin (SUCRA 69.7%), which ranked second. Bexagliflozin had the highest likelihood of AKI (SUCRA 29.4%) (Figure 4; Supplementary Appendix 16b).

3.5 Additional analyses

Assessment of outcome measures showed that 100% of the evidence was rated as low or very low (Supplementary Appendix 20). The funnel plot and Egger's test indicated publication bias for eGFR (P = 0.007) (Supplementary Appendix 17). Heterogeneity was observed in HbA1c, body weight, and eGFR. Subgroup analyses suggested that study country, medication timing, numbers of participants and drug category may contribute to this heterogeneity (Supplementary Appendix 21).

4 Discussion

4.1 Principal findings

SGLT-2 inhibitors, GLP-1 receptor agonists, and Finereone are recommended as preferred pharmacotherapies for T2DM patients with CKD; however, the guidelines do not specify a clear hierarchy of preference among these options (Chinese Diabetes Society, 2025; American Diabetes Association Professional Practice Committee, 2023). Given the widespread clinical application of these drugs, we assert that comparing their clinical efficacy holds significant importance. Therefore, in the absence of direct comparative evidence, we conducted an indirect comparison to determine the clinical efficacy and safety of SGLT-2 inhibitors, GLP-1 receptor agonists and Finerenone in patients with T2DM and non-dialysis CKD. The study has identified several significant findings. The drug Semaglutide (a GLP-1 receptor agonist) appears to be the optimal choice among all drug classes for patients with high HbA1c and poor eGFR, when compared to the other two drugs. The efficacy of SGLT-2 inhibitors and GLP-1 receptor agonists in reducing body weight and HbA1c surpasses that of Finerenone. Canagliflozin, Empagliflozin (SGLT-2 inhibitors), and Semaglutide are considered the most suitable medications for patients with high body weight and elevated HbA1c levels, specially Canagliflozin. Specifically, Empagliflozin, Dapagliflozin and Canagliflozin (SGLT-2 inhibitors) demonstrate remarkable effectiveness in managing high SBP. The use of Empagliflozin and Canagliflozin may be considered for patients presenting with high DBP as the primary accompanying symptom. Liraglutide (a GLP-1 receptor agonist) may be prioritized for patients with elevated LDL-C levels. Although there is no obvious advantage in reducing the indicators and related risk factors in patients with T2DM with non-dialysis CKD, Finerenone has less damage to eGFR, and has a convincing reduction in the occurrence of Hypoglycemia events, and the incidence of adverse events is low, so it can still be used as the first choice for some patients.

The efficacy and safety of these drugs have been extensively deliberated. In an NMA comprising 816 randomized controlled trials with 471,038 participants, SGLT-2 inhibitors, GLP-1 receptor agonists, and Finerenone exhibited favorable outcomes in terms of reducing all-cause mortality and enhancing cardiorenal results among patients diagnosed with T2DM (Shi et al., 2023). 3 studies confirmed that SGLT-2 inhibitors have a positive effect on cardio-renal outcomes in T2DM patients with CKD compared with GLP-1 receptor agonists or Finerenone (Yamada et al., 2021; Nguyen et al., 2023; Apperloo et al., 2024). However, these studies either focused solely on T2DM patients or had a limited number of RCTs and study indicators, leaving the impact of SGLT-2 inhibitors, GLP-1 receptor agonists, and Finerenone on non-dialysis CKD patients with T2DM unclear. Therefore, we designed an NMA to evaluate the effects of several drugs on various clinically accessible indicators.

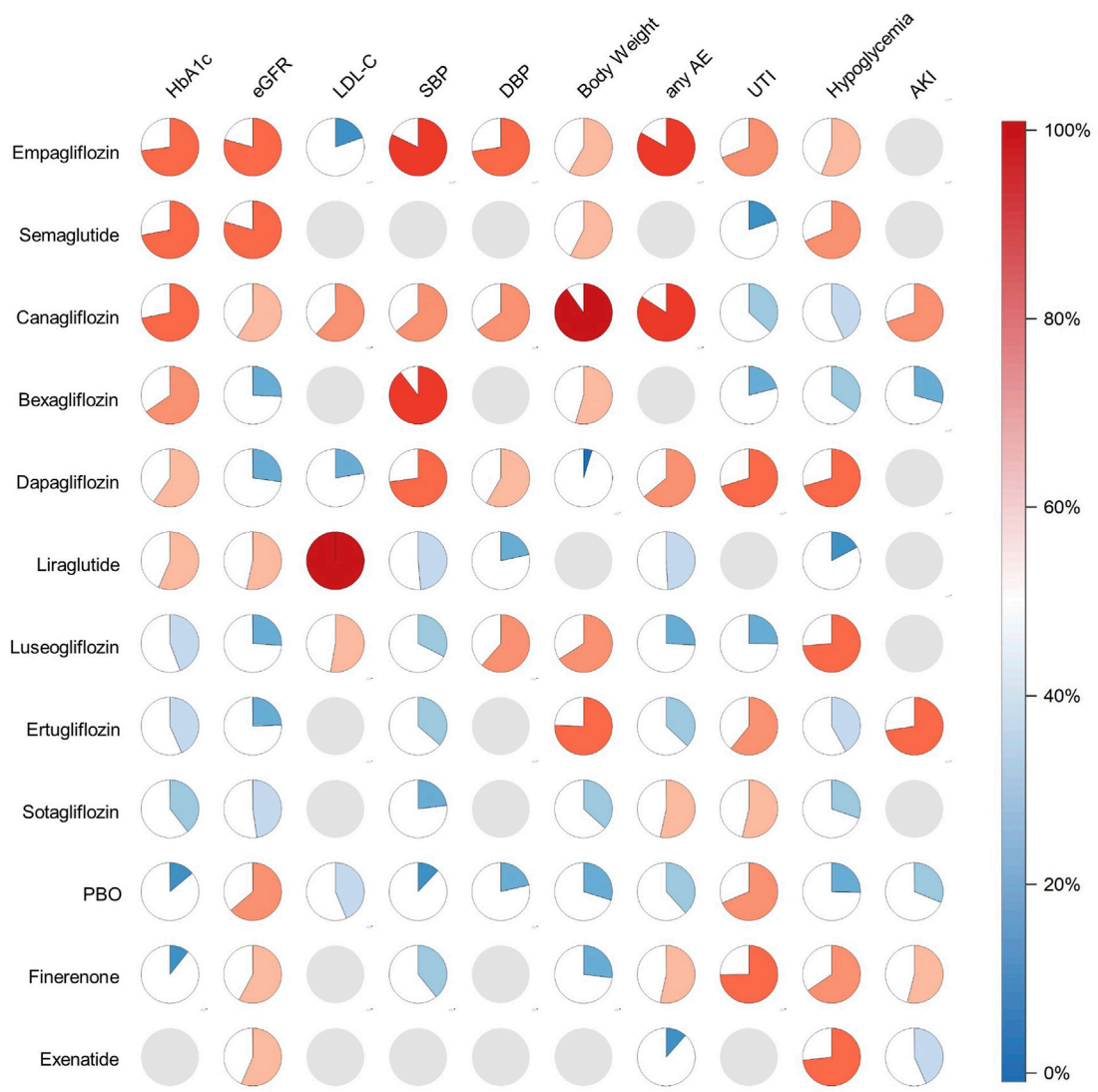


FIGURE 4
Pie charts of SUCRA value. Filling proportion and color: SUCRA value. Gray pie: not measured.

Benefits of most SGLT-2 inhibitors include renal safety, reduction in HbA1c, blood pressure, and body weight. Hyperglycemia is linked to an increase in eGFR as compensation (Thomas, 2014). Hypertension and overweight increase the risk of T2DM. Therefore, the optimal antidiabetic drug should not only have good glucose-lowering ability, but also be beneficial for body weight, blood pressure, and renal function (Leehey et al., 2015; Garvey, 2022; American Diabetes Association, 2020). The renal protective mechanism of SGLT-2 inhibitors may be dominated by direct effects on renal vessels. Empagliflozin decreases proximal tubular sodium reabsorption, increases distal sodium delivery to the macula densa, and activates glomerular feedback. This process reduces hyperfiltration and regulates vascularization (Wanner, 2017). In addition, SGLT-2 inhibitors can directly act on the kidney, reduce renal fibrosis by inhibiting oxidative stress in the kidney, (Woods et al., 2019), attenuate the increase of angiotensinogen, and reduce NLRP3 inflammasome activity (Yaribeygi et al., 2019), these mechanisms may explain how SGLT-2 inhibitors reduce blood pressure. The short-term use of SGLT-2 inhibitors may temporarily decrease eGFR, but it will gradually recover, indicating a long-term protective effect on eGFR (Barnett et al., 2014). This may be due to reduced uric acid, inhibited inflammatory response, and decreased vascular stiffness (Heerspink et al., 2016; Chilton et al., 2015). Although previous studies have suggested that angiotensin converting enzyme inhibitors (ACEI) or angiotensin II receptor blocker (ARB) are the preferred antihypertensive drugs for T2DM patients with CKD, they do not significantly reduce blood glucose levels and their effects remain limited (Bakris et al., 2000). The potential of using SGLT-2 inhibitors as the first choice after ACEI and ARB drugs, considering its high cardio-renal safety and hypoglycemic function, needs further discussion.

Unexpectedly, we found Canagliflozin, an SGLT-2 inhibitor agent, to be the most effective for weight reduction (SUCRA = 90.2%), superior to GLP-1 receptor agonists. The reason for this may be due to different principles of action. The hormone GLP-1,

produced in cells lining the intestines, slows down digestion and reduces food intake by inhibiting neural activity in the brain (Drucker, 2016). SGLT-2 inhibitors can also lead to weight loss depending on the dosage, as excess glucose is eliminated from the body (Brown et al., 2019). At the same time, it can also accelerate fat burning by lipolysis and fatty acid oxidation (Vallon et al., 2017). Therefore, it can be hypothesized that the progressive increase in blood glucose levels and concurrent decrease in body weight may account for the superior weight reduction observed with SGLT-2 inhibitors compared to GLP-1 receptor agonists.

GLP-1 receptor agonists, especially Semaglutide and Liraglutide, showed the greatest advantage in reducing LDL-C (SUCRA = 100%) and HbA1c (SUCRA = 72.0% and 56.4%), and had a higher safety profile against eGFR (SUCRA = 79.5% and 53.4%). The management of LDL-C is crucial in preventing cardiovascular events caused by atherosclerotic plaque formation (Giglio et al., 2021). The existing NMA highlights the remarkable cardiovascular benefits of GLP-1 receptor agonists (Zhang Y. et al., 2022). However, our study suggests that these advantages may be attributed to specific pathways that lower LDL-C and reduce lipid deposition in the cardiovascular system. Nevertheless, further testing is required to confirm these pathways.

Finerenone is a novel non-steroidal mineralocorticoid receptor antagonist (MRA), and its safety in cardiorenal prognosis has been proved (Agarwal et al., 2022b; Zhang M-Z. et al., 2022), this may be due to the reduction of proteinuria and tissue inflammation and fibrosis (Wish and Pablo, 2022). Our study confirmed Finerenone's safety and highlighted its significant advantage in reducing SBP, while showing no notable effect on blood glucose reduction. The combination of Finerenone with SGLT-2 inhibitors or GLP-1 receptor agonists have been suggested to enhance anti-inflammatory, anti-oxidative stress, and endothelial protection effects (Lv et al., 2023). At the same time, SGLT-2 inhibitors inhibit sodium reabsorption, while Finerenone promotes sodium retention (Lv et al., 2023; Kidokoro et al., 2019). Whether the pathways of SGLT-2 inhibitors and Finerenone partially coincide, lead to water and salt metabolism disorders, and increase additional adverse reactions still needs more clinical trials to prove its safety.

Certain limitations should be noted when interpreting our study. The results of our study indicate a higher risk of urinary tract infections in patients treated with GLP-1 receptor agonists compared to those receiving SGLT-2 inhibitors therapy, contradicting previous research findings (Tanrıverdi et al., 2023; Drucker, 2016). The effect of GLP-1 receptor agonists on UTI occurrence was only reported in one literature, which included a small number of patients and specified clear inclusion criteria as "T2DM and albuminuria" (Sivalingam et al., 2024). The presence of proteinuria may influence UTI incidence. The eGFR safety profile of most GLP-1 receptor agonists are good, except for Exenatide. From April 2005 to August 2008, 78 cases of renal disease caused by Exenatide were reported to the FDA. Gastrointestinal adverse reactions are the most common side effects of GLP-1 receptor agonists, which can cause significant fluid loss and pre-renal acute failure (Bzowyckyj, 2020). At the same time, GLP-1 receptor agonists can simultaneously enhance renal sodium efficacy, leading to renal hypoperfusion and AKI development (Skov et al., 2013). Interestingly, our study does not support a higher likelihood of Exenatide causing eGFR damage (SUCRA = 56.4%), possibly due to its ability to reduce renal pathological material deposition. Animal experiments have demonstrated that Exenatide reduces inflammatory and apoptotic cell infiltration in the glomerulus of mice, as well as lipid content (Park et al., 2007). Activation of the GLP-1 receptor stimulates adenylyl cyclase, leading to increased cAMP (a key mediator of GLP-1-induced insulin secretion) production (Fujita et al., 2014). In conclusion, Exenatide remains a controversial drug and should be minimized in patients with severe renal insufficiency.

4.2 Strengths and limitations

The advantages of this network meta-analysis are as follows: first, we included a large number of literatures with a large sample size and reliable data; secondly, on top of the existing analysis, we provided additional evidence to specifically analyze the advantages and disadvantages of different SGLT-2 inhibitors and GLP-1 receptor agonists and Finerenone in the treatment of T2DM patients with non-dialysis CKD to facilitate clinical decision making by physicians. Last but not least, the ADA recommends that individuals with T2DM and CKD should use SGLT-2 inhibitors or GLP-1 receptor agonists as their first choice, but it does not specify which medicine to use for patients with different clinical priorities (Committee, 2024). Our study identified the efficacy of both drugs in improving HbA1c and eGFR, while also providing more favorable evidence regarding the benefits and limitations of various drugs in other indicators such as LDL-C, SBP, DBP, as well as the safety profile of Finerenone.

Limitations of our NMA are largely driven by the available evidence. Firstly, it is acknowledged that the heterogeneity and inherent bias within the literature are objective realities, which may potentially compromise the accuracy of research outcomes. For instance, several included trials lacked baseline data, and variations existed in population characteristics, duration of pharmacological treatment, and follow-up periods. This may have led to some bias. Third, the dose of the study drug and the level of detail in our study were not considered. Finally, the literatures included in this paper provide only indirect comparisons between drugs, lacking direct comparison evidence, which affects the credibility of the results. More detailed studies are needed to supplement this aspect in the future.

5 Conclusion

In conclusion, this network meta-analysis provides compelling evidence regarding the effects of SGLT-2 inhibitors, GLP-1 receptor agonists, and Finerenone on patients with T2DM complicated by non-dialysis CKD. Based on robust evidence from indirect comparisons, the safety profile of Finerenone in patients with T2DM and non-dialysis. CKD outweighs its limited efficacy in improving HbA1c levels, while demonstrating an added advantage of reducing SBP. The GLP-1 receptor agonists are beneficial for T2DM patients with non-dialysis CKD, effectively reducing HbA1c, LDL-C, and body weight without significantly impacting renal function, expect Exenatide. The most recommended treatment for patients with T2DM and non-dialysis CKD, along

with high levels of HbA1c, SBP, and DBP, may be Empagliflozin and Canagliflozin, as they have a lesser impact on eGFR. Dapagliflozin demonstrated lower efficacy in reducing HbA1c compared to the aforementioned medications; however, its effect on body weight reduction was less significant, and it had a lower likelihood of causing Hypoglycemia. Except for Ertugliflozin, Luseogliflozin, and Sotagliflozin, other SGLT-2 inhibitors medications demonstrated a superior impact on reducing SBP compared to Finerenone; however, they also entailed an elevated risk of UTI. Our NMA enriches the existing body of research by providing substantial evidence to assess the benefits and risks associated with various drugs. In summary, it is imperative to consider the potential adverse effects of these treatments when formulating personalized treatment plans for individual patients.

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

JG: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Software, Supervision, Validation, Writing–original draft, Writing–review and editing. MW: Conceptualization, Methodology, Validation, Writing–review and editing. WZ: Methodology, Writing–review and editing. YJ: Data curation, Investigation, Writing–review and editing. AL: Writing–review and editing, Software. CW: Writing–review and editing, Software. DY: Writing–review and editing, Data curation. AS: Writing–review and editing, Data curation. YG: Conceptualization, Funding acquisition, Supervision, Writing–original draft, Writing–review and editing, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources.

Funding

The author(s) declare that financial support was received for the research and/or publication of this article. The authors acknowledge support from the National Administration of Traditional Chinese Medicine Young Qi Huang Scholars support project (National Traditional Chinese Medicine Human Education Development (2020) No. 7), the Leading Talent Training Program Project of Dongzhimen Hospital of Beijing University of Chinese Medicine(No. DZMG-LJRC0004), the Fundamental Research Funds for the Central Universities (2023-JYB-JBZD-010), Postdoctoral Fellowship Program of China Postdoctoral Science Foundation (GZC20230324) and China Postdoctoral Science Foundation (2024M750263).

Acknowledgments

The authors thank Dongzhimen Hospital and Beijing University of Chinese Medicine for its support of this work and the reviewers for allowing the authors to improve the manuscript.

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 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.2025.1517272/full#supplementary-material

References

Afkarian, M., Zelnick, L. R., Hall, Y. N., Heagerty, P. J., Tuttle, K., Weiss, N. S., et al. (2016). Clinical manifestations of kidney disease among US adults with diabetes, 1988-2014. *JAMA - J. Am. Med. Assoc.* 316 (6), 602–610. doi:10.1001/jama.2016.10924

Agarwal, R., Anker, S. D., Filippatos, G., Pitt, B., Rossing, P., Ruilope, L. M., et al. (2022a). Effects of Canagliflozin versus Finerenone on cardiorenal outcomes: exploratory *post hoc* analyses from FIDELIO-DKD compared to reported CREDENCE results. *Nephrol. Dial. Transplant.* 37 (7), 1261–1269. doi:10.1093/ndt/gfab336

Agarwal, R., Filippatos, G., Pitt, B., Anker, S. D., Rossing, P., Joseph, A., et al. (2022b). Cardiovascular and kidney outcomes with Finerenone in patients with type 2 diabetes and chronic kidney disease: the FIDELITY pooled analysis. *Eur. Heart J.* 43 (6), 474–484A. doi:10.1093/eurheartj/ehab777

Agarwal, R., Ruilope, L. M., Ruiz-Hurtado, G., Haller, H., Schmieder, R. E., Anker, S. D., et al. (2023). Effect of Finerenone on ambulatory blood pressure in chronic kidney disease in type 2 diabetes. *J. Hypertens.* 41 (2), 295–302. doi:10.1097/HJH.0000000000003330

Allegretti, A. S., Zhang, W., Zhou, W., Thurber, T. K., Rigby, S. P., Bowman-Stroud, C., et al. (2019). Safety and effectiveness of Bexagliflozin in patients with type 2 diabetes mellitus and stage 3a/3b CKD. *Am. J. Kidney Dis.* 74 (3), 328–337. doi:10.1053/j.ajkd.2019.03.417

American Diabetes Association Professional Practice Committee (2023). 11. Chronic kidney disease and risk management: standards of care in diabetes—2024. *Diabetes Care* 47 (Suppl. 1), S219–S230. doi:10.2337/dc24-S011

Apperloo, E. M., Neuen, B. L., Fletcher, R. A., Jongs, N., Anker, S. D., Bhatt, D. L., et al. (2024). Efficacy and safety of SGLT2 inhibitors with and without glucagon-like peptide 1 receptor agonists: a smart-C collaborative meta-analysis of randomised controlled trials. *Lancet Diabetes and Endocrinol.* 12 (8), 545–557. doi:10.1016/S2213-8587(24)00155-4

American Diabetes Association (2020). 2. Classification and diagnosis of diabetes: standards of medical care in diabetes—2021. *Diabetes Care* 44 (Suppl. 1), S15–S33. doi:10.2337/dc21-S002

Bakris, G., Oshima, M., Mahaffey, K. W., Agarwal, R., Cannon, C. P., Capuano, G., et al. (2020b). Effects of Canagliflozin in patients with baseline EGFR <30 Ml/min per 1.73 M2: subgroup analysis of the randomized CREDENCE trial. *Clin. J. Am. Soc. Nephrol.* 15 (12), 1705–1714. doi:10.2215/CJN.10140620

Bakris, G. L., Agarwal, R., Anker, S. D., Pitt, B., Ruilope, L. M., Rossing, P., et al. (2020a). Effect of Finerenone on chronic kidney disease outcomes in type 2 diabetes. *N. Engl. J. Med.* 383 (23), 2219–2229. doi:10.1056/nejmoa2025845

Bakris, G. L., Agarwal, R., Chan, J. C., Cooper, M. E., Gansevoort, R. T., Haller, H., et al. (2015). Effect of Finerenone on albuminuria in patients with diabetic nephropathy a randomized clinical trial. *JAMA - J. Am. Med. Assoc.* 314 (9), 884–894. doi:10.1001/jama.2015.10081

Bakris, G. L., Williams, M., Dworkin, L., Elliott, W. J., Epstein, M., Toto, R., et al. (2000). Preserving renal function in adults with hypertension and diabetes: a consensus approach. National kidney foundation hypertension and diabetes executive committees working group. *Am. J. Kidney Dis.* 36 (3), 646–661. doi:10.1053/ajkd.2000.16225

Barnett, A. H., Mithal, A., Manassie, J., Jones, R., Rattunde, H., Woerle, H. J., et al. (2014). Efficacy and safety of Empagliflozin added to existing antidiabetes treatment in patients with type 2 diabetes and chronic kidney disease: a randomised, double-blind, placebo-controlled trial. *Lancet Diabetes Endocrinol.* 2 (5), 369–384. doi:10.1016/S2213-8587(13)70208-0

Bhatt, D. L., Szarek, M., Pitt, B., Cannon, C. P., Leiter, L. A., McGuire, D. K., et al. (2021). Sotagliflozin in patients with diabetes and chronic kidney disease. *N. Engl. J. Med.* 384 (2), 129–139. doi:10.1056/nejmoa2030186

Brown, E., Rajeev, S. P., Cuthbertson, D. J., and Wilding, J. P. H. (2019). A review of the mechanism of action, metabolic profile and haemodynamic effects of sodium-glucose Co-transporter-2 inhibitors. *Diabetes, Obes. Metabolism* 21 (S2), 9–18. doi:10.1111/dom.13650

Bzowyckyj, A. (2020). Managing the multifaceted nature of type 2 diabetes using once-weekly injectable GLP-1 receptor agonist therapy. *J. Clin. Pharm. Ther.* 45 (S1), 7–16. doi:10.1111/jcpt.13229

Chen, Z. (2016). *To analyze the efficacy and safety of liraglutide in the treatment of type 2 diabetes mellitus with mild to moderate chronic kidney disease*.

Cherney, D., Lund, S. S., Perkins, B. A., Henrik Groop, P., Cooper, M. E., Kaspers, S., et al. (2016). The effect of sodium glucose cotransporter 2 inhibition with Empagliflozin on microalbuminuria and macroalbuminuria in patients with type 2 diabetes. *Diabetologia* 59 (9), 1860–1870. doi:10.1007/s00125-016-4008-2

Cherney, D. Z. I., Ferrannini, E., Umpierrez, G. E., Peters, A. L., Rosenstock, J., Carroll, A. K., et al. (2021). Efficacy and safety of Sotagliflozin in patients with type 2 diabetes and severe renal impairment. *Diabetes, Obes. Metabolism* 23 (12), 2632–2642. doi:10.1111/dom.14513

Cherney, D. Z. I., Ferrannini, E., Umpierrez, G. E., Peters, A. L., Rosenstock, J., Powell, D. R., et al. (2023). Efficacy and safety of Sotagliflozin in patients with type 2 diabetes and stage 3 chronic kidney disease. *Diabetes, Obes. Metabolism* 25 (6), 1646–1657. doi:10.1111/dom.15019

Chilton, R., Tikkanen, I., Cannon, C. P., Crowe, S., Woerle, H. J., Broedl, U. C., et al. (2015). Effects of Empagliflozin on blood pressure and markers of arterial stiffness and vascular resistance in patients with type 2 diabetes. *Diabetes, Obes. Metabolism* 17 (12), 1180–1193. doi:10.1111/dom.12572

Chinese Diabetes Society (2025). Guideline for the prevention and treatment of diabetes mellitus in China (2024 edition). *Chin. J. Diabetes Mellitus* 17 (1), 16–139. doi:10.3760/cma.j.cn115791-20241203-00705

Cristian, G., Juan, P. F., Lisa, S., Elise, H., Galina, M., and Sjöström, C. D. (2020). Safety and efficacy of exenatide once weekly in participants with type 2 diabetes and stage 2/3 chronic kidney disease. *Diabetes Ther.* 11 (12), 3011–3013. doi:10.6084/m9.figshare.12059103

Curovic, V. R., Eickhoff, M. K., Rönkkä, T., Frimodt-Møller, M., Hansen, T. W., Mischak, H., et al. (2022). Dapagliflozin improves the urinary proteomic kidney-risk classifier CKD273 in type 2 diabetes with albuminuria: a randomized clinical trial. *Diabetes Care* 45 (11), 2662–2668. doi:10.2337/dc22-1157

Dagogo-Jack, S., Pratley, R. E., Cherney, D. Z. I., McGuire, D. K., Cosentino, F., Shih, W. J., et al. (2021). Glycemic efficacy and safety of the SGLT2 inhibitor Ertugliflozin in patients with type 2 diabetes and stage 3 chronic kidney disease: an analysis from the VERTIS CV randomized trial. *BMJ Open Diabetes Res. Care* 9 (1), e002484. doi:10.1136/bmjdrc-2021-002484

Drucker, D. J. (2016). The cardiovascular biology of glucagon-like peptide-1. *Cell Metab.* 24 (1), 15–30. doi:10.1016/j.cmet.2016.06.009

Filippatos, G., Anker, S. D., Agarwal, R., Pitt, B., Ruilope, L. M., Rossing, P., et al. (2021). Finerenone and cardiovascular outcomes in patients with chronic kidney disease and type 2 diabetes. *Circulation* 143 (6), 540–552. doi:10.1161/CIRCULATIONAHA.120.051898

Filippatos, T. D., and Elisaf, M. S. (2013). Effects of glucagon-like peptide-1 receptor agonists on renal function. *World J. Diabetes* 4 (5), 190–201. doi:10.4239/wjd.v4.i5.190

Fioretto, P., Del Prato, S., Buse, J. B., Goldenberg, R., Giorgino, F., Reyner, D., et al. (2018). Efficacy and safety of Dapagliflozin in patients with type 2 diabetes and moderate renal impairment (chronic kidney disease stage 3A): the DERIVE study. *Diabetes, Obes. Metabolism* 20 (11), 2532–2540. doi:10.1111/dom.13413

Fujita, H., Morii, T., Fujishima, H., Sato, T., Shimizu, T., Hosoba, M., et al. (2014). The protective roles of GLP-1R signaling in diabetic nephropathy: possible mechanism and therapeutic potential. *Kidney Int.* 85 (3), 579–589. doi:10.1038/ki.2013.427

Gao, W. (2022). *To observe the clinical effect of liraglutide in the treatment of type 2 diabetes mellitus with mild to moderate chronic kidney disease*, 162–164.

Garvey, W. T. (2022). New horizons. A new paradigm for treating to target with second-generation obesity medications. *J. Clin. Endocrinol. Metabolism* 107 (4), e1339–e1347. doi:10.1210/clinem/dgab848

George, G., Sarah, C., Jeremy, J., Susan, H., Steven, G. T., James, P. M., et al. (2018). Ertugliflozin in patients with stage 3 chronic kidney disease and type 2 diabetes mellitus: the VERTIS RENAL randomized study. *Diabetes Ther.* 9 (1), 49–66. doi:10.1007/s13300-017-0337-5

Giglio, R. V., Stoian, A. P., Al-Rasadi, K., Banach, M., Patti, A. M., Ciaccio, M., et al. (2021). Novel therapeutical approaches to managing atherosclerotic risk. *Int. J. Mol. Sci.* 22 (9), 4633. doi:10.3390/ijms22094633

Haneda, M., Seino, Y., Inagaki, N., Kaku, K., Sasaki, T., Fukatsu, A., et al. (2016). Influence of renal function on the 52-week efficacy and safety of the sodium glucose cotransporter 2 inhibitor Luseogliflozin in Japanese patients with type 2 diabetes mellitus. *Clin. Ther.* 38 (1), 66–88.e20. doi:10.1016/j.clinthera.2015.10.025

Heerspink, H. J. L., Perkins, B. A., Fitchett, D. H., Husain, M., and Cherney, D. Z. I. (2016). Sodium glucose cotransporter 2 inhibitors in the treatment of diabetes mellitus: cardiovascular and kidney effects, potential mechanisms, and clinical applications. *Circulation* 134 (10), 752–772. doi:10.1161/CIRCULATIONAHA.116.021887

Hutton, B., Salanti, G., Caldwell, D. M., Chaimani, A., Schmid, C. H., Cameron, C., et al. (2015). The PRISMA extension statement for reporting of systematic reviews incorporating network meta-analyses of health care interventions: checklist and explanations. *Ann. Intern. Med.* 162 (11), 777–784. doi:10.7326/M14-2385

Kidokoro, K., Cherney, D. Z. I., Bozovic, A., Nagasu, H., Satoh, M., Kanda, E., et al. (2019). Evaluation of glomerular hemodynamic function by Empagliflozin in diabetic mice using *in vivo* imaging. *Circulation* 140 (4), 303–315. doi:10.1161/CIRCULATIONAHA.118.037418

Kohan, D. E., Fioretto, P., Tang, W., and List, J. F. (2014). Long-term study of patients with type 2 diabetes and moderate renal impairment shows that Dapagliflozin reduces weight and blood pressure but does not improve glycemic control. *Kidney Int.* 85 (4), 962–971. doi:10.1038/ki.2013.356

Koya, D., Anker, S. D., Ruilope, L. M., Rossing, P., Liu, Z. H., Lee, B. W., et al. (2023). Cardiorenal outcomes with Finerenone in asian patients with chronic kidney disease and type 2 diabetes: a FIDELIO-DKD *post hoc* analysis. *Am. J. Nephrol.* 54 (9–10), 370–378. doi:10.1159/000532102

Leehey, D. J., Zhang, J. H., Emanuele, N. V., Whaley-Connell, A., Palevsky, P. M., Reilly, R. F., et al. (2015). BP and renal outcomes in diabetic kidney disease: the veterans affairs nephropathy in diabetes trial. *Clin. J. Am. Soc. Nephrol.* 10 (12), 2159–2169. doi:10.2215/CJN.02850315

Liu, C., Xu, H., Yuan, F., Chen, H., Sheng, L., Chen, W., et al. (2023). Evaluating the bioequivalence and safety of Liraglutide injection versus Victoza® in healthy Chinese subjects: a randomized, open, two-cycle, self-crossover phase I clinical trial. *Front. Pharmacol.* 14 (December), 1326865. doi:10.3389/fphar.2023.1326865

Lv, R., Xu, L., Lin, C., Liu, S., Wang, Y., and Dong, B. (2023). Cardiovascular-renal protective effect and molecular mechanism of Finerenone in type 2 diabetic mellitus. *Front. Endocrinol.* 14 (February), 1125693. doi:10.3389/fendo.2023.1125693

Mahaffey, K. W., Jardine, M. J., Bompoint, S., Cannon, C. P., Neal, B., Heerspink, H. J. L., et al. (2019). Canagliflozin and cardiovascular and renal outcomes in type 2 diabetes mellitus and chronic kidney disease in primary and secondary cardiovascular prevention groups: results from the randomized CREDENCE trial. *Circulation* 140 (9), 739–750. doi:10.1161/CIRCULATIONAHA.119.042007

Mann, J. F. E., Fonseca, V. A., Poulter, N. R., Raz, I., Idorn, T., Rasmussen, S., et al. (2020). Safety of Liraglutide in type 2 diabetes and chronic kidney disease. *Clin. J. Am. Soc. Nephrol.* 15 (4), 465–473. doi:10.2215/CJN.11881019

Moher, D., Liberati, A., Tetzlaff, J., Altman, D. G., and PRISMA Group (2009). Preferred reporting Items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Med.* 6 (7), e1000097. doi:10.1371/journal.pmed.1000097

Nguyen, B.-N., Nguyen, L., Mital, S., Bugden, S., and Nguyen, H. V. (2023). Comparative efficacy of sodium-glucose Co-transporter-2 inhibitors, glucagon-like peptide-1 receptor agonists and non-steroidal mineralocorticoid receptor antagonists in chronic kidney disease and type 2 diabetes: a systematic review and network meta-analysis. *Diabetes, Obes. Metabolism* 25 (6), 1614–1623. doi:10.1111/dom.15009

Park, C. W., Kim, H. W., Ko, S. H., Lim, Ji H., Ryu, G. R., Chung, H. W., et al. (2007). Long-term treatment of glucagon-like peptide-1 analog exendin-4 ameliorates diabetic

nephropathy through improving metabolic anomalies in Db/Db mice. *J. Am. Soc. Nephrol.* 18 (4), 1227–1238. doi:10.1681/ASN.2006070778

Perakakis, N., Bornstein, S. R., Birkenfeld, A. L., Linkermann, A., Demir, M., Anker, S. D., et al. (2024). Efficacy of Finerenone in patients with type 2 diabetes, chronic kidney disease and altered markers of liver steatosis and fibrosis: a fidelity subgroup analysis. *Diabetes, Obes. Metabolism* 26 (1), 191–200. doi:10.1111/dom.15305

Perkovic, V., Jardine, M. J., Neal, B., Bompoint, S., Heerspink, H. J. L., Charytan, D. M., et al. (2019). Canagliflozin and renal outcomes in type 2 diabetes and nephropathy. *N. Engl. J. Med.* 380 (24), 2295–2306. doi:10.1056/nejmoa1811744

Pitt, B., Filippatos, G., Agarwal, R., Anker, S. D., Bakris, G. L., Rossing, P., et al. (2021). Cardiovascular events with Finerenone in kidney disease and type 2 diabetes. *N. Engl. J. Med.* 385 (24), 2252–2263. doi:10.1056/nejmoa2110956

Pollock, C., Stefánsson, B., Reyner, D., Rossing, P., David Sjöström, C., Wheeler, D. C., et al. (2019). Albuminuria-lowering effect of Dapagliflozin alone and in combination with saxagliptin and effect of Dapagliflozin and saxagliptin on glycaemic control in patients with type 2 diabetes and chronic kidney disease (delight): a randomised, double-blind, placebo-controlled trial. *Lancet Diabetes Endocrinol.* 7 (6), 429–441. doi:10.1016/S2213-8587(19)30086-5

Rosas, S. E., Ruilope, L. M., Anker, S. D., Pitt, B., Rossing, P., Bonfanti, A. A. C., et al. (2023). Finerenone in hispanic patients with CKD and type 2 diabetes: a *post hoc* fidelity analysis. *Kidney Med.* 5 (10), 100704. doi:10.1016/j.xkme.2023.100704

Salanti, G., Del Giovane, C., Chaimani, A., Caldwell, D. M., and Higgins, J. P. T. (2014). Evaluating the quality of evidence from a network meta-analysis. *PLoS ONE* 9 (7), e99682. doi:10.1371/journal.pone.0099682

Sarafidis, P., Agarwal, R., Pitt, B., Wanner, C., Filippatos, G., Boletis, J., et al. (2023). Outcomes with Finerenone in participants with stage 4 CKD and type 2 diabetes A fidelity subgroup analysis. *Clin. J. Am. Soc. Nephrol.* 18 (5), 602–612. doi:10.2215/CJN.0000000000000149

Scilletta, S., Marco, M. D., Miano, N., Filippello, A., Di Mauro, S., Scamporrino, A., et al. (2023). Update on diabetic kidney disease (DKD): focus on non-albuminuric DKD and cardiovascular risk. *Biomolecules* 13 (5), 752. doi:10.3390/biom13050752

Shah, M., and Vella, A. (2014). Effects of GLP-1 on appetite and weight. *Rev. Endocr. Metabolic Disord.* 15 (3), 181–187. doi:10.1007/s11154-014-9289-5

Shi, Q., Nong, K., Olav Vandvik, P., Guyatt, G. H., Schnell, O., Rydén, L., et al. (2023). Benefits and harms of drug treatment for type 2 diabetes: systematic review and network meta-analysis of randomised controlled trials. *BMJ* 381, e074068. doi:10.1136/bmj-2022-074068

Sivalingam, S., Wasehuus, V. S., Curovic, V. R., Blond, M. B., Hansen, T. W., Persson, F., et al. (2024). Albuminuria-lowering effect of adding Semaglutide on top of Empagliflozin in individuals with type 2 diabetes: a randomized and placebo-controlled study. *Diabetes, Obes. Metabolism* 26 (1), 54–64. doi:10.1111/dom.15287

Skov, J., Dejgaard, A., Frøkiær, J., Holst, J. J., Jonassen, T., Rittig, S., et al. (2013). Glucagon-like peptide-1 (GLP-1): effect on kidney hemodynamics and renin-angiotensin-aldosterone system in healthy men. *J. Clin. Endocrinol. and Metabolism* 98 (4), E664–E671. doi:10.1210/jc.2012-3855

Sterne, J. A. C., Savović, J., Page, M. J., Elbers, R. G., Blencowe, N. S., Boutron, I., et al. (2019). RoB 2: a revised tool for assessing risk of bias in randomised trials. *BMJ* 366, l4898. doi:10.1136/bmj.l4898

Sun, F., Wu, S., Guo, S., Yu, K., Yang, Z., Li, L., et al. (2015). Impact of GLP-1 receptor agonists on blood pressure, heart rate and hypertension among patients with type 2 diabetes: a systematic review and network meta-analysis. *Diabetes Res. Clin. Pract.* 110 (1), 26–37. doi:10.1016/j.diabres.2015.07.015

Takashima, H., Yoshida, Y., Nagura, C., Furukawa, T., Tei, R., Maruyama, T., et al. (2018). Renoprotective effects of Canagliflozin, a sodium glucose cotransporter 2 inhibitor, in type 2 diabetes patients with chronic kidney disease: a randomized open-label prospective trial. *Diabetes Vasc. Dis. Res.* 15 (5), 469–472. doi:10.1177/1479164118782872

Tanrıverdi, M., Baştemir, M., Demirbakan, H., Ünalan, A., Türkmen, M., and Tanrıverdi, G. Ö. (2023). Association of SGLT-2 inhibitors with bacterial urinary tract infection in type 2 diabetes. *BMC Endocr. Disord.* 23 (1), 211. doi:10.1186/s12902-023-01464-6

Thomas, M. C. (2014). Renal effects of Dapagliflozin in patients with type 2 diabetes. *Ther. Adv. Endocrinol. Metabolism* 5 (3), 53–61. doi:10.1177/2042018814544153

Tuttle, K. R., Levin, A., Nangaku, M., Kadowaki, T., Agarwal, R., Hauske, S. J., et al. (2022). Safety of Empagliflozin in patients with type 2 diabetes and chronic kidney disease: pooled analysis of placebo-controlled clinical trials. *Diabetes Care* 45 (6), 1445–1452. doi:10.2337/dc21-2034

Vallon, V., and Thomson, S. C. (2017). Targeting renal glucose reabsorption to treat hyperglycaemia: the pleiotropic effects of SGLT2 inhibition. *Diabetologia* 60 (2), 215–225. doi:10.1007/s00125-016-4157-3

Wada, T., Mori-Anai, K., Kawaguchi, Y., Katsumata, H., Tsuda, H., Iida, M., et al. (2022a). Renal, cardiovascular and safety outcomes of Canagliflozin in patients with type 2 diabetes and nephropathy in east and south-east asian countries: results from the Canagliflozin and renal events in diabetes with established nephropathy clinical evaluation trial. *J. Diabetes Investigation* 13 (1), 54–64. doi:10.1111/jdi.13624

Wada, T., Mori-Anai, K., Takahashi, A., Matsui, T., Inagaki, M., Iida, M., et al. (2022b). Effect of Canagliflozin on the decline of estimated glomerular filtration rate in chronic kidney disease patients with type 2 diabetes mellitus: a multicenter, randomized, double-blind, placebo-controlled, parallel-group, phase III study in Japan. *J. Diabetes Investigation* 13 (12), 1981–1989. doi:10.1111/jdi.13888

Wanner, C. (2017). EMPA-REG OUTCOME: the nephrologist's point of view. *Am. J. Cardiol.* 120 (1), S59–S67. doi:10.1016/j.amjcard.2017.05.012

Wanner, C., Lachin, J. M., Inzucchi, S. E., Fitchett, D., Mattheus, M., George, J., et al. (2018). Empagliflozin and clinical outcomes in patients with type 2 diabetes mellitus, established cardiovascular disease, and chronic kidney disease. *Circulation* 137 (2), 119–129. doi:10.1161/CIRCULATIONAHA.117.028268

Wish, J. B., and Pablo, P. (2022). Evolution of mineralocorticoid receptor antagonists in the treatment of chronic kidney disease associated with type 2 diabetes mellitus. *Mayo Clin. Proc. Innovations, Qual. and Outcomes* 6 (6), 536–551. doi:10.1016/j.mayocpiqo.2022.09.002

Woods, T. C., Satou, R., Miyata, K., Katsurada, A., Dugas, C. M., Klingenberg, N. C., et al. (2019). Canagliflozin prevents intrarenal angiotensinogen augmentation and mitigates kidney Injury and hypertension in mouse model of type 2 diabetes mellitus. *Am. J. Nephrol.* 49 (4), 331–342. doi:10.1159/000499597

Yale, J. F., Bakris, G., Cariou, B., Yue, D., David-Neto, E., Xi, L., et al. (2013). Efficacy and safety of Canagliflozin in subjects with type 2 diabetes and chronic kidney disease. *Diabetes, Obes. Metabolism* 15 (5), 463–473. doi:10.1111/dom.12090

Yamada, T., Wakabayashi, M., Bhalla, A., Chopra, N., Miyashita, H., Mikami, T., et al. (2021). Cardiovascular and renal outcomes with SGLT-2 inhibitors versus GLP-1 receptor agonists in patients with type 2 diabetes mellitus and chronic kidney disease: a systematic review and network meta-analysis. *Cardiovasc. Diabetol.* 20 (1), 14. doi:10.1186/s12933-020-01197-z

Yamout, H., Perkovic, V., Davies, M., Woo, V., De Zeeuw, D., Mayer, C., et al. (2014). Efficacy and safety of Canagliflozin in patients with type 2 diabetes and stage 3 nephropathy. *Am. J. Nephrol.* 40 (1), 64–74. doi:10.1159/000364909

Yaribeygi, H., Butler, A. E., Atkin, S. L., Katsiki, N., and Sahebkar, A. (2019). Sodium–glucose cotransporter 2 inhibitors and inflammation in chronic kidney disease: possible molecular pathways. *J. Cell. Physiology* 234 (1), 223–230. doi:10.1002/jcp.26851

Zhang, H., Xie, J., Chuanming, H., Li, X., Zhu, D., Zheng, H., et al. (2023). Finerenone in patients with chronic kidney disease and type 2 diabetes: the FIDELIO-DKD subgroup from China. *Kidney Dis.* 9 (6), 498–506. doi:10.1159/000531997

Zhang, M.-Z., Bao, W., Zheng, Q.-Y., Wang, Y.-H., and Sun, L.-Y. (2022). Efficacy and safety of Finerenone in chronic kidney disease: a systematic review and meta-analysis of randomized clinical trials. *Front. Pharmacol.* 13 (February), 819327. doi:10.3389/fphar.2022.819327

Zhang, Y., Jiang, L., Wang, J., Wang, T., Chien, C., Huang, W., et al. (2022). Network meta-analysis on the effects of Finerenone versus SGLT2 inhibitors and GLP-1 receptor agonists on cardiovascular and renal outcomes in patients with type 2 diabetes mellitus and chronic kidney disease. *Cardiovasc. Diabetol.* 21 (1), 232. doi:10.1186/s12933-022-01676-5

Zoja, C., Xinaris, C., and Macconi, D. (2020). Diabetic nephropathy: novel molecular mechanisms and therapeutic targets. *Front. Pharmacol.* 11 (December), 586892. doi:10.3389/fphar.2020.586892

frontiers | Frontiers in Pharmacology

TYPE Original Research
PUBLISHED 04 April 2025
DOI 10.3389/fphar.2025.1506896

Check for updates

OPEN ACCESS

EDITED BY
Swayam Prakash Srivastava,
University of Michigan, United States

REVIEWED BY
Yiyi Zhou,
Second Military Medical University, China
Zhongyu Zou,
Roche, Switzerland

*CORRESPONDENCE
Xiai Wu,
20180931313@bucm.edu.cn

RECEIVED 06 October 2024
ACCEPTED 03 March 2025
PUBLISHED 04 April 2025

CITATION
Jiang L, Yu H, Jian J, Sai X, Wang Y, Zhang Y and Wu X (2025) Landscape analysis of m6A modification regulators reveals LRPPRC as a key modulator in tubule cells for DKD: a multi-omics study.
Front. Pharmacol. 16:1506896.
doi: 10.3389/fphar.2025.1506896

COPYRIGHT
© 2025 Jiang, Yu, Jian, Sai, Wang, Zhang and Wu. 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.

Landscape analysis of m6A modification regulators reveals LRPPRC as a key modulator in tubule cells for DKD: a multi-omics study

Li Jiang[1], Hongda Yu[2], Jie Jian[3], Xulin Sai[4], Yudian Wang[5], Yufei Zhang[5] and Xiai Wu[1]*

[1]Diabetes Department of integrated Chinese and Western Medicine, China National Center for Integrated Traditional Chinese and Western Medicine, China-Japan Friendship Hospital, Beijing, China, [2]Dermatology Department, China-Japan Friendship Hospital, Beijing, China, [3]General Medicine Department, Mental Health Center of Dongcheng District, Beijing, China, [4]Dongzhimen Hospital Affiliated to Beijing University of Chinese Medicine, Beijing, China, [5]College of Traditional Chinese Medicine, Hubei University of Chinese Medicine, Hubei, Wuhan, China

Background: Diabetic Kidney Disease (DKD) is a serious complication of diabetes, imposing a substantial medical burden. The significance of N6-methyladenosine (m6A) modification in the pathogenesis of DKD has become increasingly prominent.

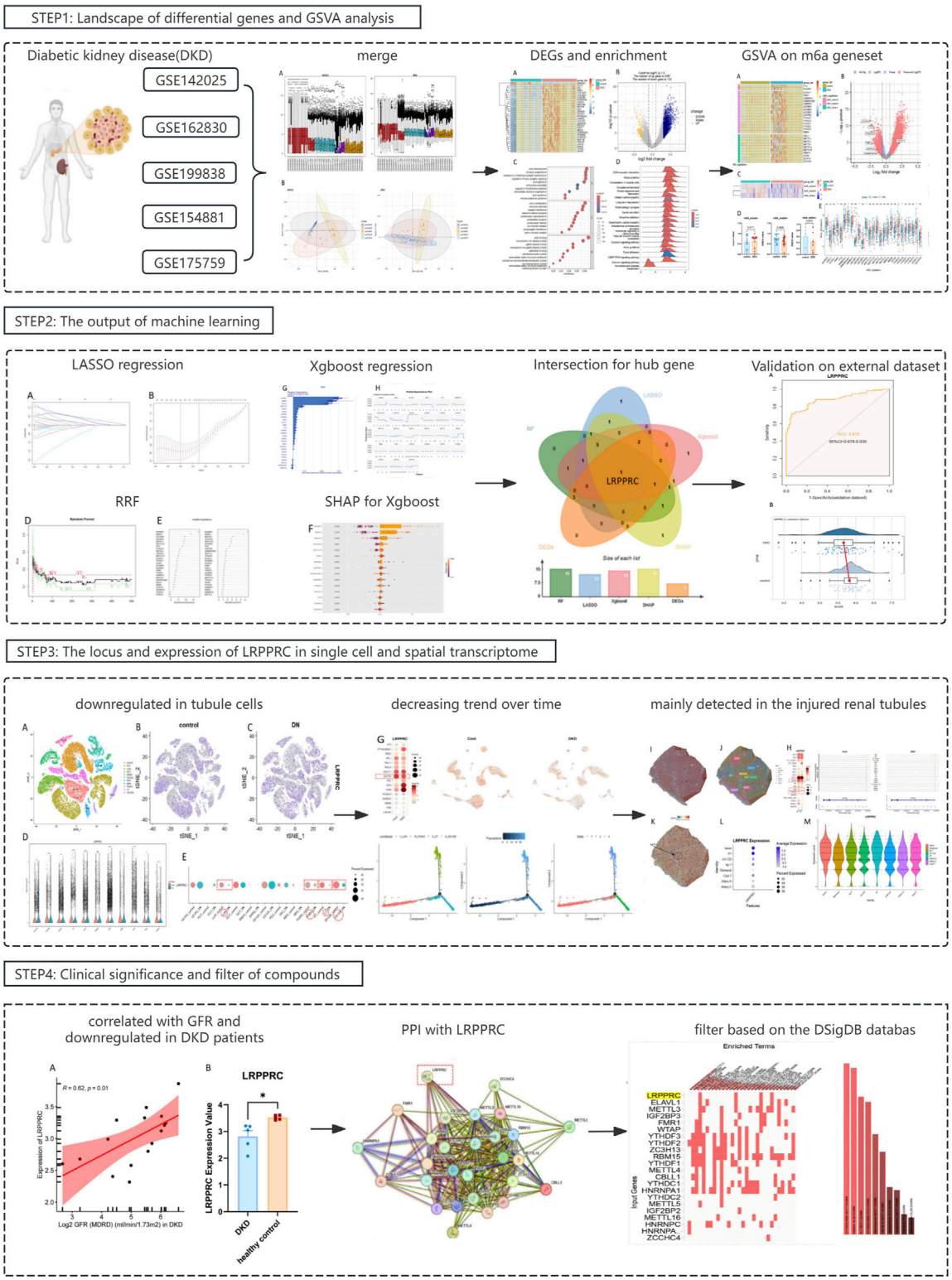
Aim: This study aimed to investigate the specific expression patterns of the m6A geneset in the pathogenesis of DKD.

Method: Bulk RNA, single-cell and spatial transcriptome were utilized to clarify the hub gene. 3 types of machine learning algorithms were applied. The possible compounds were screened based on the DSigDB database.

Result: GSEA has revealed the potential m6a-associated pathways such as cGMP-PKG pathway. GSVA showed that the two types of m6a regulation, namely m6a-readers and m6a-writers, were generally suppressed in DKD patients. The output of 3 types of machine learning algorithm and differential analysis has determined the LRPPRC as the hub gene. LRPPRC was downregulated in the LOH, PODO, CT, and CD-ICB cell populations, most of which were tubular cells. It exhibited the decreasing trend over time, particularly pronounced in LOH cells. The low activity of LRPPRC was mainly detected in the injured renal tubules. In clinical patients, the expression levels of LRPPRC mRNA in DKD showed the tendency to be downregulated and exhibited the potential correlations with Glomerular Filtration Rate (GFR) and proteinuria according to the Nephroseq database. The lobeline might be an important potential compound involved in the regulation of LRPPRC and other m6a genes. Its actual efficacy needs to be verified *in vivo* or *in vitro*.

KEYWORDS

m6A modification, diabetic kidney disease, LRPPRC, tubules, single-cell transcriptome, spatial transcriptome



GRAPHICAL ABSTRACT

1 Introduction

Diabetic kidney disease (DKD), also known as diabetic nephropathy, is a serious complication of diabetes characterized by progressive kidney damage resulting from chronic hyperglycemia and associated metabolic disturbances. Early detection and management of DKD are crucial in preventing its progression and mitigating its associated health risks (Akhtar et al., 2020; Barutta et al., 2022).

In recent years, the pathogenesis of DKD has been deeply studied. The development and progression involve a complex interplay of various signaling pathways and cellular mechanisms.The TGF-β signaling pathway is a central driver of renal fibrosis, with TGF-β1 promoting epithelial-mesenchymal transition (EMT) in renal epithelial cells, leading to the loss of epithelial characteristics and acquisition of mesenchymal traits, ultimately contributing to fibrogenesis (Chatterjee et al., 2025). Similarly, Wnt signaling, through β-catenin activation, exacerbates renal fibrosis by inducing fibroblast activation and extracellular matrix (ECM) production, promoting the endothelial-mesenchymal transition (EndMT) (Hadpech and Thongboonkerd, 2024). Notch signaling, which regulates cell fate determination, is also implicated in renal injury and fibrosis when dysregulated (Li et al., 2020). The renal angiopoietin-like protein 4 (ANGPTL4), a key protein involved in lipoprotein metabolism, interacts with Integrin β1 and exert fibrogenic effect in diabetic kidneys (Srivastava et al., 2024).

On the protective side, The activation of the endothelial glucocorticoid receptor (GR) exerts anti-inflammatory effects. The db/db mice lacking endothelial GR showed more severe fibrosis in multiple organs (Srivastava and Goodwin, 2023). The fibrogenic phenotype in the kidneys of diabetic mice lacking endothelial GR is associated with aberrant cytokine and chemokine reprogramming, augmented Wnt signaling and suppression of fatty acid oxidation, which demonstrated that endothelial GR is an essential antifibrotic molecule in diabetes (Srivastava et al., 2021). FGFR1 signaling protects against kidney fibrosis by inhibiting EndMT and suppressing TGF-β/smad signaling (Li et al., 2017). SIRT3-mediated mechanisms in endothelial cells improve mitochondrial function.The suppression in SIRT3 was associated with the induction of TGF-β/smad signaling, higher level of HIF1a accumulation and PKM2 dimer formation, led to abnormal glycolysis and linked abnormal mesenchymal transformations (Srivastava et al., 2018).

The development of drugs for DKD is also in full swing, several drugs have been evaluated for their unique therapeutic mechanisms: Linagliptin, a DPP-4 inhibitor, enhances incretin hormone activity, reducing hyperglycemia and exerting anti-inflammatory and antifibrotic effects (Nady et al., 2024). Empagliflozin, an SGLT2 inhibitor, improves DKD by inhibiting glycolysis, reducing intracellular glucose uptake, and lowering oxidative stress (Huang et al., 2024a). ACE inhibition and N-acetyl-seryl-aspartyl-lysyl-proline (AcSDKP) suppressed defective metabolism-linked mesenchymal transformations and reduced collagen-I and fibronectin accumulation in the diabetic kidneys (Srivastava et al., 2020). Mineralocorticoid antagonists like finerenone exert anti-inflammatory and antifibrotic effects, reducing proteinuria and slowing DKD progression. mice. Its effect was associated with reduced oxidative stress, mitochondrial fragmentation, and apoptosis while restoring the mitophagy via PI3K/Akt/eNOS signaling (Yao et al., 2023).

The significance of N6-methyladenosine (m6A) modification in the pathogenesis of DKD has become increasingly prominent. The m6A modification is a prevalent form of RNA methylation that plays a critical role in regulating various biological processes, including RNA stability, splicing, translation, and degradation (Oerum et al., 2021). This modification is added by methyltransferases known as "writers," removed by demethylases termed "erasers," and recognized by "readers" which facilitate the functional outcomes of m6A-modified RNA (Jiang et al., 2021). The core components involved in m6A modification include methyltransferase complexes (e.g., METTL3 and METTL14), demethylases (e.g., FTO and ALKBH5), and reader proteins (e.g., YTHDF and HNRNPA2B1). Recent studies have shown that m6A modification was involved in several essential physiological processes, such as stem cell differentiation, immune response, and metabolism (Shi et al., 2019). The dysregulation of m6A modification can be linked to various diseases, including cancer (He et al., 2019), obesity (Liu et al., 2021), and cardiovascular diseases (Xu et al., 2022), emphasizing its role in cellular homeostasis.

In the context of diabetic kidney disease (DKD), emerging evidence suggests that m6A modification plays a significant role in the pathogenesis and progression of renal damage (Ye et al., 2023; Huang et al., 2024b; Sun et al., 2023). Increased expression of METTL3 has been observed in renal tissues of diabetic models, which correlates with enhanced m6A methylation of specific target mRNAs involved in inflammation and fibrosis (Wang et al., 2024). Similarly, overexpression of m6A erasers like FTO has been associated with increased renal inflammation and fibrosis. Knockdown of FTO markedly increased SAA2 mRNA m6A modification and decreased SAA2 mRNA expression, thus alleviating the podocyte injury and inflammation (Lang et al., 2024).

This study aims to investigate the specific expression patterns of the m6A geneset in the pathogenesis of diabetic kidney disease (DKD) by integrating bulk RNA sequencing, single-cell and spatial transcriptomics, utilizing publicly available databases. The research seeks to identify core targets and uncover potential therapeutic agents, thereby providing novel insights for the clinical prevention and treatment of DKD.

2 Method

2.1 Date merge of bulk RNA transcriptomics and differential analysis

All datasets were downloaded from the Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo/). We used expression profiling by high throughput sequencing (GSE142025,GSE162830,GSE199838,GSE154881,GSE175759) (Fan et al., 2019; Eadon et al., 2021; Park et al., 2022) for initial analysis, including 45 control samples and 56 DKD samples. Detailed platform and annotation information for these datasets were provided in Supplementary Table S1. The R package "sva" was used to merge these data cohorts and address batch effects, with the ComBat function used for batch effect adjustment. The differential analysis was conducted with log2FC > 1.5 and P-value <0.05 (Wei et al., 2022). The significantly enriched m6a gene would be designated as differentially expressed genes (DEGs). The detailed m6a regulation gene was shown in Supplementary Figure S1. The GO, KEGG, GSEA/GSVA analysis was used to explore the potential pathway.

2.2 Screening of hub gene based on machine leaning

All 28 m6a regulation genes were input into 3 machine learning algorithms: the Least Absolute Shrinkage and Selection Operator (LASSO), the Regularized Random Forest (RRF)and the Extreme Gradient Boosting (XGBoost) regression (Feature importance and SHAP explanation). The m6a genes with both favourable machine

learning algorithm characteristics and DEG properties would be screened as hub genes.

2.3 Validation of hub gene

The chosen hub gene would undergo validation in an external dataset (GSE99339, GSE30529, GSE96804, GSE104954, and GSE30528, detailed in Supplementary Table S1). The receiver operating characteristic (ROC) analysis was executed. Area under the Curve (AUC) values exceeding 0.65 were deemed to exhibit commendable diagnostic efficacy (Shen et al., 2024). Correlations between the expression levels of hub gene and clinical indicators among patients diagnosed with DKD were investigated using Nephroseq (http://v5.nephroseq.org).

2.4 Single cell transcriptome analysis of LRPPRC

The expression level of LRPPRC in single cell transcriptome data would be conducted in two different platform. Firstly, the gene would be imported in the Kidney Integrative Transcriptomics (K.I.T.) platform (http://humphreyslab.com/SingleCell/) based on the original data from Human Diabetic Kidney (23980 nuclei) (Wilson et al., 2019) and Human DKD scATAC-seq (Wilson et al., 2022). Secondly, 6 signature DKD samples and 7 control samples from the GSE195460 and GSE131882 dataset would be aggregated and analyzed. The "Seurat" package was used for preprocessing and clustering. The cell pseudotime analysis was conducted using the BiocGenerics and monocle packages.

2.5 Spatial transcriptomics of LRPPRC

The spatial transcriptomics data originated from GSE261545 (Isnard et al., 2024). Detailed information was provided in Supplementary Table S1. The conditional autoregressive-based deconvolution (CARD) was applied for spatial division and inter-regional difference analysis. The LRPPRC would be mapped to specific locations in pathological sections to reflect its actual expression.

2.6 Filter of potential compounds

The PPI map of 28 m6a genes was constructed based on the STRING platform to determine the location of LRPPRC, and then possible compounds were screened based on the DSigDB database, mainly regulating LPRRC and supplemented by other m6a regulatory functions.

3 Result

3.1 Landscape of differential genes and enrichment pathway in DKD

The results of the DKD bulk RNA transcriptome analysis were shown in Figure 1. Data preprocessing was shown in Supplementary Figure S2. The heatmap showed that most of the top 30 DEGs were upregulated genes (Figure 1A). The volcano plot indicated 2397 upregulated and 122 downregulated genes (Figure 1B). The pathway enriched were most related with renal fibrosis like "extracellular matrix structural constituent" and "regulation of membrane potential" (Figure 1C). GSEA has revealed the potential m6a-associated pathways such as cGMP-PKG pathway (Figure 1D). KEGG highlighted alterations in the signaling of ECM-receptor interaction that accompany DKD (especially the renal fibrosis) (Figure 1E). Among the top five activated pathways, 2 were regulated by m6a regulation (ECM-receptor interaction, Protein digestion and absorption) (Figure 1F).

3.2 The differential gene analysis and GSVA analysis based on m6a geneset in DKD

The heatmap and volcano plot (Figures 2A, B) showed the m6a genes which expressed deferentially in DKD patients. GSVA showed that the two types of m6a regulation, namely m6a-readers and m6a-writers, were generally suppressed in DKD patients (Figure 2C). Moreover, the inhibition trend of m6a-readers was more obvious (Figure 2D). The t-test of independent samples showed that FMR1, HNRNPA2B1, IGF2BP2, LRPPRC, YTHDC2, and ZC3H13 were significantly inhibited in m6a geneset (Figure 2E).

3.3 The output of machine learning

In the LASSO regression, most m6a genes were positively correlated with DKD (Figure 3A). 12 genes were selected for output (Figure 3B) and ranked by correlation coefficient (Figure 3C). The RFF analysis used 500 decision trees (Figure 3D). 15 genes were selected for output and all ranked by accuracy value and Gini value (Figure 3E). SHAP for XGBoost regression showed the top variables as GF2BP1, RBM15 and METTL3 (Figure 3F). The feature importance and partial dependence of XGBoost showed the similar output (Figures 3G, H). The intersection of all results indicated that LRPPRC as the hub gene (Figure 3I). In validation dataset, LRPPRC was also significantly downregulated in the DKD patient. ROC analysis showed an AUC of 0.878 for LRPPRC (Supplementary Figure S3).

3.4 The expression and distribution of LRPPRC in single cell and spatial district of DKD

The quality control results of the single-cell dataset were shown in Supplementary Figure S4. Low heterogeneity was observed between samples, with no interference from mitochondrial, ribosomal, or erythrocyte RNA. A total of 59,098 nuclei and 35,618 genes were included. In the DKD group, the combined data were effectively reduced to 13 clusters and 11 cell types: collecting duct-principal cell (CD-PC), proximal convoluted tubular cell (PCT), Loop of

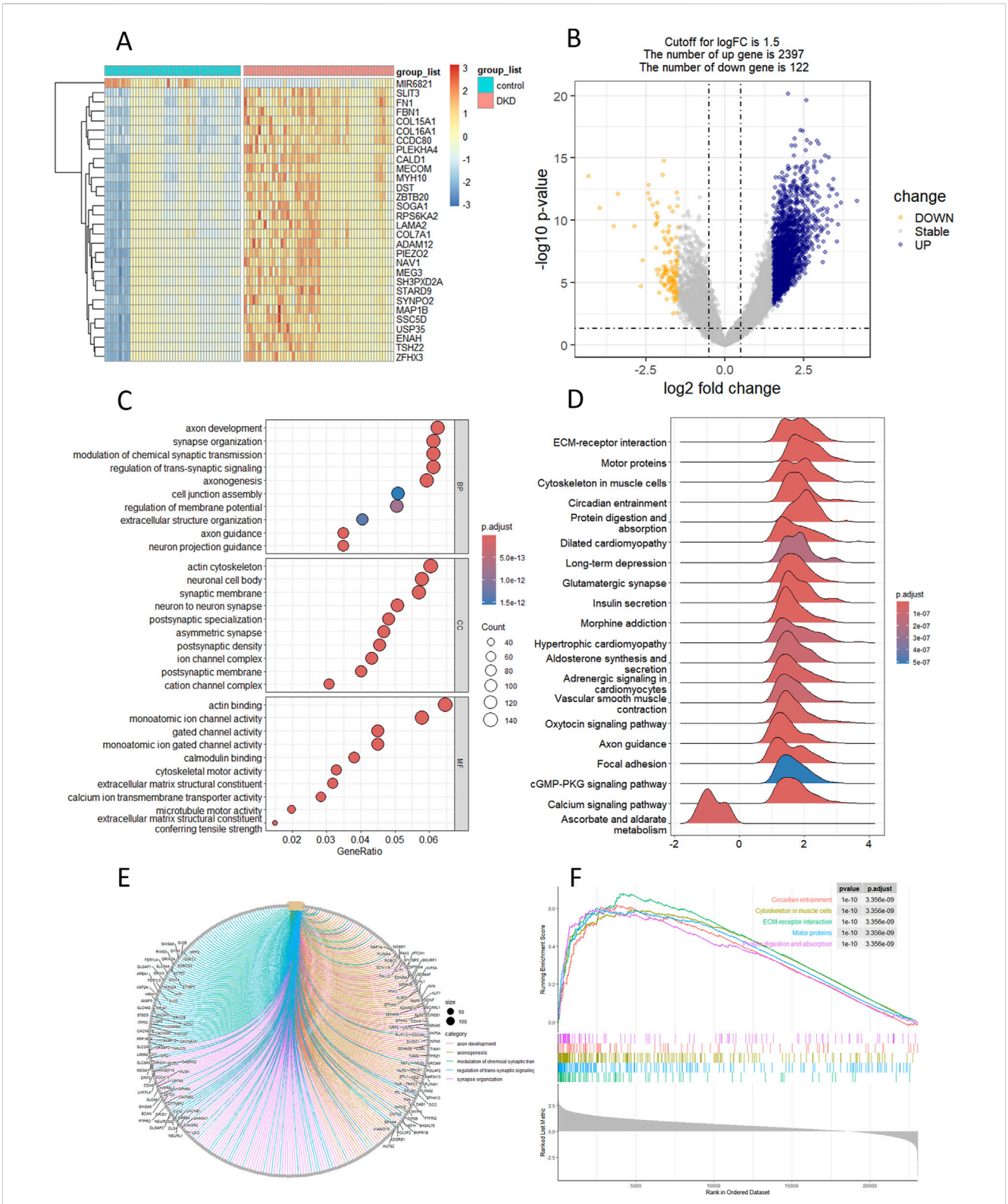


FIGURE 1
Landscape of differential genes and pathway in DKD. **(A, B)** Heatmap and volcano plot of DEGs between control and DKD groups. **(C)** GO enrichment analysis of DEGs. **(D)** GSEA analysis of hallmark genesets. **(E)** KEGG analysis. **(F)** Top-5 pathway based on GSEA analysis.

Henle cell (LOH), distal convoluted tubular cell (DCT), convoluted tubular cell (CT), collecting duct-intercalated cell type A (CD-ICA), collecting duct-intercalated cell type B(CD-ICB), glomerular parietal epithelial cell (PEC), endothelial cell (ENDO), mesenchymal cell (MES), podocyte (PODO) (Figure 4A). LRPPRC was broadly expressed across all renal

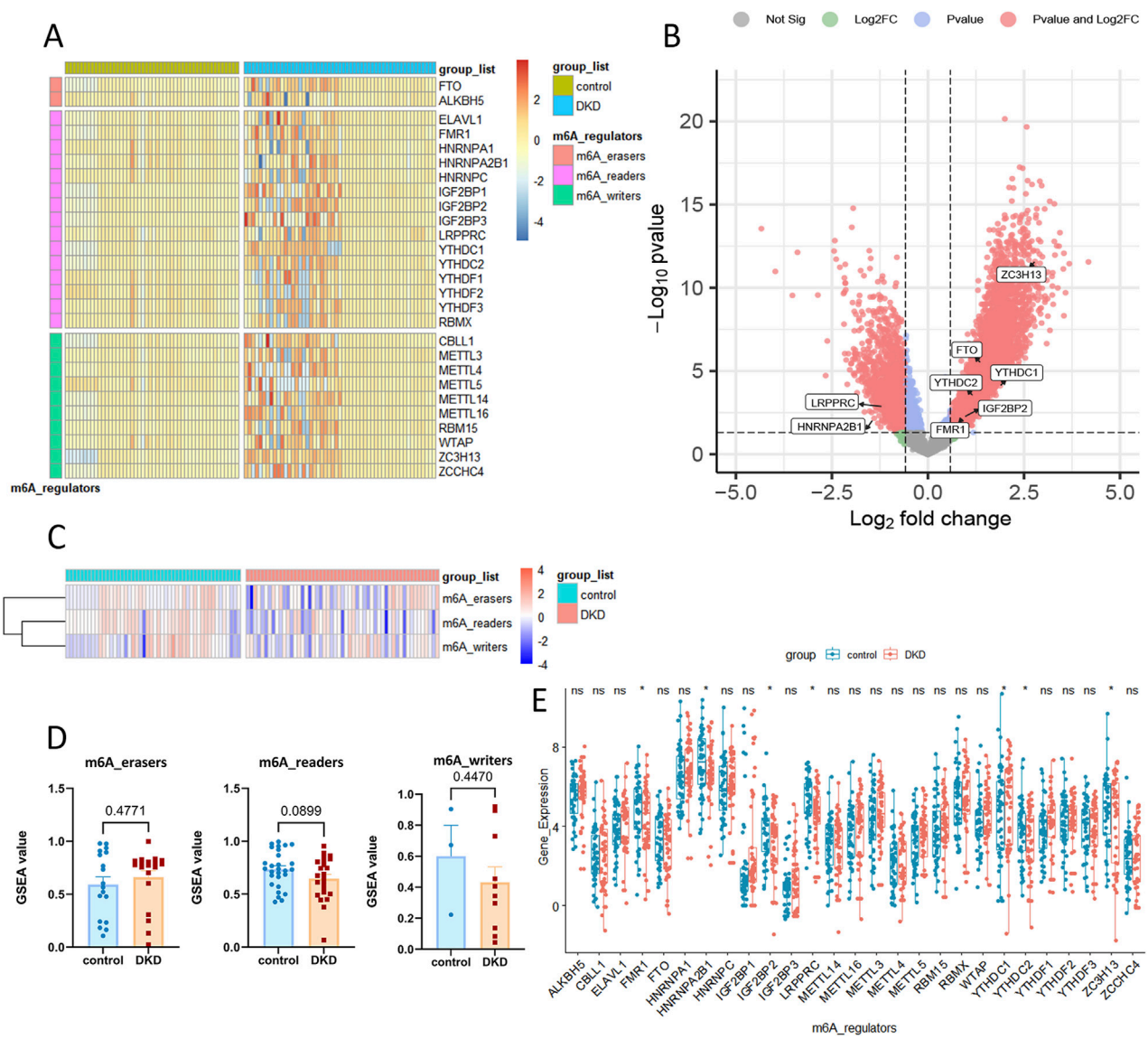


FIGURE 2
The differential gene analysis and GSVA analysis based on m6a geneset in DKD. **(A, B)** Heatmap and volcano plot of m6a geneset between control and DKD groups. **(C, D)** GSVA analysis of 3 m6a regulation. **(E)** The detailed expression of 28 m6a gene.

cell types and was downregulated in patients with diabetic kidney disease (Figures 4B, C). Particularly in the LOH, PODO, CT, and CD-ICB cell populations, it exhibited significantly lower expression levels (Figures 4D, E). Upon extracting the LOH, PODO, CT, and CD-ICB cell subpopulations from DKD patients, pseudotime analysis revealed that these four subpopulations primarily were clustered into three time points (Figure 4G). Given that the apoptosis of LOH cells representing early renal injury, LOH subpopulation was therefore designated as the starting point for differentiation. The trajectory illustrated a common differentiation direction between LOH and CT towards PODO, suggesting that as DKD progresses, tubular injury progressively impacted the podocytes in the glomeruli. A dynamic analysis of the top fifty differentially expressed genes revealed that the majority of these genes were upregulated in accordance with the direction of cell differentiation and disease progression, while LRPPRC exhibited the opposite trend, decreasing over time (Figure 4F), particularly pronounced in LOH cells (Figure 4H). In the pseudotime analysis of the LOH cell across all samples, LOH cells exhibited five distinct differentiation nodes, with a trajectory that was more diverse in DKD patients (Figure 4I).

In the K.I.T single-cell online platform, kidney cells were divided into 12 subgroups (Figure 5A). The LRPPRC was widely expressed (Figure 5B), and the expression of LRPPRC was downregulated in DKD patients compared with healthy people (Figure 5C). The main renal cells with downregulated LRPPRC expression were PODO,LOH, and DCT (Figures 5D, E). ATAC-seq analysis showed that renal cell chromatin was grouped into 20 subgroups (Figure 5F). Compared with single cells, DCT cells were subdivided into two subgroups, DCT1 and DCT2. The PT (Proximal Tubule cells) cells were subdivided into PT_CD36 and

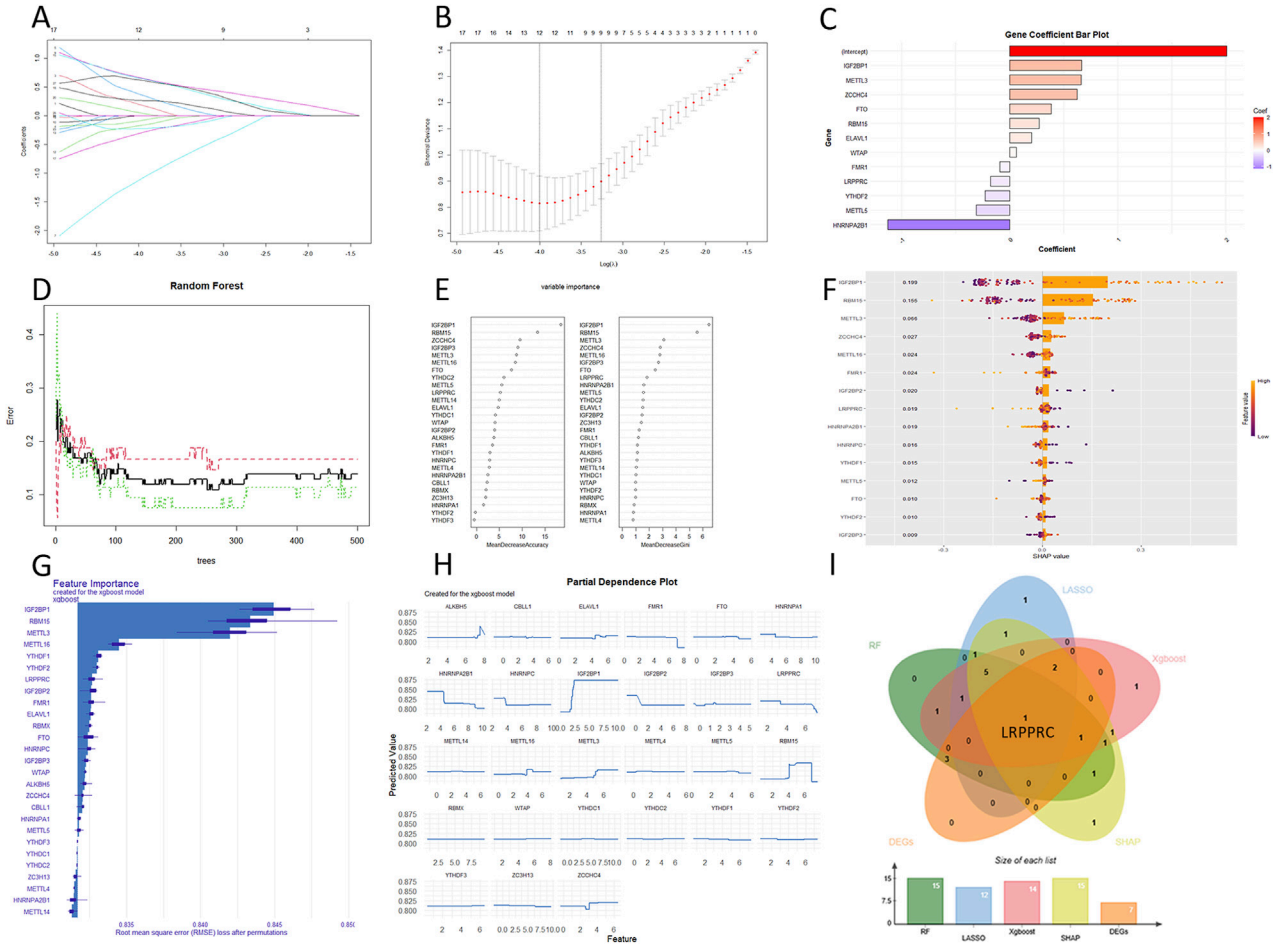


FIGURE 3
The output of 3 machine learning algorithms. **(A–C)** LASSO regression. **(D, E)** RRF. **(F)** SHAP for XGBoost regression. **(G, H)** Feature imprtace and partial dependence for XGBoost regression. **(I)** Intersetion of machine learning and DEGs.

PT_VCAM1. LOH cells are subdivided into TAL (Thick Ascending Limb) and ATL (Ascending Thin Limb). The more immune cells were distinguished, such as B cells and T cells. Fib_VSMC_MC stand for a mixture of Fibroblasts, Vascular Smooth Muscle Cells and Myofibroblasts. Gene expression showed that the distribution of LRPPRC in the open region of the genome of DKD patients was lower than that of healthy people, and it was mainly downregulated in the DCT2 subgroup (Figure 5G). In terms of gene activity and accessibility, LRPPRC was mainly downregulated in DCT2, ICB and PODO cells. It was also generally downregulated in immune cells (B cell, T cell) (Figure 5H).

The high-resolution section of kidney tissue biopsy from DKD patients was shown in Figure 6A. The spatial transcriptome included a total of 18,085 genes and 2802 sampling points. The section could be divided into 5 regions: glomeruli, injured renal tubules (Inj–T), arteries within the capsule (Artery–C), Loop of Henle and collecting ducts (LH–CD), proximal tubules (PT), tubules with endoluminal protein casts (cast-T), arteries within the renal parenchyma (arterion-K) and tumor (Figure 6B). The low activity of LRPPRC was mainly detected in the injured renal tubules (Figure 6C). The low quantitative expression of LRPPRC was reflected on glomeruli and injured renal tubules (Figures 6D, E). In all renal regions, except tumor tissue, the glomeruli produced the most cell communication, while inj-T produced the strongest cell communication (Figure 6F). The inj-T strongly interacted with all other renal regions, among which FGF-LRPPRC pathway was the most widely distributed one (Figure 6G).

3.5 The expression of LRPPRC mRNA in clinical patients

Utilizing the hub gene LRPPRC as the retrieval keyword, relevant clinical data of Chronic Didney Disease (CKD) and Diabetic Kidney Disease (DKD) in the Nephroseq platform were scrutinized. Following correction and summarization, it was observed that the expression levels of LRPPRC mRNA in CKD patients were notably downregulated, exhibiting significant positive correlations with Glomerular Filtration Rate (GFR), the significant negative correlations with serum creatinine, proteinuria and weight (Figures 7A–E). Similarly, LRPPRC

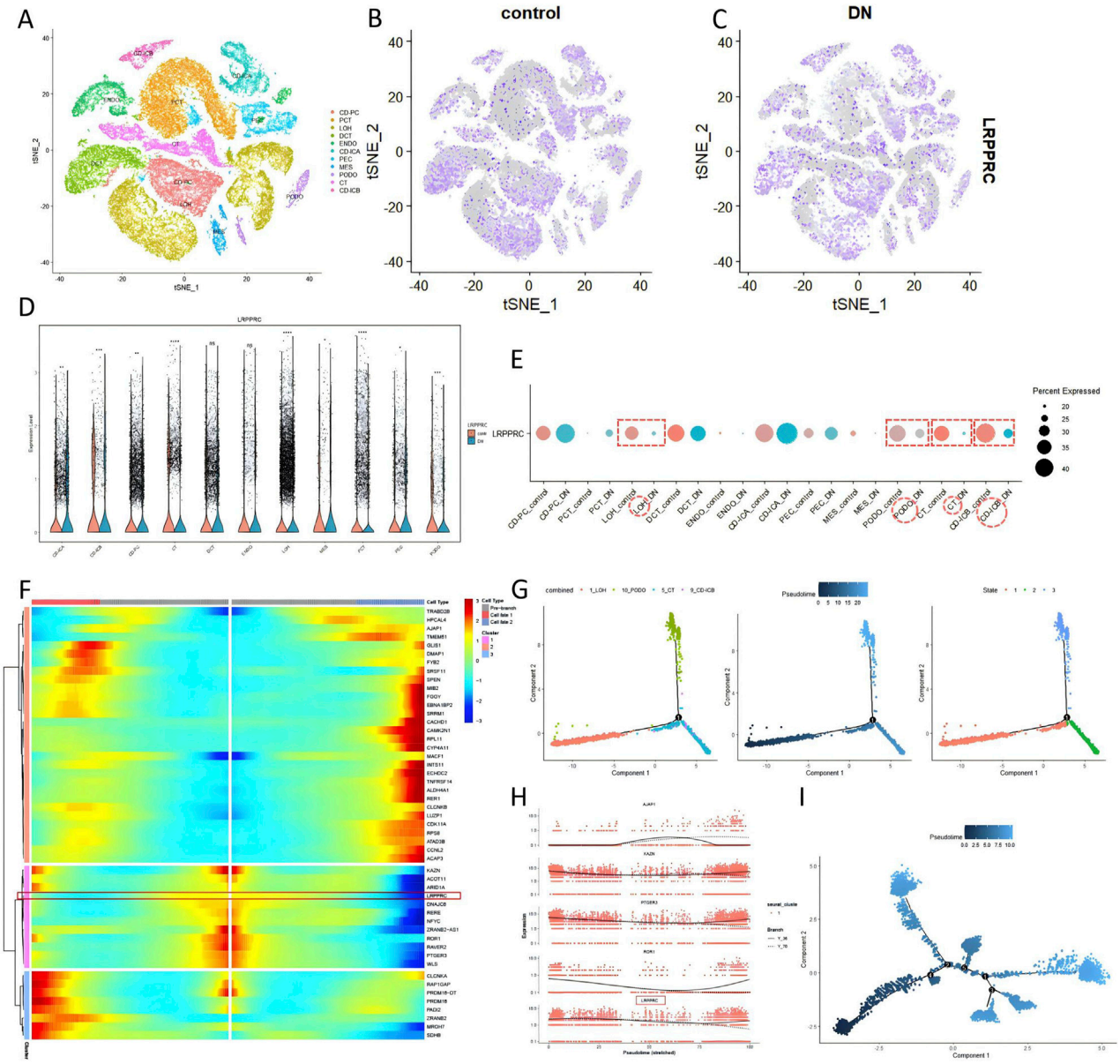


FIGURE 4
Expression of LRPPRC in DKD single-cell (GSE195460 and GSE131882). **(A–E)** The quantitative analysis of LRPPRC. **(F–I)** The pseudotime analysis.

was downregulated in DKD patients, pisitively correlated with GFR and negatively correlated with proteinuria. (Figures 7F–H).This means that the worse the kidney function, the lower the expression of LRPPRC mRNA.

3.6 The filter of compounds targeting m6a regulation

The PPI network with LRPPRC as the core was shown in Figure 8A. The 28 m6a genes were input into the drug screening platform with filtering conditions of $P < 0.05$ and Odds Ratio >5. The top 10 drug candidates were shown in Figure 8B, namely 0175029-0000(in both MCF7 and PC-3 cell line), lobeline, H-7, staurosporine, GW-8510, camptothecin (in both MCF7 and PC-3 cell line), GW-8510, glibenclamide, 4-PHENYLBUTYRIC ACID and fisetin. All drugs showed a tendency to inhibit the expression of m6a gene, which can be specifically seen in Supplementary Table S2. There were four compounds targeting LRPPRC as shown in Figure 8C lobeline, dirithromycin, glibenclamide and cimetidine. lobeline was considered as an important potential compound involved in the regulation of LRPPRC and other m6a genes (YTHDF1; YTHDF2; RBM15; YTHDF3; WTAP; FMR1; METTL3; METTL5; IGF2BP3; ELAVL1).

4 Discussion

Our study evaluated the expression patterns of the m6a geneset in the pathogenesis of DKD. GSEA has revealed the potential m6a-associated pathways such as cGMP-PKG pathway.

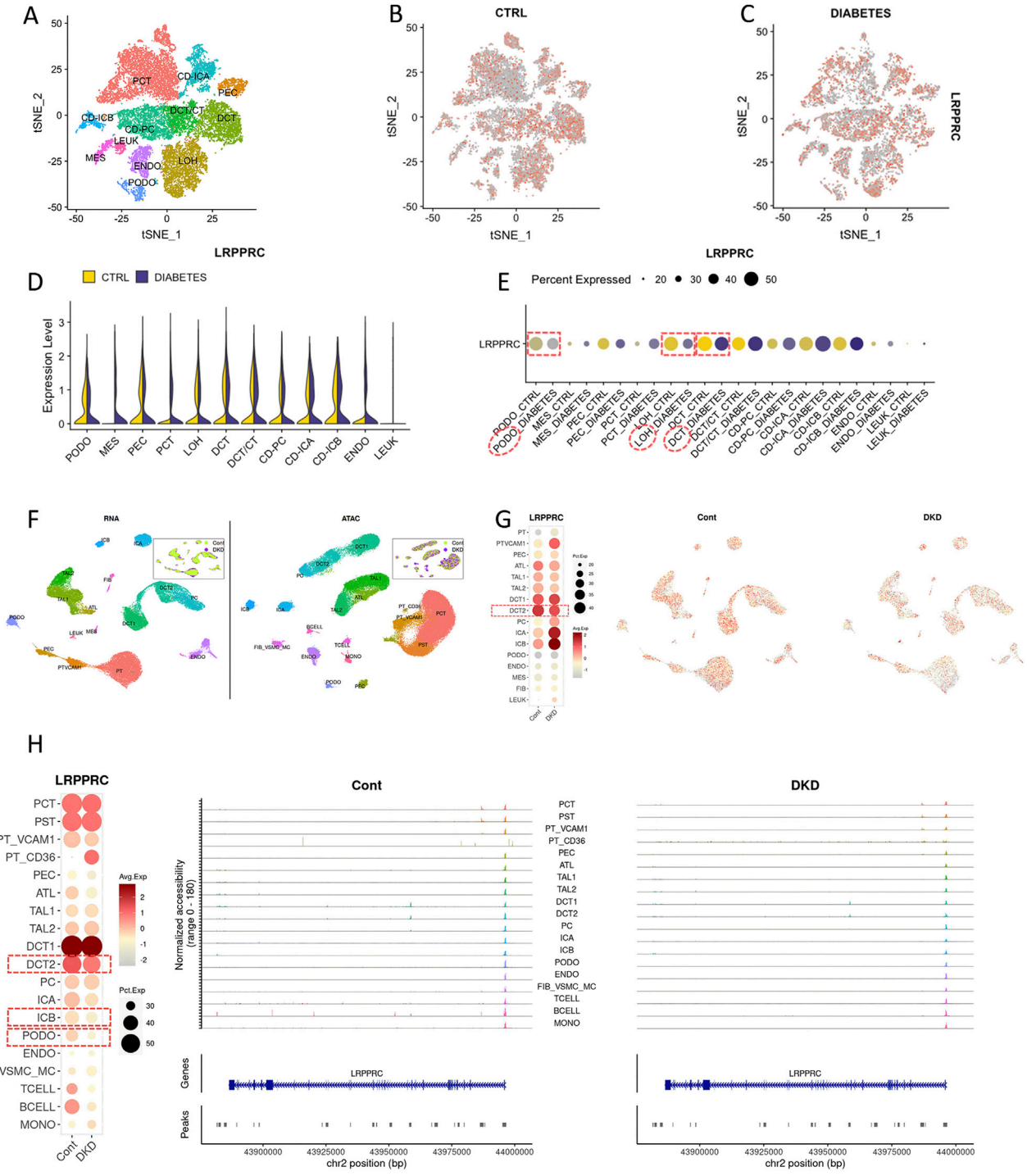


FIGURE 5
Expression of LRPPRC in DKD single-cell (K.I.T database). **(A–E)** LRPPRC in DKD single-cell. **(F–H)** LRPPRC in ATAC-seq.

GSVA showed that the two types of m6a regulation, namely m6a-readers and m6a-writers, were generally suppressed in DKD patients.

The m6A writers include a group of methyltransferases, with the methyltransferase-like 3 (METTL3) and methyltransferase-like 14 (METTL14) being the most well-characterized. They work together to catalyze the addition of methyl groups to the adenosine residues in RNA (Ries et al., 2019). Although some m6A writers, such as METTL5 and ZC3H proteins, are not directly involved in the regulation of m6a methylation (Turkalj and Vissers, 2022), However, some studies have shown that they recruit the m6A methyltransferase complex to

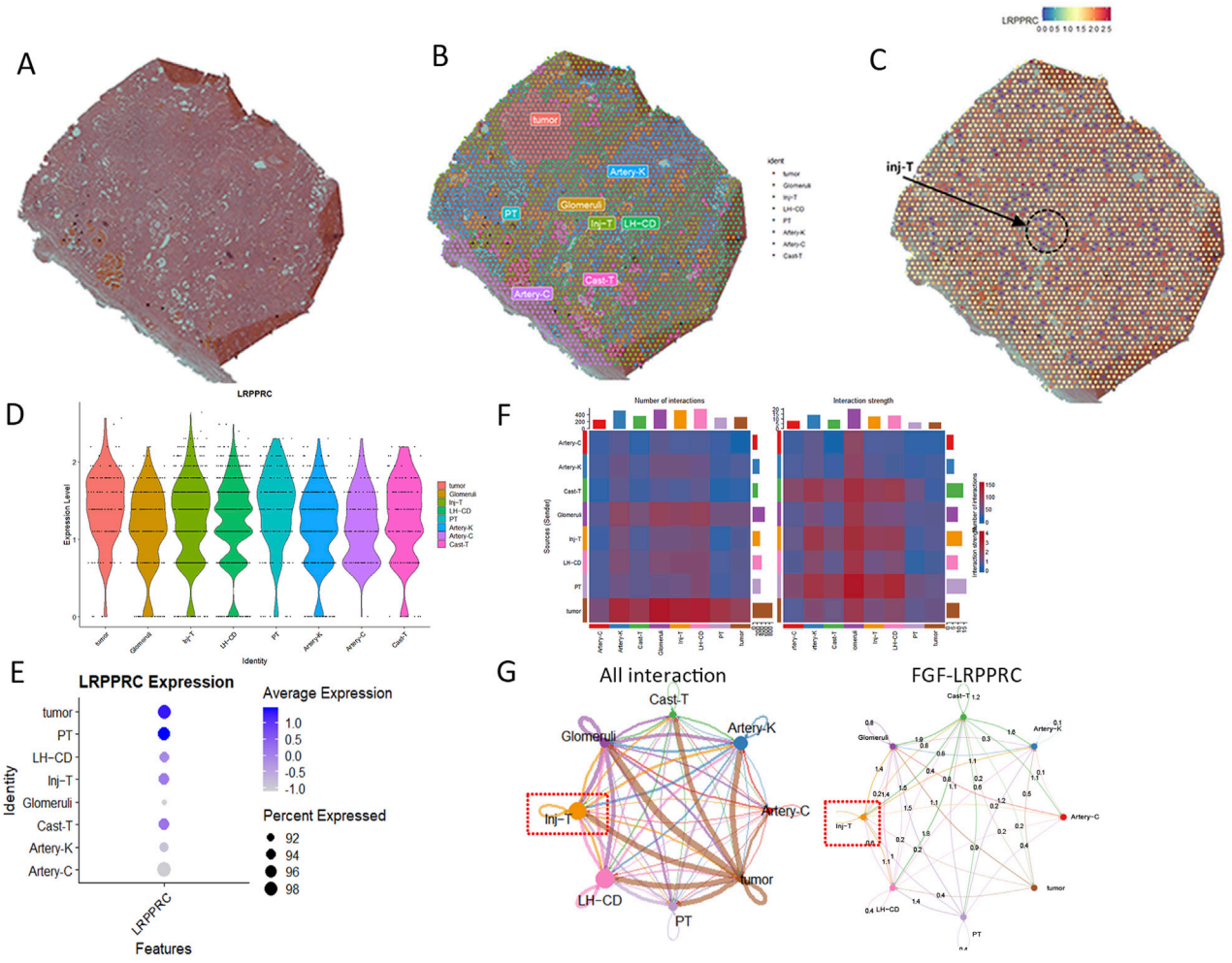


FIGURE 6
Expression of LRPPRC in spatial district of DKD (GSE261545). **(A)** raw kidney tissue slides. **(B)** The annotation of spatial section. **(C)** The distribution of LRPPRC in spatial section. **(D)** The violin plot of LRPPRC quantity in spatial section. **(E)** The dot plot of LRPPRC quantity in spatial section. **(F)** The overall cell chat between spatial section. **(G)** The role of FGF-LRPPRC in cell communication.

target mRNA, so they were widely included in bioinformatics research (Huang et al., 2022; Jiang et al., 2023). The m6A readers include proteins such as YTH domain-containing proteins (YTHDF1, YTHDF2, and YTHDF3), HNRNPA2B1, and others that recognize and bind to m6A-modified RNA. These readers play pivotal roles in interpreting the m6A signals, regulating RNA fate, and thereby influencing gene expression (Lee et al., 2021).In DKD, the suppression of m6A writers and readers suggests a dysregulation in RNA metabolism. This disorder will lead to epithelial-to-mesenchymal transition (EMT) and aggravate the progression of renal fibrosis by regulating TGF-β signaling pathway, affecting the stability of lncRNA and miRNA, and activating the expression of extracellular matrix-related genes (Liu, 2024).

The output of 3 machine learning algorithm and differential analysis has determined the LRPPRC as the hub gene. LRPPRC (Leucine-Rich Pentatricopeptide Repeat Containing Protein) is a multifunctional protein that plays crucial roles in RNA metabolism, mitochondrial function, and cellular stress responses. It is primarily involved in the stabilization, transport, and translation of mitochondrial mRNAs, ensuring proper mitochondrial protein synthesis, which is vital for energy metabolism and overall cellular health (Liu and McKeehan, 2002).The knockout of LRPPRC in mice causes an additive effect on mitochondrial translation leading to embryonic lethality and reduced growth of mouse embryonic fibroblasts (Rubalcava-Gracia et al., 2024). In T2DM, the expression of LRPPRC was differentially expressed in pancreatic, liver, adipose tissue, blood and skeletal muscle (Li et al., 2024). The predictive model containing LRPPRC (AUC = 0.806) were used as the diagnostic basis of diabetic retinopathy (Wang et al., 2023). In the context of DKD, studies have indicated that the downregulation of LRPPRC may disrupt mitochondrial mRNA stability and translation, leading to mitochondrial dysfunction (Ruzzenente et al., 2012). Mitochondrial impairment is associated with increased oxidative stress, which can exacerbate kidney injury through various mechanisms, including inflammation, fibrosis, and apoptosis of renal cells

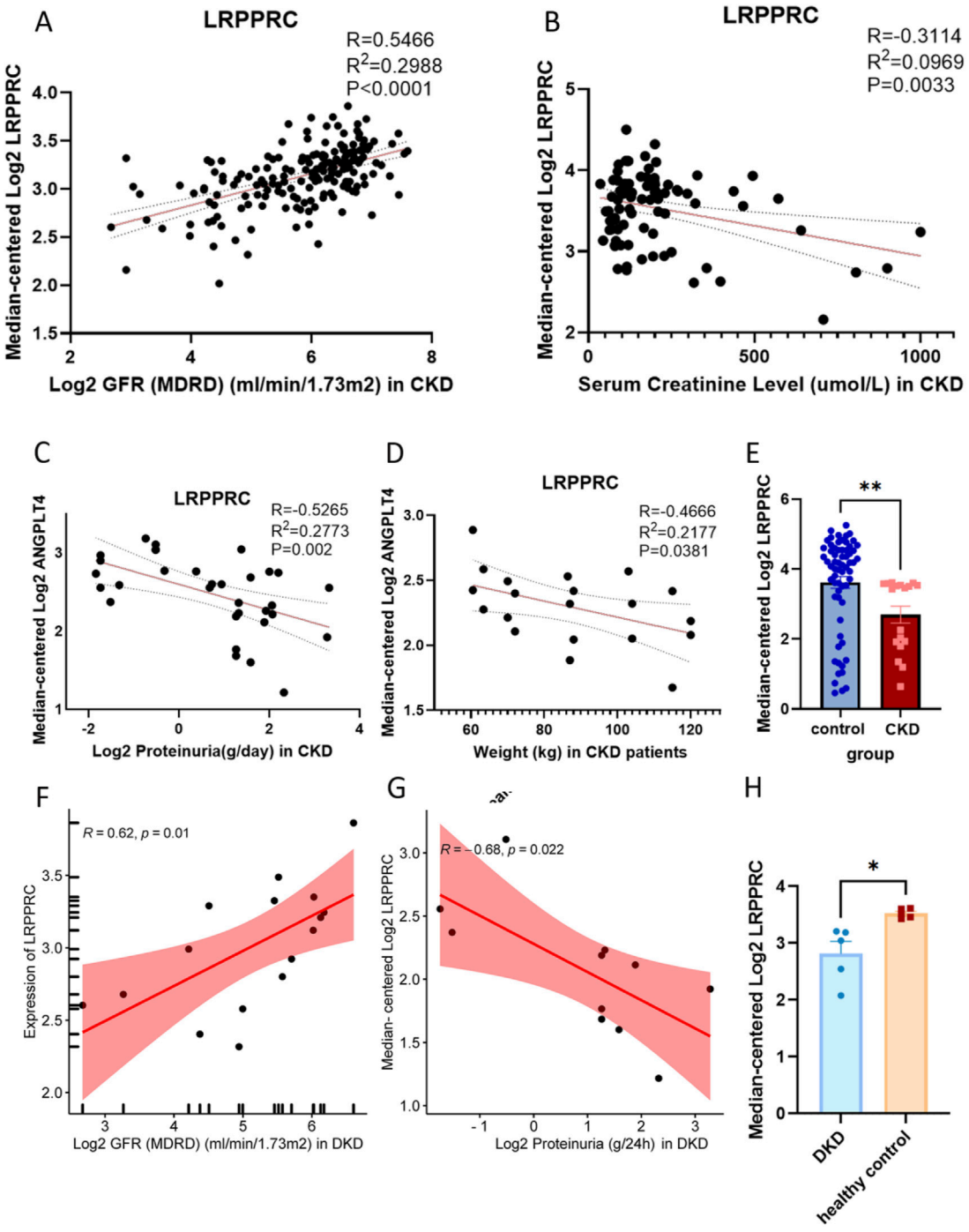


FIGURE 7
The expression of LRPPRC mRNA in clinical patients. **(A)** The association between LRPPRC mRNA and GFR in CKD patients. **(B)** The association between LRPPRC mRNA and serum creatinine level in CKD patients. **(C)** The association between LRPPRC mRNA and proteinuria in CKD patients. **(D)** The association between LRPPRC mRNA and weight in CKD patients. **(E)** The expression of LRPPRC mRNA in CKD patients. **(F)** The association between LRPPRC mRNA and GFR in DKD patients. **(G)** The association between LRPPRC mRNA and proteinuria in DKD patients. **(H)** The expression of LRPPRC mRNA in DKD patients. *P < 0.05, unpaired t-test method, **P < 0.01, unpaired t-test method.

(Liu et al., 2024). In our study, expression levels of LRPPRC mRNA were notably downregulated in DKD patients, exhibiting significant positive correlations with GFR, suggesting its protective effect on kidney function.

LRPPRC was downregulated in the LOH, PODO, CT, and CD-ICB cell populations, most of which were tubular cells. It exhibited the decreasing trend over time, particularly pronounced in LOH cells. The Loop of Henle (LOH) is a critical segment of the kidney and plays a crucial role in urine concentration and electrolyte balance, relying heavily on efficient mitochondrial function (Palm and Carlsson, 2005). The downregulation of LRPPRC in LOH cells may disrupt mitochondrial mRNA stability and exacerbate renal

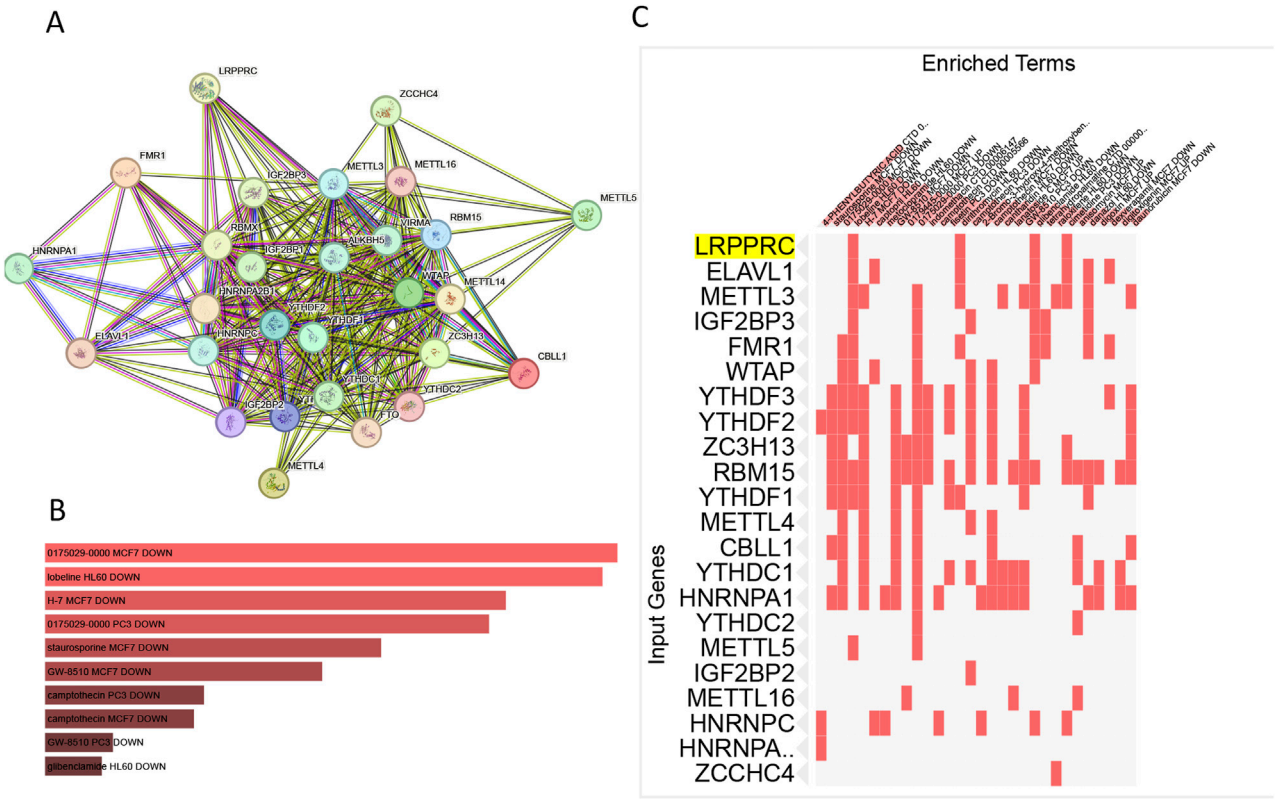


FIGURE 8
The filter of compounds targeting m6a regulation.**(A)** PPI map based on m6a geneset. **(B)** Top 10 drugs that interfere with the m6a gene. **(C)** Drugs targeting LRPPRC gene.

injury. The pseudotime analysis revealed that LRPPRC was a crucial gene during the transition from tubular injury to podocyte damage and a key gene involved in the interaction between injured tubular cells and other types of renal cells. This finding may suggested an early warning role of LRPPRC in the progression of DKD. Traditionally, DKD has been regarded as a disease primarily characterized by glomerular lesions (Wang and Chen, 2022). However, an increasing body of evidence in recent years has demonstrated that changes in tubular function may represent the "driving force" behind the development of DKD (Chatterjee et al., 2025). Tubular injury not only drives the progression of DKD but also exacerbates pre-existing glomerular damage. Some scholars have proposed the "tubule-centric hypothesis" (Zeni et al., 2017), which posits that tubular injury and functional alterations may better explain the pathogenesis and progression of DN. As a potential biomarker of injured renal tubules, LRPPRC is significantly associated with urinary protein levels and glomerular filtration rate in clinical DKD patients, potentially providing a predictive window for early tubular lesions in DKD.

Finally, the lobeline might be an important potential compound involved in the regulation of LRPPRC and other m6a genes. As a compound derived from the plant Lobelia inflata, lobeline has been shown to have various biological effects, including modulation of neurotransmitter release and potential therapeutic benefits in respiratory and neurodegenerative disorders (Remya et al., 2023). At present, there was a lack of research on this drug for endocrine or kidney disease, and its actual efficacy needs to be verified *in vivo* or *in vitro*.

5 Conclusion

In summary, we analyzed the landscape of m6A modification regulators in DKD development and progression. The m6a-readers and m6a-writers, were generally suppressed in DKD patients. the LRPPRC was identified as the hub gene and revealed the good diagnostic potential for eGFR in DKD. The lobeline might be an important potential compound involved in the regulation of LRPPRC and other m6a genes.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/supplementary material.

Author contributions

LJ: Conceptualization, Data curation, Formal Analysis, Investigation, Writing–original draft. HY: Formal Analysis, Investigation, Methodology, Writing–original draft. JJ: Conceptualization, Formal Analysis, Investigation, Methodology, Writing–original draft. XS: Conceptualization, Methodology, Software, Supervision, Validation, Writing–original draft. YW: Validation, Writing – review and editing. YZ: Validation, Writing – review and editing. XW: Funding acquisition, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing–review and editing.

Funding

The author(s) declare financial support was received for the research and/or publication of this article.

This work was supported by Capital's Funds for Health Improvement and Research (No.2024-2-4067).

Acknowledgments

All authors would like to thank the public and shared dataset from the GEO database and express gratitude for the funding.

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.2025.1506896/full#supplementary-material

References

Akhtar, M., Taha, N. M., Nauman, A., Mujeeb, I. B., and Al-Nabet, A. D. M. H. (2020). Diabetic kidney disease: past and present. *Adv. Anat. Pathol.* 27 (2), 87–97. doi:10.1097/PAP.0000000000000257

Barutta, F., Bellini, S., and Canepa, S. (2022). Novel biomarkers of diabetic kidney disease: current status and potential clinical application. *Acta Diabetol.* 59 (3), 439–441. doi:10.1007/s00592-021-01816-5

Chatterjee, A., Tumarin, J., and Prabhakar, S. (2025). Cellular cross-talk drives mesenchymal transdifferentiation in diabetic kidney disease. *Front. Med. (Lausanne)* 11, 1499473. doi:10.3389/fmed.2024.1499473

Eadon, M. T., Lampe, S., Baig, M. M., Collins, K. S., Melo Ferreira, R., Mang, H., et al. (2021). Clinical, histopathologic and molecular features of idiopathic and diabetic nodular mesangial sclerosis in humans. *Nephrol. Dial. Transpl.* 37 (1), 72–84. doi:10.1093/ndt/gfaa331

Fan, Y., Yi, Z., D'Agati, V. D., Sun, Z., Zhong, F., Zhang, W., et al. (2019). Comparison of kidney transcriptomic profiles of early and advanced diabetic nephropathy reveals potential new mechanisms for disease progression. *Diabetes* 68 (12), 2301–2314. doi:10.2337/db19-0204

Hadpech, S., and Thongboonkerd, V. (2024). Epithelial-mesenchymal plasticity in kidney fibrosis. *Genesis* 62 (1), e23529. doi:10.1002/dvg.23529

He, L., Li, H., Wu, A., Peng, Y., Shu, G., and Yin, G. (2019). Functions of N6-methyladenosine and its role in cancer. *Mol. Cancer* 18 (1), 176. doi:10.1186/s12943-019-1109-9

Huang, J., Liu, Y., Shi, M., Zhang, X., Zhong, Y., Guo, S., et al. (2024a). Empagliflozin attenuating renal interstitial fibrosis in diabetic kidney disease by inhibiting lymphangiogenesis and lymphatic endothelial-to-mesenchymal transition via the VEGF-C/VEGFR3 pathway. *Biomed. Pharmacother.* 180, 117589. doi:10.1016/j.biopha.2024.117589

Huang, J., Yang, F., Liu, Y., and Wang, Y. (2024b). N6-methyladenosine RNA methylation in diabetic kidney disease. *Biomed. Pharmacother.* 171, 116185. doi:10.1016/j.biopha.2024.116185

Huang, Q., Mo, J., Liao, Z., Chen, X., and Zhang, B. (2022). The RNA m6A writer WTAP in diseases: structure, roles, and mechanisms. *Cell Death Dis.* 13 (10), 852. doi:10.1038/s41419-022-05268-9

Isnard, P., Li, D., Xuanyuan, Q., Wu, H., and Humphreys, B. D. (2024). Histopathologic analysis of human kidney spatial transcriptomics data: toward precision pathology. *Am. J. Pathol.* 195, 69–88. doi:10.1016/j.ajpath.2024.06.011

Jiang, T., Xing, L., Zhao, L., Ye, Z., Yu, D., and Lin, S. (2023). Comprehensive analysis of m6A related gene mutation characteristics and prognosis in colorectal cancer. *BMC Med. Genomics* 16 (1), 105. doi:10.1186/s12920-023-01509-8

Jiang, X., Liu, B., Nie, Z., Duan, L., Xiong, Q., Jin, Z., et al. (2021). The role of m6A modification in the biological functions and diseases. *Signal Transduct. Target Ther.* 6 (1), 74. doi:10.1038/s41392-020-00450-x

Lang, Y., Wang, Q., Sheng, Q., Lu, S., Yang, M., Kong, Z., et al. (2024). FTO-mediated m6A modification of serum amyloid A2 mRNA promotes podocyte injury and inflammation by activating the NF-κB signaling pathway. *FASEB J.* 38 (2), e23409. doi:10.1096/fj.202301419RR

Lee, J. H., Wang, R., Xiong, F., Krakowiak, J., Liao, Z., Nguyen, P. T., et al. (2021). Enhancer RNA m6A methylation facilitates transcriptional condensate formation and gene activation. *Mol. Cell* 81 (16), 3368–3385.e9. doi:10.1016/j.molcel.2021.07.024

Li, B., Zhu, C., Dong, L., Qin, J., Xiang, W., Davidson, A. J., et al. (2020). ADAM10 mediates ectopic proximal tubule development and renal fibrosis through Notch signalling. *J. Pathol.* 252 (3), 274–289. doi:10.1002/path.5517

Li, J., Shi, S., Srivastava, S. P., Kitada, M., Nagai, T., Nitta, K., et al. (2017). FGFR1 is critical for the anti-endothelial mesenchymal transition effect of N-acetyl-seryl-aspartyl-lysyl-proline via induction of the MAP4K4 pathway. *Cell Death Dis.* 8 (8), e2965. doi:10.1038/cddis.2017.353

Li, Y. L., Zhang, Y., Chen, N., and Yan, Y. X. (2024). The role of m6A modification in type 2 diabetes: a systematic review and integrative analysis. *Gene* 898, 148130. doi:10.1016/j.gene.2024.148130

Liu, D. F., Chen, X. J., He, W. T., Lu, M., Li, Q., Zhang, S., et al. (2024). Update on the pathogenesis, diagnosis, and treatment of diabetic tubulopathy. *Integr. Med. Nephrol. Androl.* 11 (4), e23–e00029. doi:10.1097/IMNA-D-23-00029

Liu, L., and McKeehan, W. L. (2002). Sequence analysis of LRPPRC and its SEC1 domain interaction partners suggests roles in cytoskeletal organization, vesicular trafficking, nucleocytosolic shuttling, and chromosome activity. *Genomics* 79 (1), 124–136. doi:10.1006/geno.2001.6679

Liu, S., Xiu, J., Zhu, C., Meng, K., Li, C., Han, R., et al. (2021). Fat mass and obesity-associated protein regulates RNA methylation associated with depression-like behavior in mice. *Nat. Commun.* 12 (1), 6937. doi:10.1038/s41467-021-27044-7

Liu, Y. H. (2024). Kidney fibrosis: fundamental questions, challenges, and perspectives. *Integr. Med. Nephrol. Androl.* 11 (4), e24–e00027. doi:10.1097/IMNA-D-24-00027

Nady, M. E., Abd El-Raouf, O. M., and El-Sayed, E. M. (2024). Linagliptin mitigates TGF-β1 mediated epithelial-mesenchymal transition in tacrolimus-induced renal interstitial fibrosis via smad/ERK/P38 and HIF-1α/LOXL2 signaling pathways. *Biol. Pharm. Bull.* 47 (5), 1008–1020. doi:10.1248/bpb.b23-00737

Oerum, S., Meynier, V., Catala, M., and Tisné, C. (2021). A comprehensive review of m6A/m6Am RNA methyltransferase structures. *Nucleic Acids Res.* 49 (13), 7239–7255. doi:10.1093/nar/gkab378

Palm, F., and Carlsson, P. O. (2005). Thick ascending tubular cells in the loop of Henle: regulation of electrolyte homeostasis. *Int. J. Biochem. Cell Biol.* 37 (8), 1554–1559. doi:10.1016/j.biocel.2005.02.007

Park, S., Lee, H., Lee, J., Cho, S., Huh, H., et al. (2022). RNA-seq profiling of tubulointerstitial tissue reveals a potential therapeutic role of dual anti-phosphatase 1 in glomerulonephritis. *J. Cell Mol. Med.* 26 (12), 3364–3377. doi:10.1111/jcmm.17340

Remya, C., Dileep, K. V., Variyar, E. J., Omkumar, R. V., and Sadasivan, C. (2023). Lobeline: a multifunctional alkaloid modulates cholinergic and glutamatergic activities. *IUBMB Life* 75 (10), 844–855. doi:10.1002/iub.2762

Ries, R. J., Zaccara, S., Klein, P., Olarerin-George, A., Namkoong, S., Pickering, B. F., et al. (2019). m6A enhances the phase separation potential of mRNA. *Nature* 571 (7765), 424–428. doi:10.1038/s41586-019-1374-1

Rubalcava-Gracia, D., Bubb, K., Levander, F., Burr, S. P., August, A. V., Chinnery, P. F., et al. (2024). LRPPRC and SLIRP synergize to maintain sufficient and orderly mammalian mitochondrial translation. *Nucleic Acids Res.* 52, 11266–11282. doi:10.1093/nar/gkae662

Ruzzenente, B., Metodiev, M. D., Wredenberg, A., Bratic, A., Park, C. B., Cámara, Y., et al. (2012). LRPPRC is necessary for polyadenylation and coordination of translation of mitochondrial mRNAs. *EMBO J.* 31 (2), 443–456. doi:10.1038/emboj.2011.392

Shen, L., Xu, X., Yue, S., and Yin, S. (2024). A predictive model for depression in Chinese middle-aged and elderly people with physical disabilities. *BMC Psychiatry* 24 (1), 305. doi:10.1186/s12888-024-05766-4

Shi, H., Wei, J., and He, C. (2019). Where, when, and how: context-dependent functions of RNA methylation writers, readers, and erasers. *Mol. Cell.* 74 (4), 640–650. doi:10.1016/j.molcel.2019.04.025

Srivastava, S. P., and Goodwin, J. E. (2023). Loss of endothelial glucocorticoid receptor accelerates organ fibrosis in db/db mice. *Am. J. Physiol. Ren. Physiol.* 325 (4), F519–F526. doi:10.1152/ajprenal.00105.2023

Srivastava, S. P., Goodwin, J. E., Kanasaki, K., and Koya, D. (2020). Metabolic reprogramming by N-acetyl-seryl-aspartyl-lysyl-proline protects against diabetic kidney disease. *Br. J. Pharmacol.* 177 (16), 3691–3711. doi:10.1111/bph.15087

Srivastava, S. P., Li, J., Kitada, M., Fujita, H., Yamada, Y., Goodwin, J. E., et al. (2018). SIRT3 deficiency leads to induction of abnormal glycolysis in diabetic kidney with fibrosis. *Cell Death Dis.* 9 (10), 997. doi:10.1038/s41419-018-1057-0

Srivastava, S. P., Zhou, H., Setia, O., Liu, B., Kanasaki, K., Koya, D., et al. (2021). Loss of endothelial glucocorticoid receptor accelerates diabetic nephropathy. *Nat. Commun.* 12 (1), 2368. doi:10.1038/s41467-021-22617-y

Srivastava, S. P., Zhou, H., Shenoi, R., Morris, M., Lainez-Mas, B., Goedeke, L., et al. (2024). Renal Angptl4 is a key fibrogenic molecule in progressive diabetic kidney disease. *Sci. Adv.* 10 (49), eadn6068. doi:10.1126/sciadv.adn6068

Sun, Y., Liu, G., Li, M., Wang, L., He, Z., and Gu, S. (2023). Study on the correlation between regulatory proteins of N6-methyladenosine and oxidative damage in cadmium-induced renal injury. *Biol. Trace Elem. Res.* 201 (5), 2294–2302. doi:10.1007/s12011-022-03345-w

Turkalj, E. M., and Vissers, C. (2022). The emerging importance of METTL5-mediated ribosomal RNA methylation. *Exp. Mol. Med.* 54 (10), 1617–1625. doi:10.1038/s12276-022-00869-y

Wang, F., Bai, J., Zhang, X., Wang, D., Xue, J., et al. (2024). METTL3/YTHDF2 m6A axis mediates the progression of diabetic nephropathy through epigenetically suppressing PINK1 and mitophagy. *J. Diabetes Investig.* 15 (3), 288–299. doi:10.1111/jdi.14113

Wang, X., Li, X., Zong, Y., Yu, J., Chen, Y., Zhao, M., et al. (2023). Identification and validation of genes related to RNA methylation modification in diabetic retinopathy. *Curr. Eye Res.* 48 (11), 1034–1049. doi:10.1080/02713683.2023.2238144

Wang, Y. F., and Chen, H. Y. (2022). Ferroptosis in diabetic nephropathy: a narrative review. *Integr. Med. Nephrol. Androl.* 9 (1), 1. doi:10.4103/imna.imna_2_22

Wei, Y., Wei, H., Wei, Y., Tan, A., Chen, X., Liao, X., et al. (2022). Screening and identification of human endogenous retrovirus-K mRNAs for breast cancer through integrative analysis of multiple datasets. *Front. Oncol.* 12, 820883. doi:10.3389/fonc.2022.820883

Wilson, P. C., Muto, Y., Wu, H., Karihaloo, A., Waikar, S. S., and Humphreys, B. D. (2022). Multimodal single cell sequencing implicates chromatin accessibility and genetic background in diabetic kidney disease progression. *Nat. Commun.* 13 (1), 5253. doi:10.1038/s41467-022-32972-z

Wilson, P. C., Wu, H., and Kirita, Y. (2019). The single-cell transcriptomic landscape of early human diabetic nephropathy. *Proc. Natl. Acad. Sci. U. S. A.* 116(39): 19619–19625. doi:10.1073/pnas

Xu, Z., Lv, B., Qin, Y., and Zhang, B. (2022). Emerging roles and mechanism of m6A methylation in cardiometabolic diseases. *Cells* 11 (7), 1101. doi:10.3390/cells11071101

Yao, L., Liang, X., Liu, Y., Li, B., Hong, M., Wang, X., et al. (2023). Non-steroidal mineralocorticoid receptor antagonist finerenone ameliorates mitochondrial dysfunction via PI3K/Akt/eNOS signaling pathway in diabetic tubulopathy. *Redox Biol.* 68, 102946. doi:10.1016/j.redox.2023.102946

Ye, W., Lv, X., Gao, S., Li, Y., Luan, J., and Wang, S. (2023). Emerging role of m6A modification in fibrotic diseases and its potential therapeutic effect. *Biochem. Pharmacol.* 218, 115873. doi:10.1016/j.bcp.2023.115873

Zeni, L., Norden, A. G. W., Cancarini, G., and Unwin, R. J. (2017). A more tubulocentric view of diabetic kidney disease. *J. Nephrol.* 30 (6), 701–717. doi:10.1007/s40620-017-0423-9

frontiers | Frontiers in Pharmacology

TYPE Systematic Review
PUBLISHED 14 April 2025
DOI 10.3389/fphar.2025.1461792

Check for updates

OPEN ACCESS

EDITED BY
Swayam Prakash Srivastava,
University of Michigan, United States

REVIEWED BY
Osama Ashry Gheith,
Mansoura University, Egypt
Fang-Fang He,
Huazhong University of Science and Technology, China

*CORRESPONDENCE
Wenpeng Cui,
wenpengcui@163.com

RECEIVED 09 July 2024
ACCEPTED 28 February 2025
PUBLISHED 14 April 2025

CITATION
Meng L, Liu S and Cui W (2025) Renal protective effects of vitamin E for drug-induced kidney injury: a meta-analysis.
Front. Pharmacol. 16:1461792.
doi: 10.3389/fphar.2025.1461792

COPYRIGHT
© 2025 Meng, Liu, and Cui. 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.

Renal protective effects of vitamin E for drug-induced kidney injury: a meta-analysis

Lingfei Meng, Shengmao Liu and Wenpeng Cui*

Department of Nephrology, The Second Hospital of Jilin University, Changchun, Jilin Province, China

Introduction: Acute kidney injury (AKI) is a key clinical condition that has puzzled clinicians for many years since there is currently no efficient drug therapy. Vitamin E is found to exert a vital antioxidant role and can protect the kidney. However, clinical studies that analyze the correlation between vitamin E and AKI are scarce, and no consistent conclusions are reported from current studies. Therefore, this study was performed to evaluate the impact of vitamin E on treating AKI.

Methods: The PubMed, Embase, and Cochrane Library databases were comprehensively searched on 27 December 2023. Qualified studies were selected following the eligibility criteria. The incidence of AKI, serum creatinine, and urea nitrogen levels after vitamin E treatment were evaluated. Then, the data were combined with a fixed- or random-effects model, depending on the heterogeneity test results.

Results: Six eligible randomized controlled trials that used vitamin E for the prevention of kidney injury were included. According to our pooled analysis, vitamin E elevated eGFR levels [MD: 0.36; 95% CI (0.19, 0.53), p = 0.000], reduced serum creatinine levels [MD: −0.32; 95% CI (−0.48, 0.16), p = 0.000], and effectively inhibited the occurrence of AKI [RR: 0.69; 95% CI (0.49, 0.98), p = 0.036].

Conclusion: Vitamin E elevates eGFR levels, reduces serum creatinine levels, and efficiently suppresses AKI occurrence.

Systematic Review Registration: https://www.crd.york.ac.uk/PROSPERO/view/CRD42024499597, identifier CRD42024499597

KEYWORDS

vitamin E, nephrotoxin, acute kidney injury, anti-oxidation, meta

Introduction

Acute kidney injury (AKI) is a common yet severe condition that affects millions of people and causes disability and mortality in many sufferers (Kellum et al., 2013). AKI is defined by the AKI Network by at least one or more conditions: (1) elevation of serum creatinine (Scr) level by 0.3 mg/dL (26.5 mol/L) in 48 h; (2) increase of Scr level to 1.5 folds of baseline over the past week; (3) urine volume <0.5 mL/kg/h during 6 h (Mehta et al., 2007). AKI exhibits a high and growing incidence rate globally (Hoste et al., 2018; Ronco et al., 2019). It has been reported in 7.1% of patients receiving immune checkpoint inhibitor treatment (Ji et al., 2022). In addition, 22.4% of patients develop AKI within 48 h after surgery (Gameiro et al., 2020). The presence of AKI affects acute morbidity and mortality and is related to unfavorable long-time outcomes, including an increased risk of

cardiovascular complications, long-term mortality, and development of (or progression to) chronic kidney disease (CKD) (Hoste et al., 2018; Pickkers et al., 2021; James et al., 2020).

Nonetheless, effective interventions for AKI remain limited, highlighting the critical role of therapeutic drug monitoring as a cornerstone strategy in mitigating drug-associated nephrotoxicity (Ostermann et al., 2020). Based on clinical trials, early RRT implementation may not be beneficial for the survival of patients with no obvious urgent indications (Zarbock et al., 2016; Bagshaw, 2020; Gaudry et al., 2020). Implementing care bundles of AKI among inpatients can decrease the risk of moderate to severe AKI (Schaubroeck et al., 2021). AKI may be cured with early detection and treatment (Yue et al., 2023). Until now, there have been no drugs and treatments for specifically preventing or treating AKI in humans (Pickkers et al., 2021; Fang et al., 2021a).

As a fat-soluble vitamin, vitamin E exerts a critical antioxidant impact on the human body. As an essential nutrient, it was first identified by Evans and Bishop (1922) and was initially called "anti-sterility factor" or "factor X," according to its effect on rat reproduction. It is composed of a variety of compounds, and there are eight isomers, including four tocopherols (α-, β-, γ-, and δ-tocopherol) and four tocotrienols (α-, β-, γ-, and δ-tocotrienol) in the vitamin E family (Miyazawa et al., 2019). Currently, most studies concerning vitamin E metabolism pay more attention to alpha- and gamma-tocopherols because these are bioavailable and can be more easily absorbed from diets than other vitamin E isomers (Wu and Croft, 2007).

Unstable free radical molecules may induce oxidative stress and damage cells and tissues in the body. Vitamin E, containing the chromanol hydroxyl group, can chemically scavenge free radical oxidants. Previous *in vitro* results indicate that alpha-tocopherol can prevent lipid peroxidation catalyzed by free radicals (Esterbauer et al., 1989). AKI may result from various stimuli, among which reactive oxygen species (ROS) are shown to exert a vital impact on AKI, which can induce injuries to proteins, DNA, and carbohydrates (Salehipour et al., 2010). Nonetheless, vitamin E may mitigate AKI symptoms (Koga et al., 2012; Kataoka et al., 2012; Liu et al., 2015). Additionally, emerging mechanistic studies indicate that vitamin E significantly suppresses ROS and ameliorates AKI (Zhang et al., 2024; Georgiev et al., 2023). Apart from its antioxidant effect, vitamin E can regulate gene expression, resist inflammation, and protect the kidneys (Zaaboul and Liu, 2022).

The conclusions drawn from current studies on the role of vitamin E in protecting AKI are still inconsistent (Cho et al., 2017; Xu et al., 2018; Monami et al., 2021). To our knowledge, there is currently no meta-analysis that explores the effect of vitamin E on drug-induced AKI. Therefore, this study aimed to investigate the correlation between vitamin E and drug-induced AKI in randomized controlled trials (RCTs) performed with humans.

Methods

Search methods

The PubMed, Cochrane Library, and Embase databases were searched from inception to 27 December 2023 to find published RCTs on the use of vitamin E in high-risk AKI patients with available kidney function outcomes. Keywords included "contrast induced kidney injury," "renal ischemia reperfusion injury," "acute renal failure," "nephrotoxicity," "AKI," "kidney damage," "renal function," "vitamin E," "tocopherols," "tocopherylquinone," "tocotrienols," and "tocophersolan." The research was limited to human studies, and only English language manuscripts were included. Abstracts of all articles were reviewed to develop a full reference list. In addition, references in related original and review studies were checked to manually identify qualified RCTs. This study is registered with PROSPERO (number CRD42024499597).

Study selection

In the current meta-analysis, two investigators (Lingfei Meng and Shengmao Liu) independently conducted title- and abstract-screening of eligible publications. To identify whether these studies satisfied our preset eligibility criteria, the full texts of those screened articles were downloaded. Any disagreements between them were settled by mutual negotiation with a third investigator (Wenpeng Cui).

Eligibility criteria

Inclusion criteria

The inclusion criteria were (1) RCTs with a crossover or parallel design; (2) studies exploring the role of vitamin E in kidney function in human; (3) studies including patients aged ≥14 years; (4) the use of vitamin E in the treatment group; (5) studies setting a placebo or control group; (6) studies with primary outcomes of Scr, blood urea nitrogen (BUN), eGFR, or AKI; (7) studies published in English.

Types of studies

RCTs evaluating the application of vitamin E in renoprotection in humans were included.

Types of study populations

The study populations included adults with AKI associated with drug application in humans.

Types of interventions

All vitamin E types compared with control or placebo were involved, with no limitation of dosage, dosage form, vitamin E application time, or duration.

Types of outcomes

Primary outcomes were probabilities of AKI, eGFR, Scr, and BUN to evaluate kidney function.

Exclusion criteria

The following exclusion criteria were applied: (1) studies in which vitamin E was accompanied by other interventions, uncontrolled clinical trials, conference abstracts, book chapters, comments, interviews, opinion pieces, position papers, methodological papers, letters, editorials, and animal or cell culture studies; (2) studies irrelevant to drugs related kidney injury; (3) studies not reporting relevant outcomes, mean or

TABLE 1 Characteristics of primary analysis of the included trials.

Year	Author	Study design	Age	Duration	Study population	Group	Intervention	Sample size	AKI	SCR(mean ± SD), μmol/L	BUN(mean ± SD), mmol/L	eGFR, mL/min
2020	Farzaneh Ashrafi	RCT	Age ≥18 years	3 weeks	Cisplatin-based chemotherapy candidates	Treatment	400 IU vitamin E daily	26		66.3 ± 8.84		107.6 ± 18
						Control	Placebo	25		75.14 ± 17.68		93.3 ± 24.6
2023	Mahdi Moradi Goudarzi	RCT	Aged 14–60	30 days	Patients with thalassemia major	Treatment	400 IU vitamin E daily	23				162.77 ± 67.6
						Control	No treatment	27				132.58 ± 35.45
2021	Maryam Samsami	RCT	Age ≥18 years old	At least 7 days	Patients underwent colistin therapy	Treatment	400 IU alpha-tocopherol daily	26	12			
						Control	No treatment	26	11			
2013	Adis Tasanarong	RCT	Adult	48 h	CKD patients with contrast media	Treatment	α-Tocopherol (350 mg/day)	102	13	140.56 ± 53.92		47 ± 16
						Treatment	γ-Tocopherol (300 mg/day)	102	16	133.48 ± 92.11		49 ± 19
						Control	Placebo	101	24	1.77 mg/dL ± 0.85		43 ± 17
2012	Thomas M. Kitzler	RCT	Age ≥18 years old	12 h and 6 h before and after the CT	CKD with contrast-induced kidney injury	Treatment control	2060 mg vitamin E emulsion	9		120.22 ± 19.45		50.9 ± 11.3
							Placebo emulsion	10		120.22 ± 9.72		45.6 ± 5.8
2009	Adis Tasanarong	RCT	Adult	48, 24 h, and the morning before coronary procedures	The patients with contrast media	Treatment	525 IU alpha-tocopherol daily	51		144.98 ± 52.16	22 ± 9	
						Control	Placebo	52		167.96 ± 76.91	27 ± 13	

10.3389/fphar.2025.1461792

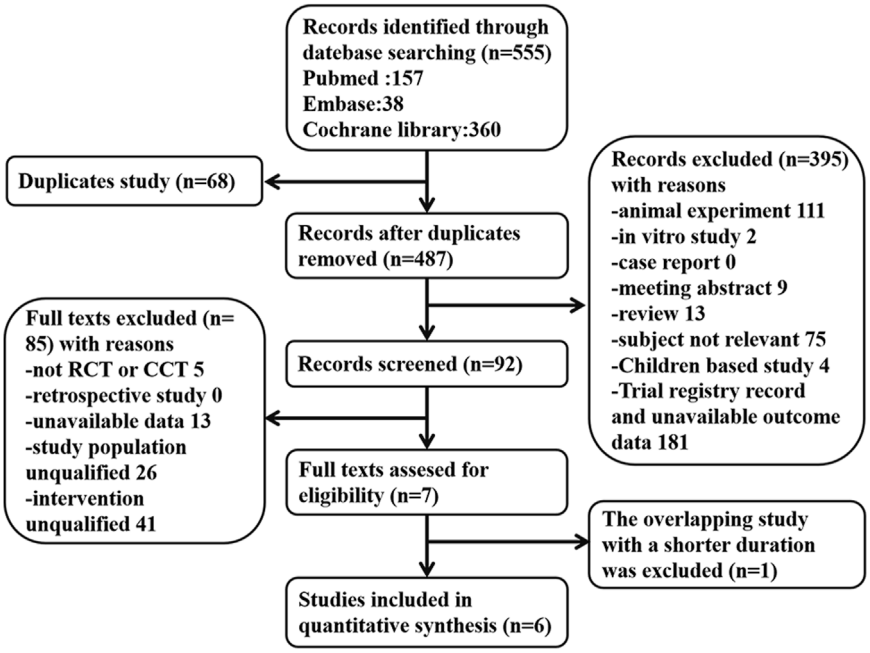


FIGURE 1
Flow diagram and description of the study selection process.

median and standard deviation (SD), and the number of AKI episodes; (3) studies with *in vitro* experiments and animal experiments alone; and (4) non-available articles.

Data collection

Data were collected from eligible RCTs by two investigators (Lingfei Meng and Shengmao Liu) independently. Any disagreements between them were settled by negotiation with a third investigator (Wenpeng Cui). These relevant data were collected from each study (Table 1): (1) study features (first author, publication year); (2) study population; (3) interventions (grouping, dosage and time, route, timing, and duration of administration); and (4) eGFR, BUN, and Scr data, as well as the number of AKI episodes. In addition, the means, SD, AKI episodes, and population size in each group were collected.

Assessment of risk of bias

To examine the overall study quality, two reviewers (Lingfei Meng and Shengmao Liu) assessed the bias risk using RevMan 5.3. Bias was marked as high, low, or "unclear" (indicating that the bias risk was unknown). Any disagreement was settled by discussion with a third reviewer (Wenpeng Cui).

Statistical analysis

If two or more studies presented the findings of an identical outcome, we summarized data and assessed the outcomes (eGFR, BUN, Scr, and AKI). Initially, we performed meta-analysis of articles comparing vitamin E with control groups, using Review Manager 5.3 software and Stata SE 15 for data analysis. The results presented in the current report were mean difference (MD) of measurements with the same unit or standardized mean difference (SMD) with different units. To evaluate the therapeutic effect of vitamin E, the pooled data were employed. In addition, I^2 statistic was used to assess heterogeneity among our enrolled articles. A fixed-effect model should be applied in the case of insignificant heterogeneity (i.e., $p > 0.1$; $I^2 \leq 50\%$); otherwise, a random-effect model was applied.

Results

Characteristics of eligible studies

A total of 555 articles were acquired through literature search and later screened following the inclusion and exclusion criteria. If two reports covered overlapping studies, the one involving more samples and/or having a longer study period was chosen. There were six eligible RCTs concerning vitamin E for the prevention of kidney injury. Of these trials, kidney function was applied as the primary or secondary outcome. Contrast media-induced kidney injury was reported in three RCTs. Other drug-induced kidney injuries were reported in three RCTs. AKI was reported in two studies, while eGFR, Scr, and BUN were reported in four, four, and one study, respectively. Figure 1 summarizes the screening process of eligible clinical trials. Finally, six RCTs involving 580 participants were included for analysis. All patients in these studies received vitamin E treatment, and the follow-up time in all studies ranged from 1 day to 2 months. Table 1 presents the characteristics of the studies we included on analyzing kidney protection.

Quality assessment

The methodological features most associated with the risk of bias below were assessed, including randomization, allocation

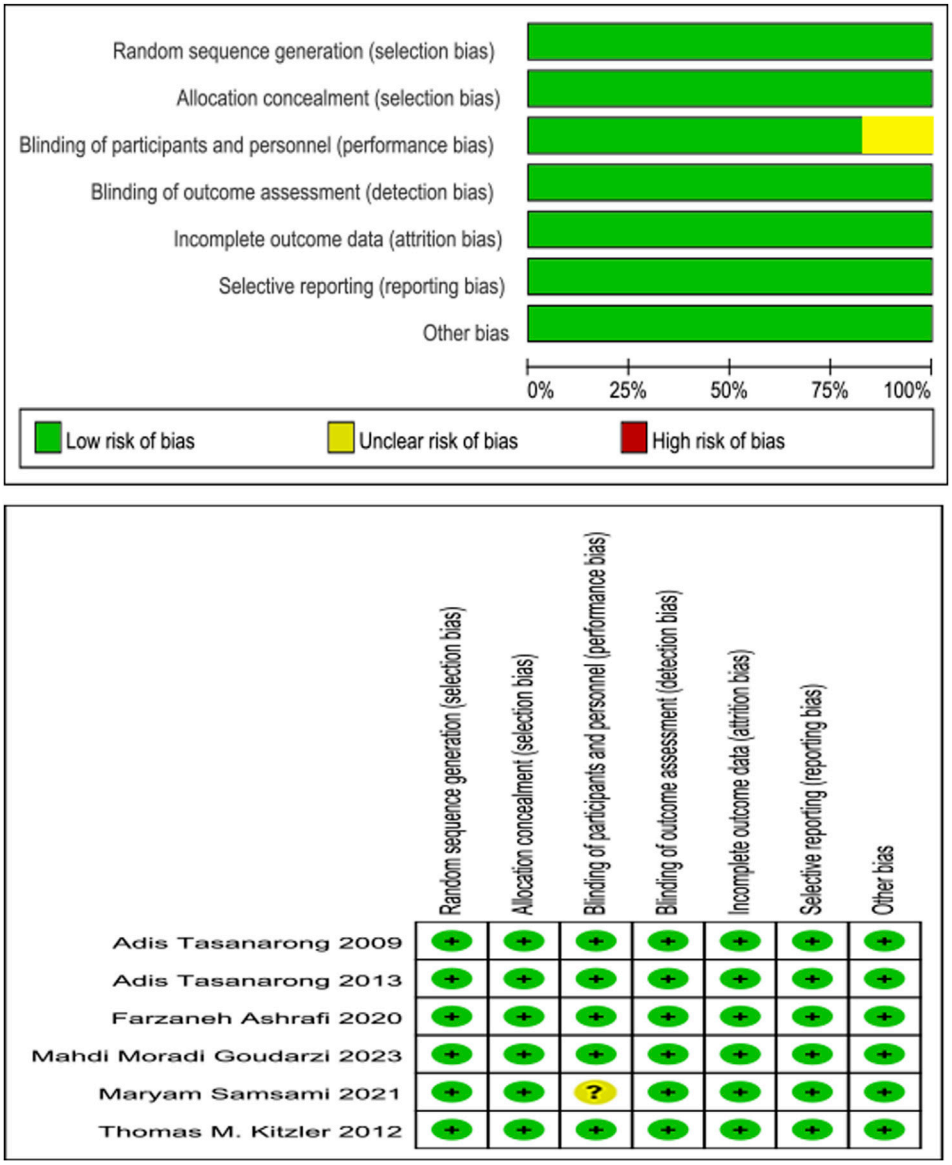


FIGURE 2
Chart and summary of the bias risk.

concealment, treatment allocation blinding, outcome assessment blinding, incomplete outcome data and selective reporting, and other biases. With study quality, five of these six enrolled RCTs met seven of the above criteria, and one of these six enrolled RCTs met six (Figure 2).

Effects of interventions

Primary outcome: AKI

Two RCTs (Tasanarong et al., 2013; Samsami et al., 2021) satisfied our inclusion criteria. One study included both α- and γ-tocopherol in the treatment group, and thus study data were extracted twice. Due to the absence of significant heterogeneity, a fixed-effects model ($p = 0.244$, $I^2 = 29.1\%$) was used. After combined analysis of these two studies, the AKI incidence of the vitamin E group significantly decreased compared to the control group [RR: 0.69; 95% CI (0.49, 0.98), $p = 0.036$] (Figure 3A). The galbr plot exhibited little heterogeneity between the three outcomes of the two studies (Figure 3B). Based on sensitivity analysis, no study exerted a disproportionate impact on the outcomes (Figure 3C).

Primary outcome: eGFR

Four RCTs (Tasanarong et al., 2013; Goudarzi et al., 2023; Ashrafi et al., 2020; Kitzler et al., 2012) met our inclusion criteria. One study included both α- and γ-tocopherol in the treatment group; thus, study data were extracted twice. A fixed-effects model was employed since no significant heterogeneity was detected ($p = 0.627$, $I^2 = 0\%$). According to pooled results from these four RCTs, the change of serum eGFR from baseline to follow-up of vitamin E group significantly decreased compared with the control group [MD: 0.36; 95%

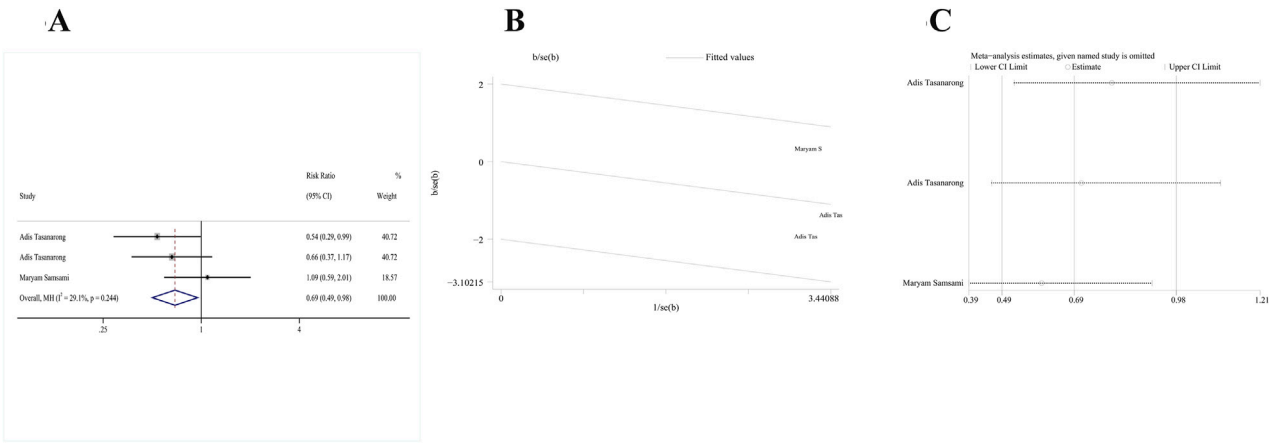


FIGURE 3
Comparison of efficacy between vitamin E and placebo in AKI. **(A)** Forest map. **(B)** Test of heterogeneity by the galbr plot. **(C)** Sensitivity analysis.

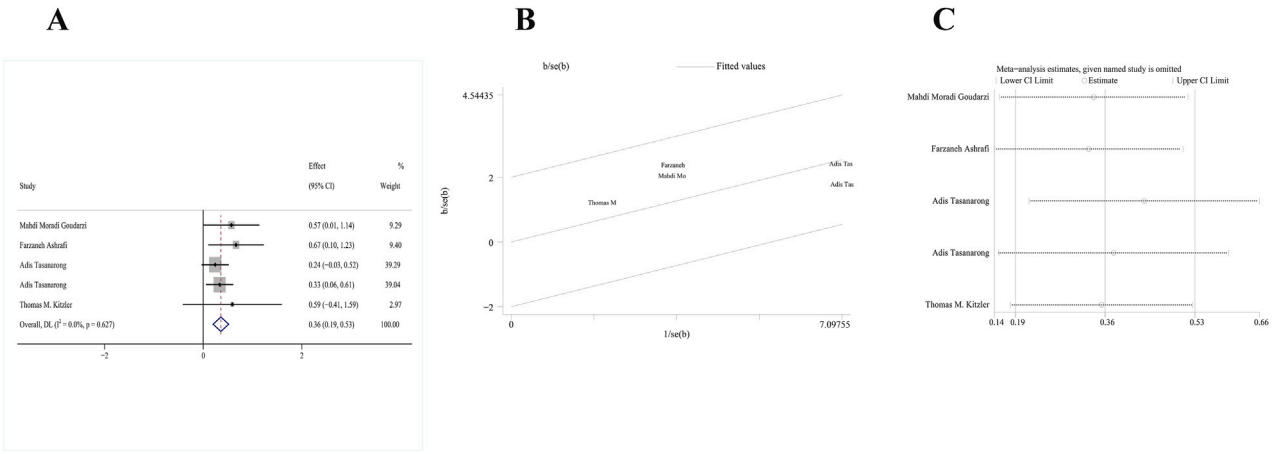


FIGURE 4
Comparison of efficacy between vitamin E and placebo in eGFR. **(A)** Forest map. **(B)** Test of heterogeneity by the galbr plot. **(C)** Sensitivity analysis.

CI (0.19, 0.53), p = 0.000] (Figure 4A). In addition, the galbr plot showed little heterogeneity between the included studies (Figure 4B). According to sensitivity analysis, no study exerted a disproportionate effect on the outcome (Figure 4C).

Primary outcome: Scr

Four RCTs (Tasanarong et al., 2013; Ashrafi et al., 2020; Kitzler et al., 2012; Tasanarong et al., 2009) mentioning the role of vitamin E in Scr as the primary outcome satisfied our inclusion criteria. The serum creatinine follow-up data were extracted. A fix-effects model was used owing to the absence of heterogeneity (p = 0.731, I^2 = 0.00%). The pooled analysis of these studies demonstrated that the change of serum creatinine level in the treatment group between baseline and follow-up significantly decreased relative to the control group [MD: −0.32; 95% CI (−0.48, 0.16), p = 0.000] (Figure 5A). The galbr plot exhibited little heterogeneity between the included studies (Figure 5B). Sensitivity analysis indicated that the serum creatinine data in the included RCTs remained stable (Figure 5C).

Primary outcome: BUN

One RCT (Tasanarong et al., 2009) mentioned the role of vitamin E in serum BUN as the primary outcome. No data extraction or analysis was performed.

Discussion

This meta-analysis enrolled six RCTs about vitamin E for AKI. The designs of these studies were reasonable, and they were of high quality. Our pooled analysis indicated that vitamin E effectively inhibited the occurrence of AKI, increased the eGFR level, and reduced the Scr level in drug-induced kidney injury.

AKI has puzzled clinicians as its pathophysiology has not been completely understood. There are multiple mechanisms involved in its process, including oxidative stress, inflammation, RAAS activation, and DNA damage (De Chiara et al., 2022; Schefold et al., 2016). Among these, oxidative stress exerts a vital role in AKI. According to related basic and clinical study results, vitamin E

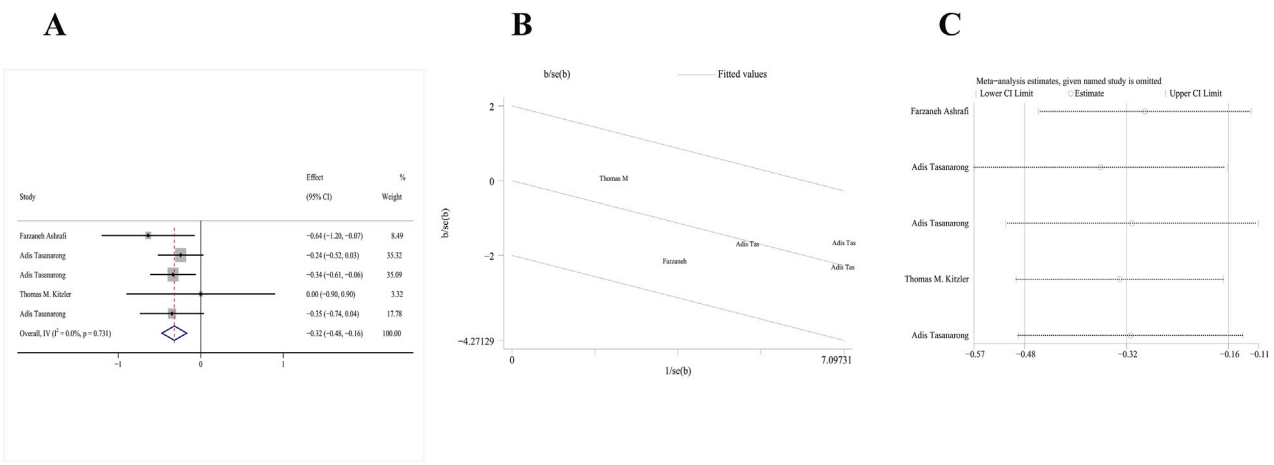


FIGURE 5
Comparison of efficacy between vitamin E and placebo in Scr. **(A)** Forest map. **(B)** Test of heterogeneity by the galbr plot. **(C)** Sensitivity analysis.

makes an anti-oxidative stress impact on AKI. It also shows a protective effect against nephrotoxicity induced by drugs like cisplatin or other nephrotoxic medicines.

Vitamin E prevents drug-mediated kidney damage. Numerous animal experiments regarding the therapeutic efficacy of vitamin E in cisplatin-related toxicities have been published (Abdel-Daim et al., 2019; Abo-Elmaaty et al., 2020; Busari et al., 2018). Cisplatin-related toxicities may mostly result from free radical generation, and the latter can induce oxidative organ injury (Hakiminia et al., 2019). ROS production in mitochondria is a major mechanism related to nephrotoxicity. ROS production and proapoptotic protein activation within an intrinsic pathway can lead to increased mitochondrial membrane permeability (Servais et al., 2008) which reduces the mitochondrial membrane potential, causes calcium homeostasis imbalance, decreases adenosine triphosphate (ATP) generation, and leads to mitochondrial respiratory chain impairment (dos Santos et al., 2012). Vitamin E can increase ATP levels in every tissue except muscle and improve drug-induced mitochondrial dysfunctions in rats (Eser Faki et al., 2020).

Vitamin E also has a protective impact on kidney damage induced by other drugs. Its use can improve oxidative stress indicators, inflammatory markers, histopathological results, and kidney functions in rats with toxic acetamiprid (Erdemli et al., 2020). Vitamin E supplementation can mitigate cadmium-mediated oxidative stress and injury in rat kidneys by suppressing renal cell apoptosis and promoting the antioxidant defense system (Fang et al., 2021b). Supplementation with vitamins E alone in rats applied with amphotericin B can improve kidney tissue structures, functional parameters, and oxidative stress status (Salehzadeh et al., 2020). Vitamin E can regulate apoptosis and autophagy to prevent $HgCl_2$-mediated kidney injury (Alhusaini et al., 2022). Furthermore, vitamin E can be used to mitigate kidney impairments induced by chronic gabapentin use, and its possible mechanism is associated with the inhibition of tissue injury and apoptosis biomarkers (Welson et al., 2021).

The use of vitamin E in treating AKI thus has a profound pathophysiological basis. Human studies on the application of vitamin E in treating AKI are limited, and no consistent conclusion has been drawn (Samsami et al., 2021; Goudarzi et al., 2023; Kitzler et al., 2012; Zhao et al., 2018; Koay et al., 2021). Although some systematic reviews on contrast-induced AKI (CIAKI) have been performed, the conclusions are also inconsistent (Cho et al., 2017; Xu et al., 2018; Monami et al., 2021; Cho et al., 2017). One meta-analysis showed that vitamin E significantly decreased the CIAKI risk ratio by 62% and reduced Scr elevation following contrast application; however, no differences were found in GFR (Cho et al., 2017). Another meta-analysis showed that α-tocopherol pretreatment prior to contrast medium-requiring imaging examinations significantly reduced contrast-induced nephropathy risk, while it exerted no impact on Scr or eGFR (Monami et al., 2021). The meta-analysis performed by Xu et al. (2018) found that vitamin E was beneficial for decreasing CIAKI risk. These studies' conclusions are probably inconsistent due to their heterogeneous timing and inclusion criteria and their outcome definitions. Our meta-analysis offers some strengths as the sample size increases as the study population focuses on patients with drug-induced AKI.

Four studies regarding the impact of vitamin E on Scr were included in our meta-analysis (Kitzler et al., 2012; Tasanarong et al., 2009; Schefold et al., 2016). In other clinical trials, one study showed that vitamin E, compared to placebo, significantly decreased Scr levels in patients with diabetic nephropathy (Tan et al., 2018), supporting our conclusion. Another study found that vitamin E and allopurinol did not significantly decrease Scr levels among patients developing pre-existing kidney failure who received coronary artery bypass grafting (CABG) surgery (Nouri-Majalan et al., 2009). This seemed inconsistent with our conclusion. The inconsistency in the findings may be related to the inclusion of interventions other than vitamin E alone and difference in the study population. Our meta-analysis only included vitamin E not accompanied by other treatments, which is more beneficial.

As was concluded in four RCTs about the function of vitamin E in eGFR, vitamin E effectively increased eGFR levels. Patients with drug-induced AKI were studied in this meta-analysis. However, most clinical trials have focused on vitamin E and eGFR in diabetic

kidney disease. One RCT showed that compared with placebo, vitamin E did not influence eGFR in patients with diabetic nephropathy at 2 months (Tan et al., 2018). Another study indicated that vitamin E application led to improved kidney function at 8 and 12 months, as evaluated through eGFR in diabetic kidney disease (Koay et al., 2021). The course of vitamin E was different in the above two studies. A longer-term application of vitamin E had better renoprotection. Nevertheless, vitamin E may have an inconsistent function in people with different genotypes of diabetes. Based on Dalan et al. (2020), vitamin E application resulted in increased eGFR levels in patients with haptoglobin 2-2 (Hp2-2) genotype diabetes compared with the non-Hp2-2 group. A newly published meta-analysis reported that eGFR of the vitamin E group did not exhibit any statistical significance in diabetic nephropathy (Jin et al., 2024). Furthermore, the pathogenesis of diabetic nephropathy involves multiple interconnected pathways, such as hemodynamic disturbances, oxidative stress, cellular apoptosis, and genetic/epigenetic regulation, with glomerular lesions representing the most prominent pathological feature (Wang et al., 2023). In contrast, drug-induced kidney injury, although it also involves oxidative stress and other mechanisms, primarily manifests as tubulointerstitial damage, a leading contributor to AKI (Perazella and Rosner, 2022). These distinct pathogenic mechanisms, combined with the heterogeneity of study populations, may explain the differential effects of vitamin E on eGFR.

In two RCTs included in this study, the vitamin E group showed a significantly reduced AKI risk, although no significant difference was found in AKI prevalence and duration between vitamin E and control groups in Samsami et al. (2021). Currently, vitamin E combined with additional drugs attracts more attention. Guo and Wang (2018) suggested that vitamin E combined with umbilical cord mesenchymal stem cells (UC-MSC) apparently suppressed the renal inflammatory response by regulating inflammatory cytokines within the kidney microenvironment in AKI rats. They also found that vitamin E combined with UC-MSC has superior efficacy in treating AKI to vitamin E or UC-MSC monotherapy. Truksa et al. (2015) reported that vitamin E analogs targeting mitochondria represent a new mitocan class, which can cause apoptosis and inhibit proliferation, transcription and normal mitochondrial activity. Regulating mitochondrial activity with the mitochondria-targeting antioxidant is the candidate way to mitigate injury of proximal tubular epithelial cells (Sakamoto et al., 2017). Site-targeting vitamin E is also a vital direction in the future of AKI. Due to the limited number of studies included, caution is warranted when generalizing the findings regarding the therapeutic effect of vitamin E on AKI. Further large-scale studies are needed to validate our conclusions.

Nevertheless, as reported in some clinical studies, high-dose α-tocopherol supplementation induces unfavorable effects (Brown et al., 2001; Ward et al., 2007), which is also supported by a meta-analysis (Miller et al., 2005). The dosage of vitamin E therapy is an important issue. Clearly, the mean dietary source of α-tocopherol is approximately 1–10 mg[68]. Oral vitamin E can be absorbed by the body and catabolized in the liver and intestine. In an intervention with 40% fat, it can be estimated that most metabolite excreted in urine is derived from the liver. In the fasting period, nearly half of metabolite is derived from the liver, while the rest is from the intestine (Traber et al., 2021). Nevertheless, research has not yet identified the clinically effective blood concentration of biologically active vitamin E for ameliorating AKI. Concerning serum levels, 13% of all recruited data points worldwide are below 12 μmol/L, which is the threshold of functional deficiency and is mainly for neonates and children. Based on some prospective observational studies, the α-tocopherol content ≥30 μmol/L in serum benefits human health (Péter et al., 2015). The excessive use of vitamin supplements containing high-dose vitamins A, D, and E may lead to AKI (De Francesco Daher et al., 2017). Both the dose of vitamin E therapy and target serum concentration will be directions of future studies.

The strength of our study is that all the included studies were high-quality RCTs with a relatively large population. As far as we know, this is the first meta-analysis exploring the effect of vitamin E on drug-induced AKI in RCTs performed in humans. Nonetheless, this study does have the following limitations: differences in the agents causing AKI, the inclusion of patients with varying underlying conditions, variations in dosing regimens, interventions in the control groups, and follow-up periods that may have contributed to heterogeneity. Due to the limiting data from the included RCTs, the side effects of vitamin E were not analyzed systematically. Therefore, more RCTs are needed to study the therapeutic effects of different vitamin E doses, the effect of vitamin E on markers of oxidative stress, and the side effects of vitamin E.

Conclusion

Vitamin E can effectively inhibit the occurrence of AKI, increase eGFR levels, and decrease Scr levels. Further well-designed RCTs should be performed to verify the protection, side effects, and appropriate dosing of vitamin E.

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

LM: writing–original draft and writing–review and editing. SL: data curation and writing–review and editing. WC: conceptualization and 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.

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

Abdel-Daim, M. M., Aleya, L., El-Bialy, B. E., Abushouk, A. I., Alkahtani, S., Alarifi, S., et al. (2019). The ameliorative effects of ceftriaxone and vitamin E against cisplatin-induced nephrotoxicity. *Environ. Sci. Pollut. Res. Int.* 26, 15248–15254. doi:10.1007/s11356-019-04801-2

Abo-Elmaaty, A. M. A., Behairy, A., El-Naseery, N. I., and Abdel-Daim, M. M. (2020). The protective efficacy of vitamin E and cod liver oil against cisplatin-induced acute kidney injury in rats. *Environ. Sci. Pollut. Res. Int.* 27, 44412–44426. doi:10.1007/s11356-020-10351-9

Alhusaini, A. M., Alhumaidan, S. A., Alharbi, G. M., Alzahrani, E. A., Sarawi, W. S., Alomar, H. A., et al. (2022). Cross-regulation between autophagy and apoptosis induced by vitamin E and lactobacillus plantarum through beclin-1 Network. *Int. J. Mol. Sci.* 23, 15305. doi:10.3390/ijms232315305

Ashrafi, F., Tabiei, M. N., Mousavi, S., Nematbakhsh, M., Sotoodehnasab, P., and Janbabaei, G. (2020). Does vitamin E mitigate cisplatin-induced nephrotoxicity in cancer patients: results from a randomized placebo-controlled clinical trial. *Middle East J. cancer* 11, 174–184. doi:10.30476/mejc.2019.78710.0

Bagshaw, S. M. (2020). Timing of initiation of renal-replacement therapy in acute kidney injury. *Injury* 383, 240–251. doi:10.1056/NEJMoa2000741

Brown, B. G., Zhao, X. Q., Chait, A., Fisher, L. D., Cheung, M. C., Morse, J. S., et al. (2001). Simvastatin and niacin, antioxidant vitamins, or the combination for the prevention of coronary disease. *N. Engl. J. Med.* 345, 1583–1592. doi:10.1056/NEJMoa011090

Busari, A. A., Adejare, A. A., Shodipe, A. F., Oduniyi, O. A., Ismail-Badmus, K. B., and Oreagba, I. A. (2018). Protective but non-synergistic effects of nigella sativa and vitamin E against cisplatin-induced renal toxicity and oxidative stress in wistar rats. *Animals open access J. MDPI* 68, 696–703. doi:10.1055/a-0626-7003

Cho, M., Kim, S., Park, H., Chung, S., and Kim, K. (2017). Could vitamin E prevent contrast-induced acute kidney injury? A systematic review and meta-analysis. *J. Korean Med. Sci.* 32, 1468–1473. doi:10.3346/jkms.2017.32.9.1468

Dalan, R., Goh, L. L., Lim, C. J., Seneviratna, A., Liew, H., Seow, C. J., et al. (2020). Impact of Vitamin E supplementation on vascular function in haptoglobin genotype stratified diabetes patients (EVAS Trial): a randomised controlled trial. *Nutr. Diabetes* 10, 13. doi:10.1038/s41387-020-0116-7

De Chiara, L., Lugli, G., Villa, G., Raglianti, V., Husain-Syed, F., Ravaglia, F., et al. (2022). Molecular mechanisms and biomarkers associated with chemotherapy-induced AKI. *Int. J. Mol. Sci.* 23, 2638. doi:10.3390/ijms23052638

De Francesco Daher, E., Mesquita Martiniano, L. V., Lopes Lima, L. L., Viana Leite Filho, N. C., de Oliveira Souza, L. E., Duarte Fernandes, P. H. P., et al. (2017). Acute kidney injury due to excessive and prolonged intramuscular injection of veterinary supplements containing vitamins A, D and E: a series of 16 cases. *Nefrol. publicacion Of. Soc. Espanola Nefrol.* 37, 61–67. doi:10.1016/j.nefro.2016.05.017

dos Santos, N. A., Carvalho Rodrigues, M. A., Martins, N. M., and dos Santos, A. C. (2012). Cisplatin-induced nephrotoxicity and targets of nephroprotection: an update. *Archives Toxicol.* 86, 1233–1250. doi:10.1007/s00204-012-0821-7

Erdemli, M., Zayman, E., Erdemli, Z., Gul, M., Gul, S., and Gozukara Bag, H. (2020). Protective effects of melatonin and vitamin E in acetamiprid-induced nephrotoxicity. *Environ. Sci. Pollut. Res. Int.* 27, 9202–9213. doi:10.1007/s11356-019-06754-y

Eser Faki, H., Tras, B., and Uney, K. (2020). Alpha lipoic acid and vitamin E improve atorvastatin-induced mitochondrial dysfunctions in rats. *Mitochondrion* 52, 83–88. doi:10.1016/j.mito.2020.02.011

Esterbauer, H., Striegl, G., Puhl, H., and Rotheneder, M. (1989). Continuous monitoring of *in vitro* oxidation of human low density lipoprotein. *Free Radic. Res. Commun.* 6, 67–75. doi:10.3109/10715768909073429

Evans, H. M., and Bishop, K. S. (1922). On the existence of a hitherto unrecognized dietary factor essential for reproduction. *Sci. (New York, N.Y.)* 56, 650–651. doi:10.1126/science.56.1458.650

Fang, C. Y., Lou, D. Y., Zhou, L. Q., Wang, J. C., Yang, B., He, Q. J., et al. (2021a). Natural products: potential treatments for cisplatin-induced nephrotoxicity. *Acta Pharmacol. Sin.* 42, 1951–1969. doi:10.1038/s41401-021-00620-9

Fang, J., Xie, S., Chen, Z., Wang, F., Chen, K., Zuo, Z., et al. (2021b). Protective effect of vitamin E on cadmium-induced renal oxidative damage and apoptosis in rats. *Biol. Trace Elem. Res.* 199, 4675–4687. doi:10.1007/s12011-021-02606-4

Gameiro, J., Duarte, I., Marques, F., Fonseca, J. A., Jorge, S., Rosa, R., et al. (2020). Transient and persistent AKI and outcomes in patients undergoing major abdominal surgery. *Nephron* 144, 236–244. doi:10.1159/000506397

Gaudry, S., Hajage, D., Benichou, N., Chaïbi, K., Barbar, S., Zarbock, A., et al. (2020). Delayed versus early initiation of renal replacement therapy for severe acute kidney injury: a systematic review and individual patient data meta-analysis of randomised clinical trials. *Lancet London, Engl.* 395, 1506–1515. doi:10.1016/s0140-6736(20)30531-6

Georgiev, T., Nikolova, G., Dyakova, V., Karamalakova, Y., Georgieva, E., Ananiev, J., et al. (2023). Vitamin E and silymarin reduce oxidative tissue damage during gentamycin-induced nephrotoxicity. *Pharm. (Basel).* 16, 1365. doi:10.3390/ph16101365

Goudarzi, M. M., Falahati, V., Yousefichaijan, P., and Tajerian, A. (2023). Efficacy of vitamin E on renal function and preventing proximal tubulopathy caused by iron chelation therapy in thalassemia major patients: a randomized controlled clinical trial. *Nephro-urology Mon.* 15. doi:10.5812/numonthly-139443

Guo, Q., and Wang, J. (2018). Effect of combination of vitamin E and umbilical cord-derived mesenchymal stem cells on inflammation in mice with acute kidney injury. *Immunopharmacol. Immunotoxicol.* 40, 168–172. doi:10.1080/08923973.2018.1424898

Hakiminia, B., Goudarzi, A., and Moghaddas, A. (2019). Has vitamin E any shreds of evidence in cisplatin-induced toxicity. *J. Biochem. Mol. Toxicol.* 33, e22349. doi:10.1002/jbt.22349

Hoste, E. A. J., Kellum, J. A., Selby, N. M., Zarbock, A., Palevsky, P. M., Bagshaw, S. M., et al. (2018). Global epidemiology and outcomes of acute kidney injury. *injury* 14, 607–625. doi:10.1038/s41581-018-0052-0

James, M., Bhatt, M., Pannu, N., and Tonelli, M. (2020). Long-term outcomes of acute kidney injury and strategies for improved care. *Nat. Rev. Nephrol.* 16, 193–205. doi:10.1038/s41581-019-0247-z

Ji, M., Wu, R., Feng, Z., Wang, Y. D., Wang, Y., Zhang, L., et al. (2022). Incidence, risk factors and prognosis of acute kidney injury in patients treated with immune checkpoint inhibitors: a retrospective study. *Sci. Rep.* 12, 18752. doi:10.1038/s41598-022-21912-y

Jin, Z., Sun, J., and Zhang, W. (2024). Effect of vitamin E on diabetic nephropathy: a meta-analysis. *Altern. Ther. health Med.* 30, 344–349.

Kataoka, T., Yamato, K., Nishiyama, Y., Morii, Y., Etani, R., Takata, Y., et al. (2012). Comparative study on the inhibitory effects of α-tocopherol and radon on carbon tetrachloride-induced renal damage. *Ren. Fail.* 34, 1181–1187. doi:10.3109/0886022x.2012.717496

Kellum, J. A., Lameire, N., and KDIGO AKI Guideline Work Group (2013). Diagnosis, evaluation, and management of acute kidney injury: a KDIGO summary (Part 1). *Crit. care London, Engl.* 17, 204. doi:10.1186/cc11454

Kitzler, T. M., Jaberi, A., Sendlhofer, G., Rehak, P., Binder, C., Petnehazy, E., et al. (2012). Efficacy of vitamin E and N-acetylcysteine in the prevention of contrast induced kidney injury in patients with chronic kidney disease: a double blind, randomized controlled trial. *Wien. Klin. Wochenschr.* 124, 312–319. doi:10.1007/s00508-012-0169-2

Koay, Y. Y., Tan, G. C. J., Phang, S. C. W., Ho, J. I., Chuar, P. F., Ho, L. S., et al. (2021). A phase IIb randomized controlled trial investigating the effects of tocotrienol-rich vitamin E on diabetic kidney disease. *Nutrients* 13, 258. doi:10.3390/nu13010258

Koga, H., Hagiwara, S., Mei, H., Hiraoka, N., Kusaka, J., Goto, K., et al. (2012). The vitamin E derivative, ESeroS-GS, attenuates renal ischemia-reperfusion injury in rats. *J. Surg. Res.* 176, 220–225. doi:10.1016/j.jss.2011.07.039

Liu, P., Feng, Y., Wang, Y., Zhou, Y., and Zhao, L. (2015). Protective effect of vitamin E against acute kidney injury. *Bio-medical Mater. Eng.* 26 (Suppl. 1), S2133–S2144. doi:10.3233/bme-151519

Mehta, R. L., Kellum, J. A., Shah, S. V., Molitoris, B. A., Ronco, C., Warnock, D. G., et al. (2007). Acute Kidney Injury Network: report of an initiative to improve outcomes in acute kidney injury. *Crit. care London, Engl.* 11, R31. doi:10.1186/cc5713

Miller, E. R., Pastor-Barriuso, R., Dalal, D., Riemersma, R. A., Appel, L. J., and Guallar, E. (2005). Meta-analysis: high-dosage vitamin E supplementation may increase all-cause mortality. *Ann. Intern. Med.* 142, 37–46. doi:10.7326/0003-4819-142-1-200501040-00110

Miyazawa, T., Burdeos, G., Itaya, M., Nakagawa, K., and Miyazawa, T. (2019). Vitamin E: regulatory redox interactions. *IUBMB life* 71, 430–441. doi:10.1002/iub.2008

Monami, M., Cignarelli, A., Pinto, S., D'Onofrio, L., Milluzzo, A., Miccoli, R., et al. (2021). Alpha-tocopherol and contrast-induced nephropathy: a meta-analysis of randomized controlled trials. *Int. J. Vitam. Nutr. Res.* 91, 188–196. doi:10.1024/0300-9831/a000573

Nouri-Majalan, N., Ardakani, E. F., Forouzannia, K., and Moshtaghian, H. (2009). Effects of allopurinol and vitamin E on renal function in patients with cardiac coronary artery bypass grafts. *Vasc. Health Risk Manag.* 5, 489–494. doi:10.2147/vhrm.s5761

Ostermann, M., Bellomo, R., Burdmann, E. A., Doi, K., Endre, Z. H., Goldstein, S. L., et al. (2020). Controversies in acute kidney injury: conclusions from a kidney disease: improving global outcomes (KDIGO) conference. *Kidney Int.* 98, 294–309. doi:10.1016/j.kint.2020.04.020

Perazella, M. A., and Rosner, M. H. (2022). Drug-induced acute kidney injury. *Clin. J. Am. Soc. Nephrol. CJASN* 17, 1220–1233. doi:10.2215/cjn.11290821

Péter, S., Friedel, A., Roos, F. F., Wyss, A., Eggersdorfer, M., Hoffmann, K., et al. (2015). A systematic review of global alpha-tocopherol status as assessed by nutritional intake levels and blood serum concentrations. *Int. J. Vitam. Nutr. Res.* 85, 261–281. doi:10.1024/0300-9831/a000281

Pickkers, P., Darmon, M., Hoste, E., Joannidis, M., Legrand, M., Ostermann, M., et al. (2021). Acute kidney injury in the critically ill: an updated review on pathophysiology and management. *Intensive care Med.* 47, 835–850. doi:10.1007/s00134-021-06454-7

Ronco, C., Bellomo, R., and Kellum, J. A. (2019). Acute kidney injury. *Lancet* 394, 1949–1964. doi:10.1016/s0140-6736(19)32563-2

Sakamoto, Y., Yano, T., Hanada, Y., Takeshita, A., Inagaki, F., Masuda, S., et al. (2017). Vancomycin induces reactive oxygen species-dependent apoptosis via mitochondrial cardiolipin peroxidation in renal tubular epithelial cells. *Eur. J. Pharmacol.* 800, 48–56. doi:10.1016/j.ejphar.2017.02.025

Salehipour, M., Monabbati, A., Salahi, H., Nikeghbalian, S., Bahador, A., Marvasti, V. E., and Abbasalipourkab, R. (2010). Protective effect of parenteral vitamin E on ischemia-reperfusion injury of rabbit kidney. *Urology* 75, 858–861. doi:10.1016/j.urology.2009.04.062

Salehzadeh, A., Salehzadeh, A., Maghsood, A.-H., Heidarisasan, S., Taheri-Azandaryan, M., Ghafourikhosroshahi, A., et al. (2020). Effects of vitamin A and vitamin E on attenuation of amphotericin B-induced side effects on kidney and liver of male Wistar rats. *Environ. Sci. Pollut. Res. Int.* 27, 32594–32602. doi:10.1007/s11356-020-09547-w

Samsami, M., Shabani, M., Hajiesmaeili, M., Tavakoli-Ardakani, M., Ardehali, S. H., Fatemi, A., et al. (2021). The effects of vitamin E on colistin-induced nephrotoxicity in treatment of drug-resistant gram-negative bacterial infections: a randomized clinical trial. *J. Infect. Chemother.* 27, 1181–1185. doi:10.1016/j.jiac.2021.03.013

Schaubroeck, H., Vargas, D., Vandenberghe, W., and Hoste, E. (2021). Impact of AKI care bundles on kidney and patient outcomes in hospitalized patients: a systematic review and meta-analysis. *BMC Nephrol.* 22, 335. doi:10.1186/s12882-021-02534-4

Schefold, J. C., Filippatos, G., Hasenfuss, G., Anker, S. D., and von Haehling, S. (2016). Heart failure and kidney dysfunction: epidemiology, mechanisms and management. *Nat. Rev. Nephrol.* 12, 610–623. doi:10.1038/nrneph.2016.113

Servais, H., Ortiz, A., Devuyst, O., Denamur, S., Tulkens, P. M., and Mingeot-Leclercq, M. P. (2008). Renal cell apoptosis induced by nephrotoxic drugs: cellular and molecular mechanisms and potential approaches to modulation. *Apoptosis Int. J. Program. Cell death* 13, 11–32. doi:10.1007/s10495-007-0151-z

Tan, S., Chiew, Y., Ahmad, B., and Kadir, K. (2018). Tocotrienol-rich vitamin E from palm oil (tocovid) and its effects in diabetes and diabetic nephropathy: a pilot phase II clinical trial. *Nutrients* 10, 1315. doi:10.3390/nu10091315

Tasanarong, A., Piyayotai, D., and Thitiarchakul, S. (2009). Protection of radiocontrast induced nephropathy by vitamin E (alpha tocopherol): a randomized controlled pilot study. *Chotmaihet thangphaet J. Med. Assoc. Thail.* 92, 1273–1281.

Tasanarong, A., Vohakiat, A., Hutayanon, P., and Piyayotai, D. (2013). New strategy of α- and γ-tocopherol to prevent contrast-induced acute kidney injury in chronic kidney disease patients undergoing elective coronary procedures. *Nephrol. Dial. Transplant.* 28, 337–344. doi:10.1093/ndt/gfs525

Traber, M. G., Leonard, S. W., Ebenuwa, I., Violet, P. C., Niyyati, M., Padayatty, S., et al. (2021). Vitamin E catabolism in women, as modulated by food and by fat, studied using 2 deuterium-labeled α-tocopherols in a 3-phase, nonrandomized crossover study. *Am. J. Clin. Nutr.* 113, 92–103. doi:10.1093/ajcn/nqaa298

Truksa, J., Dong, L. F., Rohlena, J., Stursa, J., Vondrusova, M., Goodwin, J., et al. (2015). Mitochondrially targeted vitamin E succinate modulates expression of mitochondrial DNA transcripts and mitochondrial biogenesis. *Antioxidants and redox Signal.* 22, 883–900. doi:10.1089/ars.2013.5594

Wang, H., Liu, D., Zheng, B., Yang, Y., Qiao, Y., Li, S., et al. (2023). Emerging role of ferroptosis in diabetic kidney disease: molecular mechanisms and therapeutic opportunities. *Int. J. Biol. Sci.* 19, 2678–2694. doi:10.7150/ijbs.81892

Ward, N. C., Wu, J. H. Y., Clarke, M. W., Puddey, I. B., Burke, V., Croft, K. D., et al. (2007). The effect of vitamin E on blood pressure in individuals with type 2 diabetes: a randomized, double-blind, placebo-controlled trial. *J. Hypertens.* 25, 227–234. doi:10.1097/01.hjh.0000254373.96111.43

Welson, N. N., Rofaeil, R. R., Ahmed, S. M., Gaber, S. S., Batiha, G. E. S., and Shahataa, M. G. (2021). Vitamin E protects against gabapentin-induced chronic hepatic and renal damage associated with the inhibition of apoptosis and tissue injury in rats. *Life Sci.* 267, 118940. doi:10.1016/j.lfs.2020.118940

Wu, J., and Croft, K. (2007). Vitamin E metabolism. *Mol. aspects Med.* 28, 437–452. doi:10.1016/j.mam.2006.12.007

Xu, Y., Zheng, X., Liang, B., Gao, J., and Gu, Z. (2018). Vitamins for prevention of contrast-induced acute kidney injury: a systematic review and trial sequential analysis. *Am. J. Cardiovasc. Drugs* 18, 373–386. doi:10.1007/s40256-018-0274-3

Yue, J., Bao, X., and Meng, L. F. (2023). Protective role of melatonin for acute kidney injury: a systematic review and meta-analysis. *Shock (Augusta, Ga.)* 61, 167–174. doi:10.1097/shk.0000000000002278

Zaaboul, F., and Liu, Y. (2022). Vitamin E in foodstuff: nutritional, analytical, and food technology aspects. *Compr. Rev. food Sci. food Saf.* 21, 964–998. doi:10.1111/1541-4337.12924

Zarbock, A., Kellum, J. A., Schmidt, C., Van Aken, H., Wempe, C., Pavenstädt, H., et al. (2016). Effect of early vs delayed initiation of renal replacement therapy on mortality in critically ill patients with acute kidney injury: the ELAIN randomized clinical trial. *Jama* 315, 2190–2199. doi:10.1001/jama.2016.5828

Zhang, J., Ren, X., Nie, Z., You, Y., Zhu, Y., Chen, H., et al. (2024). Dual-responsive renal injury cells targeting nanoparticles for vitamin E delivery to treat ischemia reperfusion-induced acute kidney injury. *J. nanobiotechnology* 22, 626. doi:10.1186/s12951-024-02894-7

Zhao, Y., Zhang, W., Jia, Q., Feng, Z., Guo, J., Han, X., et al. (2018). High dose vitamin E attenuates diabetic nephropathy via alleviation of autophagic stress. *Front. physiology* 9, 1939. doi:10.3389/fphys.2018.01939

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