

Exploring the target points and mechanisms of Compound Danshen Dripping Pills in treating diabetic nephropathy based on network pharmacology and molecular docking technology

Zhengju Du^{1,*}, Lidan Zhang^{2,*}, Ju Ran³, Lisha Gao³, Hongrong Chen³
and Honggang Ni²

¹ Guizhou University of Traditional Chinese Medicine, No. 50, Shidong Road, Guiyang, Guizhou, China

² The Second Affiliated Hospital of Guizhou University of Traditional Chinese Medicine, Guiyang, Guizhou, China

³ Guizhou University of Traditional Chinese Medicine, Guiyang, Guizhou, China

Abstract. Diabetic nephropathy is a formidable complication arising from diabetes. Compound Danshen Dripping Pills (CDDP) has demonstrated remarkable efficacy in managing diabetic retinopathy. Our study integrates network pharmacology and molecular docking methodologies to explore the therapeutic targets and mechanisms of CDDP in diabetic nephropathy. We systematically retrieved compounds and their corresponding target molecules associated with CDDP from the Traditional Chinese Medicine Systems Pharmacology (TCMSP) and UniProt databases. Our exhaustive analysis revealed 76 bioactive compounds and 203 potential targets for CDDP. Through meticulous Protein-Protein Interaction network analysis, we narrowed down on ten pivotal targets central to the therapeutic effects. Functional assessments illuminated the multifaceted impact of CDDP on metabolic homeostasis, inflammatory responses, oxidative stress modulation, and apoptotic regulation, implicating pathways such as lipid metabolism, atherosclerotic processes, and Advanced Glycation End-products receptor for Advanced Glycation End-products (AGE-RAGE) signaling cascade. Molecular docking assays further substantiated these findings by demonstrating a robust binding affinity between the bioactive constituents and their respective molecular targets. In conclusions, this study forecasts the complex interplay of components, therapeutic targets, and modulated pathways involved in CDDP's therapeutic intervention in diabetic nephropathy.

Key words: Diabetic nephropathy — Compound Danshen Dripping Pills — Network pharmacology — Molecular docking mechanism

Introduction

Diabetes mellitus, amidst a striking global escalation in incidence (Federation 2021), severely jeopardizes patient health and diminishes the quality of life, notably through the pervasive microvascular sequela, diabetic nephropathy

(DN) (Anders et al. 2018; Cole and Florez 2020; Gupta et al. 2023). As a paramount contributor to the development of end-stage renal disease (ESRD) (Kanwar et al. 2011), DN instigates a detrimental sequence of pathophysiological events, hallmarked by chronic hyperglycemia-induced perturbations affecting a myriad of renal cellular compartments. This insidious decline in cellular function progressively deteriorates renal capability, accentuating the pressing requirement for efficacious therapeutic interventions. Historically, the therapeutic landscape for DN has been notably constricted (Tuttle and Cherney 2021). Contemporary therapeutic modalities primarily revolve around manipulat-

* These authors contributed equally to this work.

Correspondence to: Honggang Ni, The Second Affiliated Hospital of Guizhou University of Traditional Chinese Medicine, 83 Flying Hill Street, Guiyang, Guizhou, 550001, China
E-mail: nihonggang0624@163.com

ing the renin-angiotensin-aldosterone system (RAAS) in an attempt to mitigate proteinuria and retard the progression of renal fibrosis, thereby aiming to decelerate the disease's advancement (Alicic et al. 2017; Rao et al. 2019). Nonetheless, the clinical benefits of these approaches have fallen short of optimal, necessitating an urgent exploration of more potent and efficacious strategies to combat DN.

Investigations have illuminated the substantial therapeutic potential of Compound Danshen Dripping Pills (CDDP) in addressing diabetic microvascular complications (Deng et al. 2024). CDDP has been demonstrated to be effective in treating diabetic retinopathy through the following mechanisms: preservation and restoration of vascular endothelium integrity, alongside exertion of anticoagulation and anti-inflammatory activities, enhancement of blood fluid dynamics, modulation of the lipid profiles, and improvement of retinal microarchitecture (Liu L et al. 2022; Xu X et al. 2024). The overlapping pathogenic mechanisms linking diabetic retinopathy with DN are poor blood sugar control and microvascular disease caused by diabetes (Yang and Liu 2022; Liu Z et al. 2023; Wang et al. 2024). Therefore, it is postulated that CDDP might similarly prove efficacious in the treatment of DN. Nevertheless, the intricate constitution of CDDP poses challenges to conventional pharmacological approaches in thoroughly deciphering its operative mechanisms.

Consequently, the objective of this study is to harness the power of network pharmacology and molecular docking methodologies in elucidating the prospective therapeutic targets and underlying mechanisms of CDDP within the context of DN management. By doing so, our endeavor lays a robust theoretical groundwork and provides substantial data support, thereby facilitating informed directions for subsequent experimental inquiries. This pioneering research endeavor is poised to catalyze advancements in the discovery and development of novel therapeutic strategies tailored to combat DN.

Materials and Methods

Data collection

Acquisition of bioactive constituents in CDDP

In this investigation, focusing on the individual botanical ingredients of CDDP, namely *Radix Salviae*, *Borneolum Syntheticum*, and *Panax Notoginseng*, we employed the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP, accessible at <https://old.tcmsp-e.com/index.php>) to comprehensively discern all constituent compounds alongside their respective biological targets. Following established procedures, compounds underwent additional screening based on criteria of oral

bioavailability (OB) $\geq 30\%$ and drug-likeness (DL) ≥ 0.18 to identify effective chemical constituents of each herbal component and their potential targets.

Prediction of potential targets for CDDP

Compound data were retrieved in Excel format from the UniProt database (<https://www.uniprot.org/>). Data optimization was carried out using the "TRIM" function to clean the data, and the "VLOOKUP" function was employed to match target gene names. Subsequently, annotation of target proteins associated with the chemical constituents obtained through these processes was performed using the UniProt database. Ineffective components lacking target data were filtered out.

Disease-related target prediction

To obtain a list of targets associated with DN, the GeneCards database (<http://www.genecards.org/>) and the Online Mendelian Inheritance in Man (OMIM) database (<http://www.omim.org/>) were searched using the keyword "diabetes nephropathy." After removing duplicate entries, a comprehensive list of disease targets for DN was compiled.

Overlap between bioactive compounds and DN targets

The Venny tool facilitated identifying potential crucial targets for managing the disease through the herbal components of the formulation.

Construction of protein-protein interaction (PPI) networks and analysis of network topology

Gene targets identified from the overlap of two datasets were input into the STRING database, specifying *Homo sapiens* as the organism and setting the confidence threshold to 0.90. Disconnected gene nodes were excluded to generate a protein interaction network.

Following that, the network of potential key targets was built utilizing Cytoscape 3.9.0 software, which was succeeded by a comprehensive analysis of network parameters. The thickness of the edges corresponds to the combined score, indicating higher combined scores with thicker edges. The core targets ranking within the top 10 were determined based on their degree values.

GO and KEGG enrichment analyses

Bioinformatics investigations utilizing the open-source software suite Bioconductor (<http://www.bioconductor.org/>) were conducted within the environment provided by R software. The packages clusterProfiler, Stringin, and

Pathview were installed and executed for performing functional enrichment analyses involving Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG), with visualization facilitated by the Bioinformatics platform (<https://www.bioinformatics.com.cn/>). GO analysis encompasses three domains: Biological Processes (BP), Molecular Functions (MF), and Cellular Components (CC), annotating the roles of drug treatment target proteins within gene function. Meanwhile, KEGG analysis focuses on pathway analysis to elucidate the primary signaling pathways involved in drug-mediated disease treatment. Raw p values were calculated using the hypergeometric distribution test, with a screening condition of $p < 0.05$. Benjamini-Hochberg method was used to control the false discovery rate (FDR), and the corrected q -value threshold was set to 0.1.

Molecular docking verification

Based on the ranking of topological parameters, the top five compounds from the core formulation of CDDP were selected for docking, with the top five proteins among the core targets. The protein crystal structures were obtained *via* the AlphaFold database (<https://alphafold.ebi.ac.uk/>). Traditional Chinese medicine compounds were sourced from the PubChem database to establish the small molecule library. The docking process involved the following steps: initially, protein crystal structures underwent preparation, which included removing water molecules and adding hydrogen atoms using AutoDockTools 5.6. The grid center coordinates (X, Y, Z) were defined based on the target active site residual

matrix center, and the grid size covered the binding pocket extension 5 Å region ($20 \times 20 \times 20 \text{ Å}^3$). Subsequently, preparatory tasks such as splitting the small molecule library were conducted using Open Babel and the AutoDock program. Finally, the AutoDock program executed the docking operation (Exhaustiveness = 20 to enhance the comprehensiveness of the conformation search, and AutoDock default values were used for the remaining parameters), visualizing results in PyMOL.

Results

Active ingredients and prospective targets of CDDP

Radix Salviae (RS), Panax Notoginseng (PN), and Borneolum Syntheticum (BS) contain 65, 8, and 3 active compounds, respectively (Supplementary Table S1: Basic information of active compounds in CDDP). A Venn diagram distinctly displays the number of active compounds in each herbal component of CDDP based on the intersection of active compounds from the three medicinal herbs (Fig. 1A). By importing the active compounds and their corresponding targets into Cytoscape 3.7.1 for network analysis, the findings unveiled key nodes: MOL000098-Quercetin (Degree:45), MOL000006-Luteolin (Degree:30), MOL007154-Tanshinone IIA (Degree:24), MOL007156-Tanshinone VI (Degree:7), and MOL007088-Cryptotanshinone (Degree:6) (Fig. 1B). Leveraging the nomenclature of these active compounds, we curated 203 targets from the Uniprot database

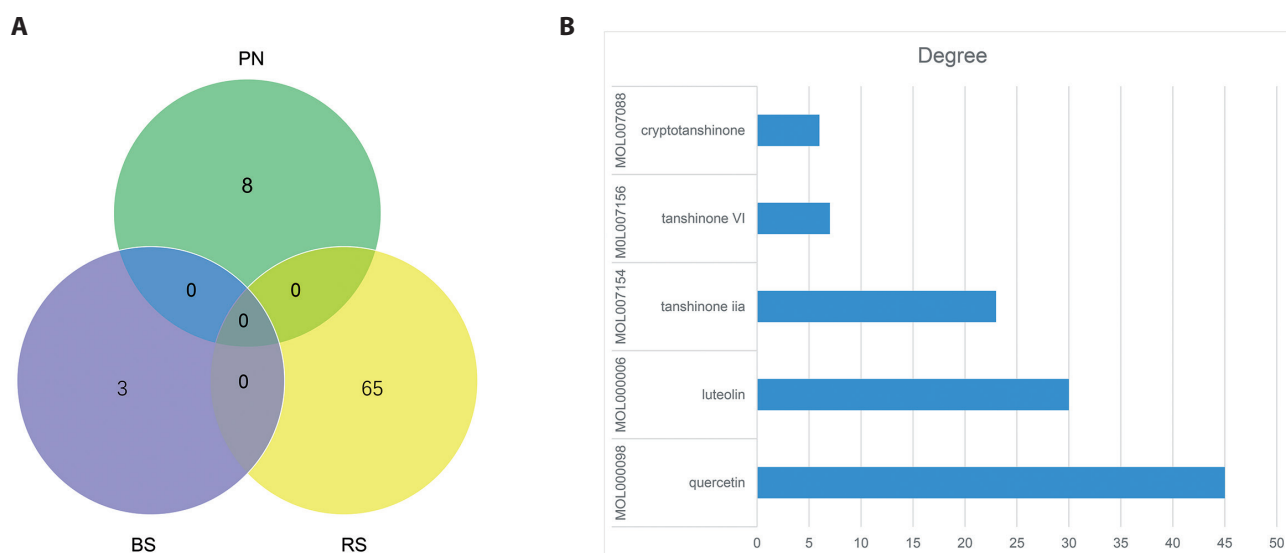


Figure 1. Identification of active compounds and targets in CDDP. **A.** Venn diagram illustrating the quantity of active compounds within each herbal component of CDDP. Yellow color represents Radix Salviae (RS) with 65 compounds, green denotes Panax Notoginseng (PN) with 8 compounds, and purple indicates Borneolum Syntheticum (BS) with 3 compounds. **B.** Critical nodes of active compounds. (See online version for color figure.)

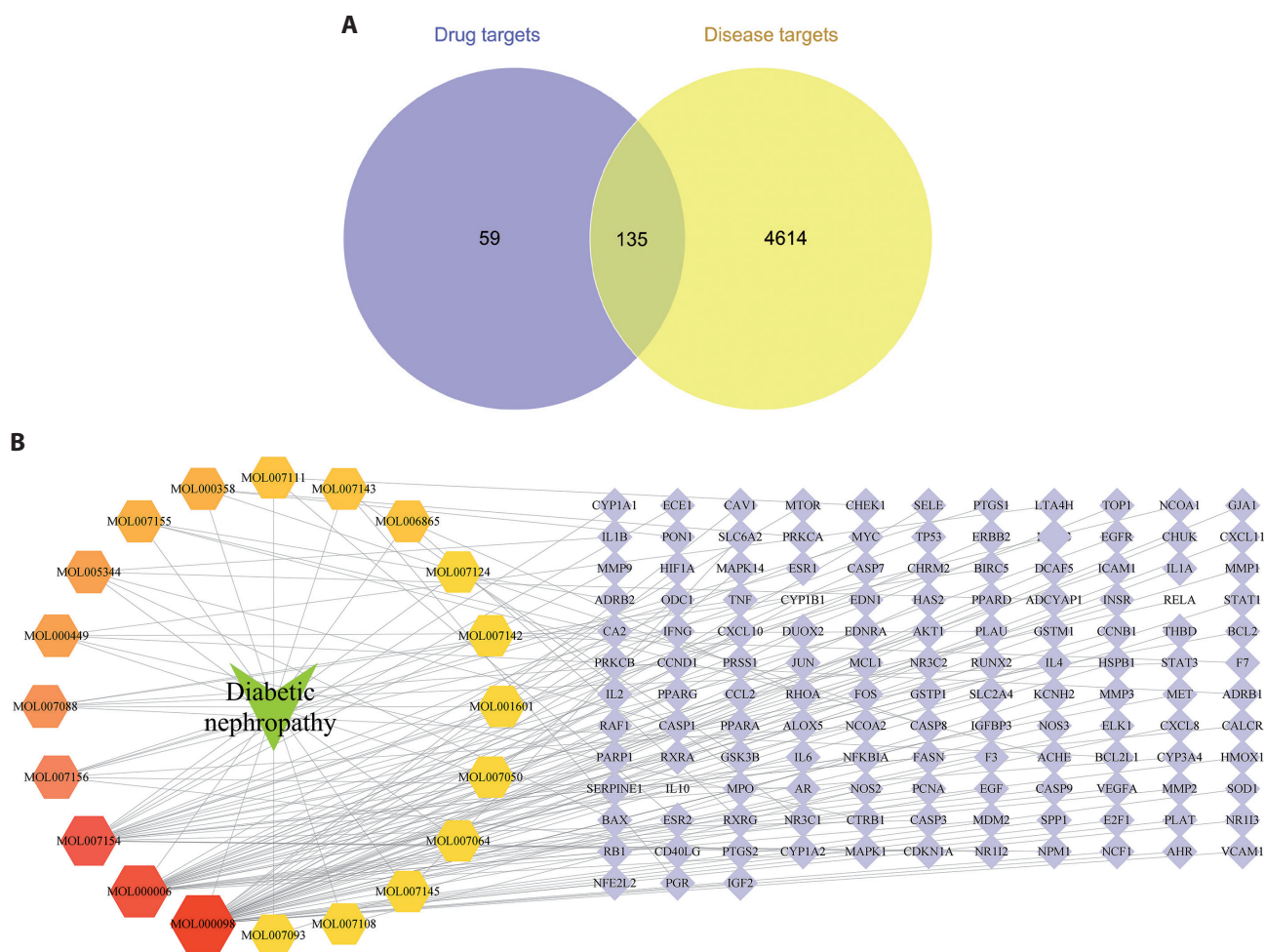


Figure 2. Identification of overlapping goals between compounds and DN. **A.** Venn chart illustrating the overlap of 135 common targets shared by the active compounds of CDDP and the targets associated with DN. **B.** Network of core components targets disease.

(Table S2: Information of 203 potential targets of CDDP active compounds).

Disease targets associated with DN

We synthesized disease targets sourced from GeneCards and OMIM, excluding duplicates, and identified 4716 disease targets specific to DN. Detailed information on DN disease targets is displayed in Table S3 (Information of DN disease targets).

Analysis and construction of the “Active Compound-Disease-Shared Target Network”

After eliminating targets with the slightest degree of non-contributory, an intersection analysis was conducted between CDDP active compounds and those associated with DN, identifying 135 potential targets for CDDP's

therapeutic action in DN (Fig. 2); these filtered targets are considered critical for evaluating the efficacy of CDDP compounds in treating DN.

Shared target PPI network

We uploaded 135 overlapping targets to the STRING database to generate a PPI network, aiming to elucidate the mechanisms by which CDDP applies its therapeutic effects on DN. This network comprised 54 nodes and 292 edges, where the thickness of the lines denotes the intensity of target interactions, and the nodes' depth of color and size indicate higher node degree values. Upon importing the PPI network into Cytoscape, we proceeded with additional analysis to determine the top 10 targets ranked by node degree and betweenness centrality. These targets include tumor protein p53 (TP53), signal transducer and activator of transcription 3 (STAT3), AKT serine/threonine kinase 1 (AKT1), jun

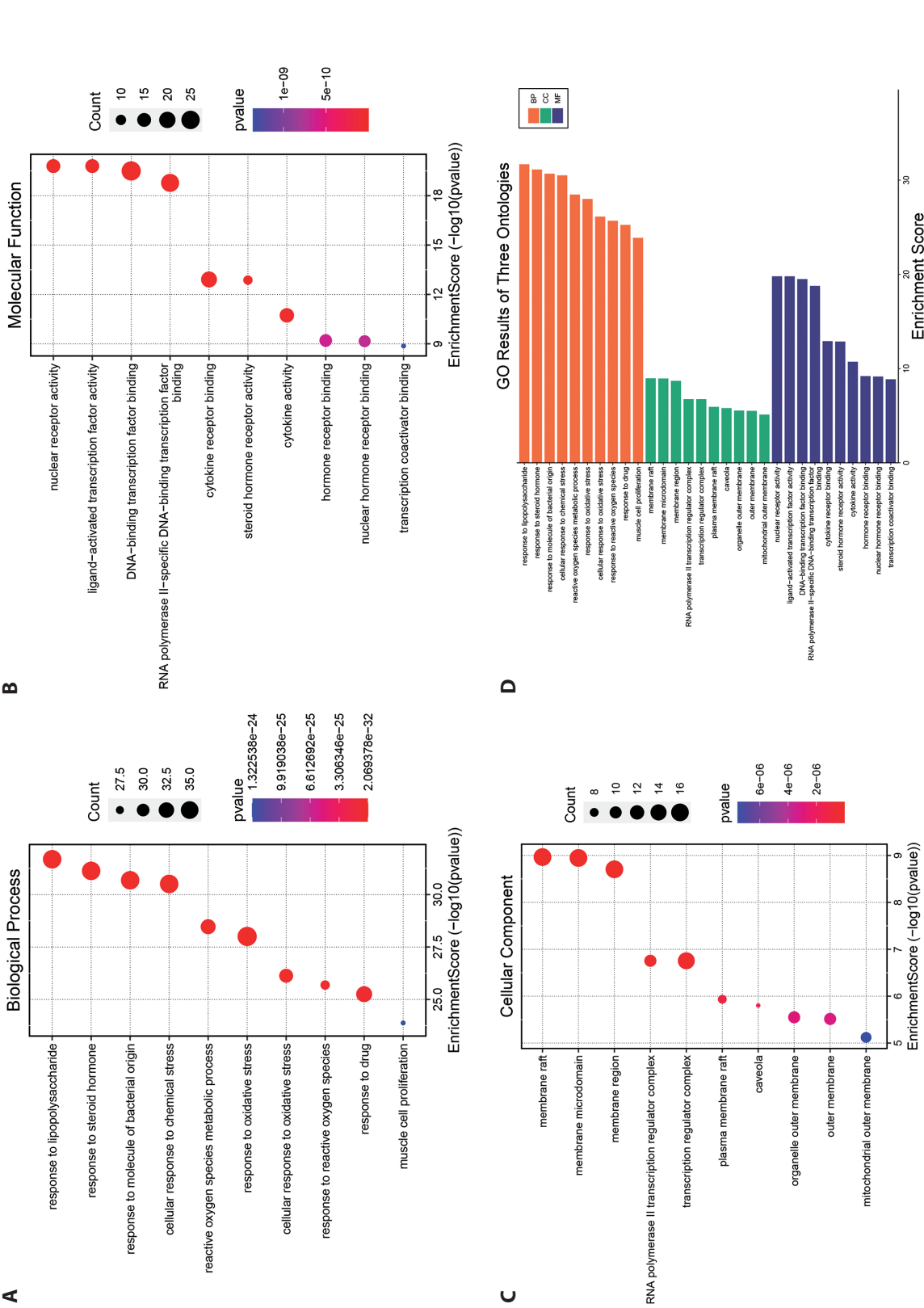


Figure 3. Results of the GO enrichment analysis. **A.** Graphical representation of BP terms derived from GO enrichment analysis. **B.** Bubble diagram illustrating MF categories identified through GO enrichment analysis. **C.** Bubble diagram depicting CC categories derived from GO enrichment analysis. The *p*-value is depicted along the horizontal axis, while the full names of the processes are displayed on the vertical axis. Each bubble's color and size indicate the *p*-value and gene count. Subsequent bubble charts follow a similar presentation format. **D.** The top 10 BP, CC, and MF from GO enrichment analysis are showcased with orange, green, and deep blue bars, respectively. (See online version for color figure.)

Table 1. Information of 10 core targets

No.	UniProt ID	Gene	Protein name	Degree
1	GC17M007661	TP53	Tumor protein P53	40
2	GC17M042313	STAT3	Signal transducer and activator of transcription 3	29
3	GC14M104769	AKT1	AKT serine/threonine kinase 1	29
4	GC01M058780	JUN	Jun proto-oncogene, AP-1 transcription factor subunit	28
5	GC06P100202	TNF	Tumor necrosis factor	24
6	GC06P151656	ESR1	Estrogen receptor 1	23
7	GC07P022725	IL6	Interleukin 6	21
8	GC22M021759	MAPK1	Mitogen-activated protein kinase 1	20
9	GC02M112829	IL1 β	Interleukin 1 β	19
10	GC11P069641	CCND1	Cyclin D1	16

proto-oncogene, AP-1 transcription factor subunit (JUN), tumor necrosis factor (TNF), estrogen receptor 1 (ESR1), interleukin-6 (IL6), mitogen-activated protein kinase 1 (MAPK1), interleukin-1 β (IL1- β), and cyclin D1 (CCND1). For more details, see Table 1.

GO enrichment analysis

We performed GO enrichment analysis on 135 commonly targeted genes to explore the intricate mechanisms underlying CDDP's effectiveness in treating DN. This analysis revealed 4112 biological processes (BP), 468 molecular functions (MF), and 273 cellular components (CC). Visual representations in bubble charts (Fig. 3A–C) and bar graphs (Fig. 3D) highlight a selection of enriched terms in each category, totaling 30. Notably, biological processes include responses to lipopolysaccharides, steroid hormones, molecules of bacterial origin, chemical stress, and reactive oxygen species. Molecular functions encompass nuclear receptor activity, ligand-activated transcription factor activity, DNA-binding transcription factor binding, RNA polymerase II-specific DNA-binding transcription factor binding, and cytokine receptor binding. Within cellular components, enriched terms include membrane raft, membrane microdomain, membrane region, RNA polymerase II transcription regulator complex, and transcription regulator complex. Detailed summaries of these findings are provided in Table S4 (Results of the biological process category terms from GO enrichment analysis), Table S5 (Results of the cellular component category terms from GO enrichment analysis), and Table S6 (Results of the molecular function category terms from GO enrichment analysis).

Analysis of KEGG pathways

A KEGG pathway enrichment analysis was performed on 135 commonly targeted genes, with a significance level set at $p < 0.05$. The analysis revealed significant enrichment of these

targets across 236 pathways, with detailed findings in Supplementary Table S7 (Information of the top 236 pathways based on KEGG enrichment analysis). The top 20 pathways exhibiting high enrichment were identified based on gene counts (Fig. 4).

Molecular docking

For validation of the network pharmacology results, we used molecular docking to assess the interactions between selected pharmaceutical compounds and their corresponding targets. Focusing on the top five critical targets associated with DN, namely TP53, AKT1, STAT3, JUN, and TNF, and the five compounds identified as having the highest degree values in the analysis of the herbal compound-target network: quercetin, luteolin, tanshinone IIA, tanshinone VI, and cryptotanshinone. The binding energies between these targets and active compounds are shown in Figure 5, where a binding energy < -1 kcal/mol indicates binding activity and < -5 kcal/mol suggests binding stability. Notably, quercetin exhibited binding energy of -7.4 kcal/mol with AKT1, -7.3 kcal/mol with TNF; luteolin showed -7.3 kcal/mol with JUN, and tanshinone IIA had -7.1 kcal/mol with TP53. Additionally, AutodockTools 1.5.6 software revealed strong binding affinities between tanshinone IIA and tanshinone VI with JUN and luteolin with TNF, indicating their potential as drug molecules (Table 2: Binding energy of the key target to the active compound). Molecular docking and visualization of compound-target interactions using PyMOL provide valuable insights into binding patterns between herbal compounds and disease-relevant targets (Fig. 6A–D).

Discussion

DN, a prevalent complication of diabetes, frequently advances to ESKD, necessitating life-sustaining treatments such as dialysis or kidney transplantation (Reidy et al. 2014; Anders et al.

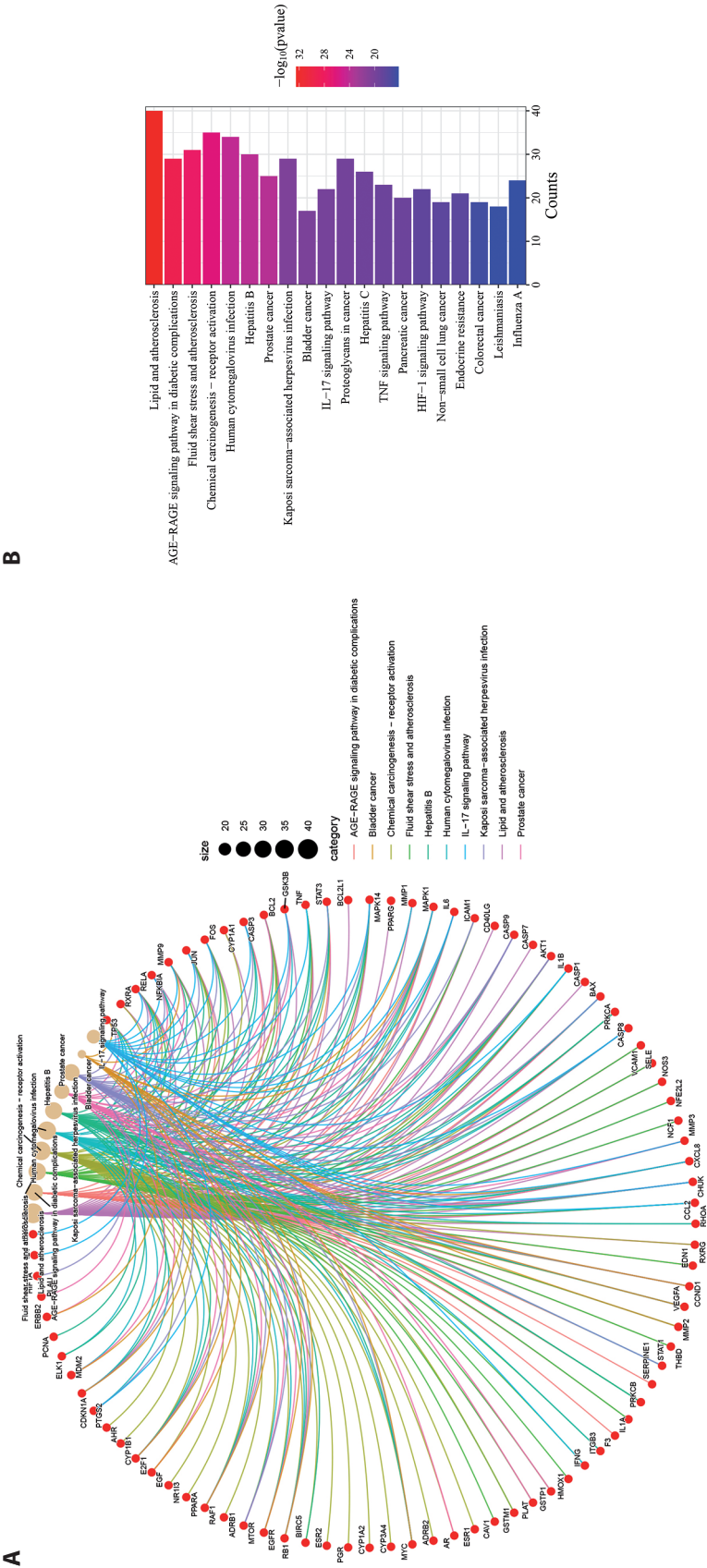


Figure 4. KEGG enrichment analysis and the establishment of a pivotal pathway network. **A.** Network diagram of key pathways. **B.** According to the findings of the KEGG enrichment analysis, we identified the primary 20 pathways categorized by KEGG classification.

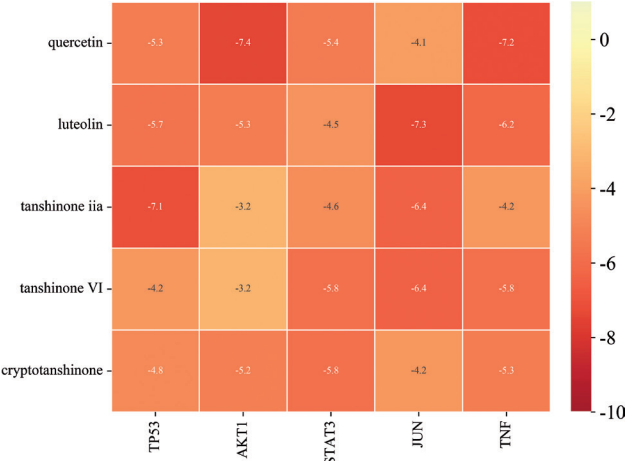


Figure 5. Heatmap of molecular docking scores, showcasing the binding affinities between critical targets of herbal compounds and active agents (kcal/mol).

2018). Traditional Chinese Medicine has gained recognition as a comprehensive therapeutic strategy for DN, providing holistic adjustments and multi-target modulation to regulate blood glucose levels, enhance renal function, and alleviate proteinuria (Liu Z et al. 2022; Zhang et al. 2022; Liu Y et al. 2023; Chen et al. 2024). CDDP enhances cardiovascular health by dilating coronary arteries and improving blood flow (Liu Y et al. 2023). Recent studies emphasize CDDP’s antioxidative, anti-inflammatory, and endothelial protective properties, especially in microvascular complications associated with diabetes, such as retinopathy (Liu L et al. 2022; Fang et al. 2023; Tang et al. 2024; Wang et al. 2024; Xu X et al. 2024). Given the shared pathological basis between diabetic retinopathy and DN, further elucidation of CDDP’s specific bioactive components and mechanisms in DN treatment is warranted. Consequently, this study utilizes network pharmacology and molecular docking to pinpoint potential targets and elucidate the pathways through which CDDP impacts DN.

In this study, 76 pharmacologically active compounds in CDDP were identified from the TCMSP database, while

135 potential targets associated with both CDDP and DN were retrieved from the GeneCards and OMIM databases. The top five bioactive constituents of CDDP comprise quercetin, luteolin, tanshinone IIa, tanshinone VI, and cryptotanshinone, have been extensively validated in numerous studies for their pharmacological efficacy. For instance, Quercetin has shown promising effects in improving renal function and alleviating oxidative stress and inflammation in DN animal models (Hu et al. 2022; Yan et al. 2023). Its antioxidant, anti-inflammatory, lipid-regulatory, and anti-fibrotic properties contribute to these benefits (Hickson et al. 2019; Li et al. 2022). Additionally, Quercetin can reactivate the Hippo pathway to inhibit mesangial cell proliferation in DN, thereby improving renal fibrosis, function, and lipid levels (Ding et al. 2018; Lei et al. 2019; Feng et al. 2023). Luteolin exhibits anti-inflammatory and antioxidant properties, mitigating podocyte injury, glomerulosclerosis, and interstitial fibrosis in DN mouse models (Zhang et al. 2021; Pradhan and Kulkarni 2024). Tanshinone IIa inhibits the release of inflammatory mediators and renal interstitial fibrosis, thus improving DN progression (Xu et al. 2020; Liu et al. 2021). It diminishes cellular stress within the endoplasmic reticulum and co-modulates the PI3K/Akt/NF-κB pathway, thereby ameliorating renal injury and metabolic abnormalities in early-stage DN rats (Xu Z et al. 2024). Additionally, Tanshinone IIa delays DN progression by regulating the Txnip/NLRP3 inflammasome-mediated glomerular endothelial cell pyroptosis and improves DN-induced renal fibrosis through the miRNA-34a-5p axis (Xue et al. 2018; Zhang and Yang 2022). These mechanisms of action are intimately intertwined with the pathophysiological processes of DN, thereby furnishing a robust theoretical underpinning for the application of CDDP in the treatment of this condition.

GO analysis highlighted the multifaceted mechanisms of CDDP in DN, implicating processes such as metabolic regulation, inflammation, oxidative stress, and apoptosis. Central to CDDP’s therapeutic impact are pathways related to lipid metabolism, oxidative stress, and inflammation, including the AGE-RAGE axis, which is pivotal in diabetic complications. Prior research underscores the AGE-RAGE system’s role in DN progression, where it activates nuclear factor-κB (NF-κB), promoting vascular endothelial growth factor (VEGF) and transforming growth factor-β1 (TGF-β1) synthesis, monocyte chemotactic protein-1 (MCP-1) up-regulation, and renal dysfunction *via* the renin-angiotensin system and NADPH oxidase-mediated oxidative stress (Rabani and Thornalley 2018; Tang et al. 2021). The AGE-RAGE interaction activates NF-κB, instigating a cascade of events: increased adhesion molecules and growth factors, oxidative stress, pro-inflammatory cytokine secretion, tissue injury, and VEGF-driven proteinuria. This also activates NADPH oxidase, exacerbating oxidative stress and renal damage, ultimately leading to DN (Bangalore et al. 2016; Deng et al.

Table 2. Binding energy of the key target to the active compound (kcal/mol)

	TP53	AKT1	STAT3	JUN	TNF
Quercetin	-5.3	-7.4*	-5.4	-4.1	-7.2*
Luteolin	-5.7	-5.3	-4.5	-7.3*	-6.2
Tanshinone iia	-7.1*	-3.2	-4.6	-6.4	-4.2
Tanshione VI	-4.2	-3.2	-5.8	-6.4	-5.8
Cryptotanshinone	-4.8	-5.2	-5.8	-4.2	-5.3

* The binding thermal energy was in the range of < -6.5 kcal/mol, indicating that the binding was relatively stable.

2018; Yang et al. 2019; Sridhar et al. 2021; Gutiérrez et al. 2022; Apte et al. 2024; Yu et al. 2024). Moreover, TGF- β 1 release *via* this pathway triggers extracellular matrix fibrosis, advancing renal fibrosis (Hills and Squires 2011; Kim et al. 2021; Schrauben et al. 2021; Hong et al. 2022; Wang et al. 2023). In a hyperglycemic state, renal lipid accumulation exacerbates dyslipidemia and atherosclerosis, further compromising renal function through endothelial dysfunction, inflammation, and oxidative stress. This leads to renal arte-

riosclerosis, membrane injury, and structural abnormalities, accelerating DN (Poznyak et al. 2020; Kon et al. 2021; Elbarbary et al. 2023). The PI3K/Akt pathway, closely tied to DN pathogenesis, regulates cellular responses under high glucose conditions, including cell growth, epithelial-mesenchymal transition, and lipid metabolism (Hao et al. 2014). Modulating this pathway fosters mitochondrial autophagy and HDAC1 suppression, effectively countering oxidative stress and apoptosis in diabetic kidneys, thereby alleviating renal

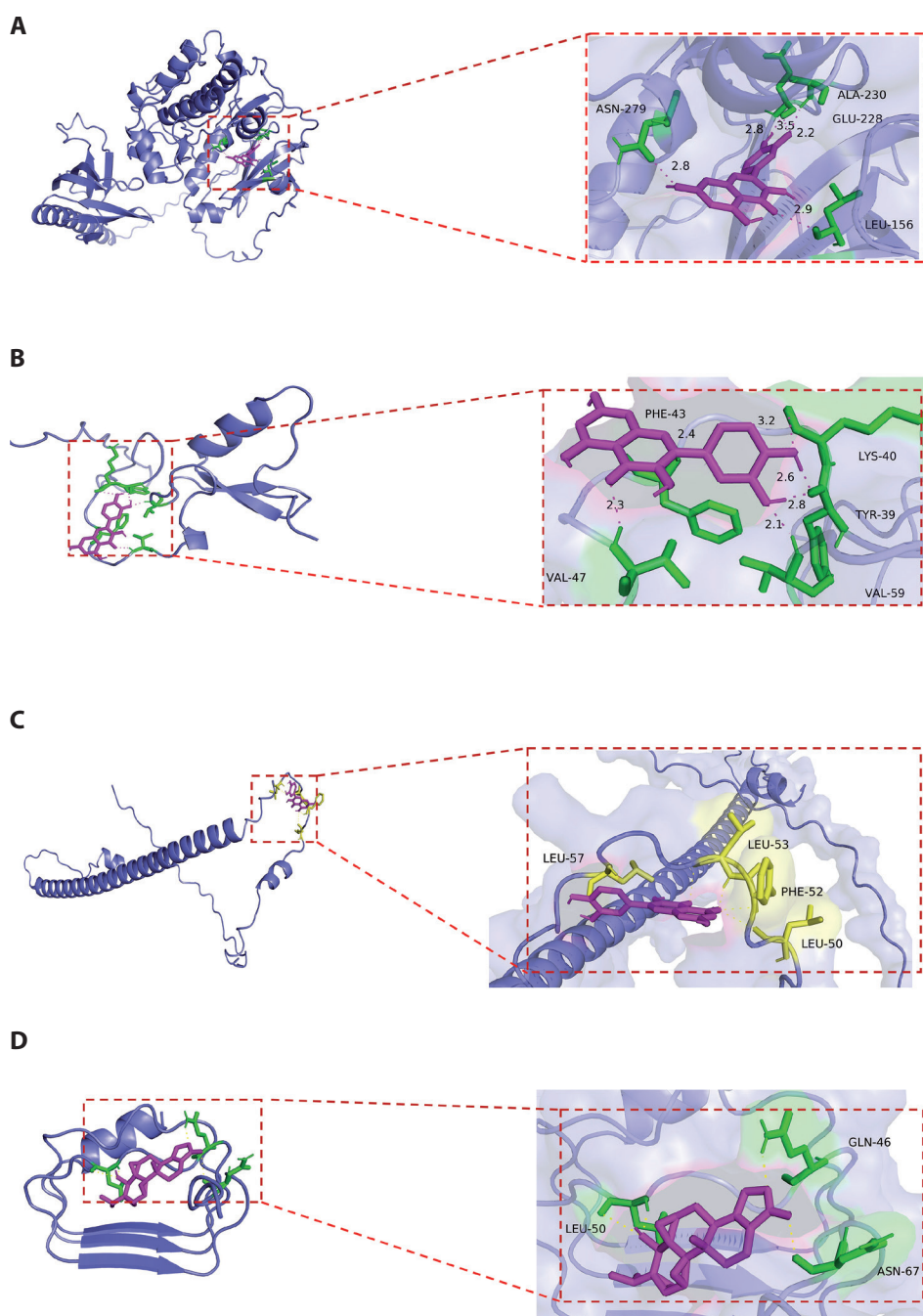


Figure 6. Docking patterns between critical targets and active compounds: quercetin-AKT1 (A), quercetin-TNF (B), luteolin-JUN (C) and tanshinone II A-TP53 (D).

injury (Hou et al. 2020; Lei et al. 2021; Su et al. 2021; Xu et al. 2021; Ni et al. 2022; Dong et al. 2023; Kong et al. 2023; Yao et al. 2023).

Additionally, through the application of sophisticated bioinformatics methodologies, we have successfully identified ten pivotal molecular targets (Table 1), which are intricately entwined with the pathogenic mechanisms of DN. Notably, these targets demonstrate a substantial degree of enrichment in network clusters, highlighting their pivotal roles as central hubs in the disease's pathobiological framework. Furthermore, molecular docking analyses were performed to evaluate the binding affinity between a quintet of paramount target proteins (TP53, AKT1, STAT3, JUN, and TNF) and the top five bioactive constituents of CDDP: Quercetin, Luteolin, Tanshinone IIA, Tanshinone VI, and cryptotanshinone. The binding energy profiles, ranging from -3.2 to -7.4 kcal/mol, denote a substantial docking capacity, implying a high likelihood of these specific molecular interactions contributing to the therapeutic efficacy of CDDP against DN.

Limitations of this study

Firstly, it is crucial to acknowledge that the foundation of our analysis rests upon the collation of data on bioactive compounds and targets sourced from literature and databases; thus, the veracity of our predictive outcomes is inherently tied to the quality and reliability of these sources, and may have database bias. Second, given the inherent nature of our data-mining-based approach, the experimental validation is lacking. The necessity arises for supplementary validation through both clinical trials and animal experimentation. Third, assumptions exist in docking simulations, which may lead to Results bias. Fourth, there may be potential false positives/negatives in target prediction. Recognizing these limitations, we embark on human subject validation endeavors, all the while remaining vigilant to address these methodological constraints, thereby fortifying the scientific grounding for CDDP's utilization in DN therapy.

Conclusion

Our study reveals the complex pharmacodynamics and molecular foundations of CDDP in treating DN using an unprecedented integration of bioinformatics, network pharmacology, and molecular docking. We've identified quercetin, luteolin, tanshinones IIA and VI, plus cryptotanshinone as key CDDP components with therapeutic potential against DN. CDDP's mechanisms target diverse pathways, including metabolic regulation, inflammation control, oxidative stress mitigation, and apoptosis modulation, with a focus on AGE-RAGE interactions and lipid-atherosclerotic signaling.

This work emphasizes CDDP's multitarget, multipathway therapeutic profile in DN therapy, the further clinical treatment could focus on these identified key CDDP components, and explore the drugs effective on these targets and pathways. This study also serves as a valuable framework for exploring the composition and therapeutic targets of other Chinese herbal medicines. Investigations have illuminated the substantial therapeutic potential of CDDP in addressing diabetic microvascular complications, but not in DN, especially from the molecular targets due to the intricate constitution of CDDP. This study has successfully identified ten pivotal molecular targets through the application of sophisticated bioinformatics methodologies, which might be helpful for further treatment explorations. CDDP can be applied in a more definite clinical position in the preventive treatment of diabetic nephropathy of different stages in the future. Through the research pathway of component analysis – target prediction – pathway validation – clinical validation, it is possible to achieve the transition from empirical medication to precise treatment in patients with diabetic nephropathy.

Data availability. The datasets analysed during the current study are available from the corresponding author on reasonable request.

Funding. This study was fund by the China International Medical Foundation (Z-2017-26-2302).

Conflict of interests. The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author contributions. HN, guarantor of integrity of the entire study, study concepts; ZD, study design, definition of intellectual content, and manuscript preparation and editing; LZ, literature research and data analysis; LG, clinical studies and manuscript review; JR, experimental studies and data acquisition; HC, statistical analysis.

References

- Alicic RZ, Rooney MT, Tuttle KR (2017): Diabetic kidney disease: Challenges, progress, and possibilities. *Clin. J. Am. Soc. Nephrol.* **12**, 2032-2045
<https://doi.org/10.2215/CJN.11491116>
- Anders HJ, Huber TB, Isermann B, Schiffer M (2018): CKD in diabetes: diabetic kidney disease versus nondiabetic kidney disease. *Nat. Rev. Nephrol.* **14**, 361-377
<https://doi.org/10.1038/s41581-018-0001-y>
- Apte MM, Khattar E, Tupe RS (2024): Mechanistic role of *Syzygium cumini* (L.) Skeels in glycation induced diabetic nephropathy via RAGE-NF- κ B pathway and extracellular proteins modifications: A molecular approach. *J. Ethnopharmacol.* **322**, 117573
<https://doi.org/10.1016/j.jep.2023.117573>

- Bangalore S, Fakheri R, Toklu B, Messerli FH (2016): Diabetes mellitus as a compelling indication for use of renin angiotensin system blockers: systematic review and meta-analysis of randomized trials. *BMJ* **352**, i438
<https://doi.org/10.1136/bmj.i438>
- Chen Y, Huang G, Qin T, Zhang Z, Wang H, Xu Y, Shen X (2024): Ferroptosis: A new view on the prevention and treatment of diabetic kidney disease with traditional Chinese medicine. *Biomed. Pharmacother.* **170**, 115952
<https://doi.org/10.1016/j.biopha.2023.115952>
- Cole JB, Florez JC (2020): Genetics of diabetes mellitus and diabetes complications. *Nat. Rev. Nephrol.* **16**, 377-390
<https://doi.org/10.1038/s41581-020-0278-5>
- Deng X, Mo Y, Zhu X, Liang S (2024): The effectiveness and safety of Chinese patent medicines plus calcium dobesilate for the treatment of diabetic retinopathy: A network meta-analysis. *Heliyon* **10**, e24533
<https://doi.org/10.1016/j.heliyon.2024.e24533>
- Deng X, Sun L, Lai X, Xiang L, Li Q, Zhang W, Zhang L, Sun S (2018): Tea Polypeptide ameliorates diabetic nephropathy through RAGE and NF- κ B signaling pathway in type 2 diabetes mice. *J. Agric. Food Chem.* **66**, 11957-11967
<https://doi.org/10.1021/acs.jafc.8b04819>
- Ding T, Wang S, Zhang X, Zai W, Fan J, Chen W, Bian Q, Luan J, Shen Y, Zhang Y, et al (2018). Kidney protection effects of dihydroquercetin on diabetic nephropathy through suppressing ROS and NLRP3 inflammasome. *Phytomedicine* **41**, 45-53
<https://doi.org/10.1016/j.phymed.2018.01.026>
- Dong R, Zhang X, Liu Y, Zhao T, Sun Z, Liu P, Xiang Q, Xiong J, Du X, Yang X, et al (2023): Rutin alleviates EndMT by restoring autophagy through inhibiting HDAC1 via PI3K/AKT/mTOR pathway in diabetic kidney disease. *Phytomedicine* **112**, 154700
<https://doi.org/10.1016/j.phymed.2023.154700>
- Elbarbary NS, Ismail EaR, Mohamed SA (2023): Omega-3 fatty acids supplementation improves early-stage diabetic nephropathy and subclinical atherosclerosis in pediatric patients with type 1 diabetes: A randomized controlled trial. *Clin. Nutr.* **42**, 2372-2380
<https://doi.org/10.1016/j.clnu.2023.10.007>
- Fang J, Luo C, Zhang D, He Q, Liu L (2023): Correlation between diabetic retinopathy and diabetic nephropathy: a two-sample Mendelian randomization study. *Front. Endocrinol. (Lausanne)* **14**, 1265711
<https://doi.org/10.3389/fendo.2023.1265711>
- Federation ID (2021). International Diabetes Federation. IDF Diabetes Atlas, 10th edn. Brussels, Belgium: International Diabetes Federation
- Feng Q, Yang Y, Qiao Y, Zheng Y, Yu X, Liu F, Wang H, Zheng B, Pan S, Ren K, et al (2023): Quercetin ameliorates diabetic kidney injury by inhibiting ferroptosis via activating Nrf2/HO-1 signaling pathway. *Am. J. Chin. Med.* **51**, 997-1018
<https://doi.org/10.1142/S0192415X23500465>
- Gupta S, Dominguez M, Golestaneh L (2023): Diabetic kidney disease: An Update. *Med. Clin. North. Am.* **107**, 689-705
<https://doi.org/10.1016/j.mcna.2023.03.004>
- Gutiérrez OM, Shlipak MG, Katz R, Waikar SS, Greenberg JH, Schrauben SJ, Coca S, Parikh CR, Vasan RS, Feldman HI, et al (2022): Associations of plasma biomarkers of inflammation, fibrosis, and kidney tubular injury with progression of diabetic kidney disease: A cohort study. *Am. J. Kidney Dis.* **79**, 849-857
<https://doi.org/10.1053/j.ajkd.2021.09.018>
- Hao J, Li F, Liu W, Liu Q, Liu S, Li H, Duan H (2014). Phosphorylation of PRAS40-Thr246 involved in renal lipid accumulation of diabetes. *J. Cell. Physiol.* **229**, 1069-1077
<https://doi.org/10.1002/jcp.24533>
- Hickson LJ, Langhi Prata LGP, Bobart SA, Evans TK, Giorgadze N, Hashmi SK, Herrmann SM, Jensen MD, Jia Q, Jordan KL, et al (2019): Senolytics decrease senescent cells in humans: Preliminary report from a clinical trial of Dasatinib plus Quercetin in individuals with diabetic kidney disease. *EBioMedicine* **47**, 446-456
<https://doi.org/10.1016/j.ebiom.2019.08.069>
- Hills CE, Squires PE (2011): The role of TGF- β and epithelial-to-mesenchymal transition in diabetic nephropathy. *Cytokine Growth Factor Rev.* **22**, 131-139
<https://doi.org/10.1016/j.cytogfr.2011.06.002>
- Hong Q, Cai H, Zhang L, Li Z, Zhong F, Ni Z, Cai G, Chen XM, He JC, Lee K (2022): Modulation of transforming growth factor- β -induced kidney fibrosis by leucine-rich α -2 glycoprotein-1. *Kidney Int.* **101**, 299-314
<https://doi.org/10.1016/j.kint.2021.10.023>
- Hou B, Li Y, Li X, Zhang C, Zhao Z, Chen Q, Zhang N, Li H (2020): HGF protected against diabetic nephropathy via autophagy-lysosome pathway in podocyte by modulating PI3K/Akt-GSK3 β -TFEB axis. *Cell. Signal.* **75**, 109744
<https://doi.org/10.1016/j.cellsig.2020.109744>
- Hu T, Yue J, Tang Q, Cheng KW, Chen F, Peng M, Zhou Q, Wang M (2022): The effect of quercetin on diabetic nephropathy (DN): a systematic review and meta-analysis of animal studies. *Food Funct.* **13**, 4789-4803
<https://doi.org/10.1039/D1FO03958J>
- Joo YS, Kim HW, Lee S, Nam KH, Yun HR, Jhee JH, Han SH, Yoo TH, Kang SW, Park JT (2021): Dietary zinc intake and incident chronic kidney disease. *Clin. Nutr.* **40**, 1039-1045
<https://doi.org/10.1016/j.clnu.2020.07.005>
- Kanwar YS, Sun L, Xie P, Liu FY, Chen S (2011): A glimpse of various pathogenetic mechanisms of diabetic nephropathy. *Annu. Rev. Pathol.* **6**, 395-423
<https://doi.org/10.1146/annurev.pathol.4.110807.092150>
- Kim S, Kang SW, Joo J, Han SH, Shin H, Nam BY, Park J, Yoo TH, Kim G, Lee P, et al (2021): Characterization of ferroptosis in kidney tubular cell death under diabetic conditions. *Cell. Death. Dis.* **12**, 160
<https://doi.org/10.1038/s41419-021-03667-y>
- Kon V, Yang HC, Smith LE, Vickers KC, Linton MF (2021): High-density lipoproteins in kidney disease. *Int. J. Mol. Sci.* **22**, 8201
<https://doi.org/10.3390/ijms22158201>
- Kong Z, Xiao M, Wang B, Zhang W, Che K, Lv W, Wang Y, Huang Y, Zhao H, Zhao Y, et al (2023): Renoprotective effect of isoorientin in diabetic nephropathy via activating autophagy and inhibiting the PI3K-AKT-TSC2-mTOR pathway. *Am. J. Chin. Med.* **51**, 1269-1291
<https://doi.org/10.1142/S0192415X23500581>
- Lei D, Chengcheng L, Xuan Q, Yibing C, Lei W, Hao Y, Xizhi L, Yuan L, Xiaoxing Y, Qian L (2019): Quercetin inhibited mesangial

- cell proliferation of early diabetic nephropathy through the Hippo pathway. *Pharmacol. Res.* **146**, 104320
<https://doi.org/10.1016/j.phrs.2019.104320>
- Lei L, Zhao J, Liu XQ, Chen J, Qi XM, Xia LL, Wu YG (2021): Wogonin alleviates kidney tubular epithelial injury in diabetic nephropathy by inhibiting PI3K/Akt/NF- κ B signaling pathways. *Drug. Des. Devel. Ther.* **15**, 3131-3150
<https://doi.org/10.2147/DDDT.S310882>
- Li Z, Deng H, Guo X, Yan S, Lu C, Zhao Z, Feng X, Li Q, Wang J, Zeng J, Ma X (2022): Effective dose/duration of natural flavonoid quercetin for treatment of diabetic nephropathy: A systematic review and meta-analysis of rodent data. *Phytomedicine* **105**, 154348
<https://doi.org/10.1016/j.phymed.2022.154348>
- Liu H, Zhan X, Xu G, Wang Z, Li R, Wang Y, Qin Q, Shi W, Hou X, Yang R, et al (2021): Cryptotanshinone specifically suppresses NLRP3 inflammasome activation and protects against inflammasome-mediated diseases. *Pharmacol. Res.* **164**, 105384
<https://doi.org/10.1016/j.phrs.2020.105384>
- Liu L, Li X, Cai W, Guo K, Shi X, Tan L, Zhan Y, Jing X, Wang W, Zhou S, et al (2022): Coadministration of Compound Danshen dripping pills and bezafibrate has a protective effect against diabetic retinopathy. *Front. Pharmacol.* **13**, 1014991
<https://doi.org/10.3389/fphar.2022.1014991>
- Liu XJ, Hu XK, Yang H, Gui LM, Cai ZX, Qi MS, Dai CM (2022): A review of traditional chinese medicine on treatment of diabetic nephropathy and the involved mechanisms. *Am. J. Chin. Med.* **50**, 1739-1779
<https://doi.org/10.1142/S0192415X22500744>
- Liu Y, Wang S, Jin G, Gao K, Wang S, Zhang X, Zhou K, Cai Y, Zhou X, Zhao Z (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
<https://doi.org/10.1016/j.phymed.2023.154915>
- Liu Z, Li X, Wang Y, Song Y, Liu Q, Gong J, Fan W, Lv C, Cao C, Zhao W, et al (2023): The concordance and discordance of diabetic kidney disease and retinopathy in patients with type 2 diabetes mellitus: A cross-sectional study of 26,809 patients from 5 primary hospitals in China. *Front. Endocrinol. (Lausanne)* **14**, 1133290
<https://doi.org/10.3389/fendo.2023.1133290>
- Ni WJ, Guan XM, Zeng J, Zhou H, Meng XM, Tang LQ (2022): Berberine regulates mesangial cell proliferation and cell cycle to attenuate diabetic nephropathy through the PI3K/Akt/AS160/GLUT1 signalling pathway. *J. Cell. Mol. Med.* **26**, 1144-1155
<https://doi.org/10.1111/jcmm.17167>
- Poznyak A, Grechko AV, Poggio P, Myasoedova VA, Alfieri V, Orekhov AN (2020): The diabetes mellitus-atherosclerosis connection: The role of lipid and glucose metabolism and chronic inflammation. *Int. J. Mol. Sci.* **21**, 1835
<https://doi.org/10.3390/ijms21051835>
- Pradhan G, Kulkarni YA (2024): Diabetes and its complications: Role of luteolin, a wonder chemical from the natural source. *Curr. Diabetes. Rev.* **21**, e290224227537
<https://doi.org/10.2174/0115733998285798240217084632>
- Rabbani N, Thornalley PJ (2018): Advanced glycation end products in the pathogenesis of chronic kidney disease. *Kidney. Int.* **93**, 803-813
<https://doi.org/10.1016/j.kint.2017.11.034>
- Reidy K, Kang HM, Hostetter T, Susztak K (2014): Molecular mechanisms of diabetic kidney disease. *J. Clin. Invest.* **124**, 2333-2340
<https://doi.org/10.1172/JCI72271>
- Schrauben SJ, Shou H, Zhang X, Anderson AH, Bonventre JV, Chen J, Coca S, Furth SL, Greenberg JH, Gutierrez OM, et al (2021): Association of multiple plasma biomarker concentrations with progression of prevalent diabetic kidney disease: Findings from the Chronic Renal Insufficiency Cohort (CRIC) Study. *J. Am. Soc. Nephrol.* **32**, 115-126
<https://doi.org/10.1681/ASN.2020040487>
- Sridhar VS, Liu H, Cherney DZI (2021): Finerenone-a new frontier in renin-angiotensin-aldosterone system inhibition in diabetic kidney disease. *Am. J. Kidney Dis.* **78**, 309-311
<https://doi.org/10.1053/j.ajkd.2021.02.324>
- Su WY, Li Y, Chen X, Li X, Wei H, Liu Z, Shen Q, Chen C, Wang YP, Li W (2021): Ginsenoside Rh1 improves type 2 diabetic nephropathy through AMPK/PI3K/Akt-mediated inflammation and apoptosis signaling pathway. *Am. J. Chin. Med.* **49**, 1215-1233
<https://doi.org/10.1142/S0192415X21500580>
- Tang G, Li S, Zhang C, Chen H, Wang N, Feng Y (2021): Clinical efficacies, underlying mechanisms and molecular targets of Chinese medicines for diabetic nephropathy treatment and management. *Acta Pharm. Sin. B.* **11**, 2749-2767
<https://doi.org/10.1016/j.apsb.2020.12.020>
- Tang S, An X, Sun W, Zhang Y, Yang C, Kang X, Sun Y, Jiang L, Zhao X, Gao Q, et al (2024): Parallelism and non-parallelism in diabetic nephropathy and diabetic retinopathy. *Front. Endocrinol. (Lausanne)* **15**, 1336123
<https://doi.org/10.3389/fendo.2024.1336123>
- Tuttle KR, Cherney DZI (2021): Therapeutic transformation for diabetic kidney disease. *Kidney Int.* **99**, 301-303
<https://doi.org/10.1016/j.kint.2020.10.003>
- Vr ALBVR, Tan SH, Candasamy M, Bhattamisra SK (2019): Diabetic nephropathy: An update on pathogenesis and drug development. *Diabetes Metab. Syndr.* **13**, 754-762
<https://doi.org/10.1016/j.dsx.2018.11.054>
- Wang D, Li J, Luo G, Zhou J, Wang N, Wang S, Zhao R, Cao X, Ma Y, Liu G, et al (2023): Nox4 as a novel therapeutic target for diabetic vascular complications. *Redox. Biol.* **64**, 102781
<https://doi.org/10.1016/j.redox.2023.102781>
- Wang Q, Cheng H, Jiang S, Zhang L, Liu X, Chen P, Liu J, Li Y, Liu X, Wang L, et al (2024): The relationship between diabetic retinopathy and diabetic nephropathy in type 2 diabetes. *Front. Endocrinol. (Lausanne)* **15**, 1292412
<https://doi.org/10.3389/fendo.2024.1292412>
- Xu S, He L, Ding K, Zhang L, Xu X, Wang S, Qian X (2020): Tanshinone IIA ameliorates streptozotocin-induced diabetic nephropathy, partly by attenuating PERK pathway-induced fibrosis. *Drug Des. Devel. Ther.* **14**, 5773-5782
<https://doi.org/10.2147/DDDT.S257734>
- Xu X, Wang M, Zhang S, Wang J, Li X, Ma X, Luo Y, Sun X (2024a): Compound Danshen dripping pills prevent early diabetic retinopathy: roles of vascular protection and neuroprotection. *Front. Pharmacol.* **15**, 1294620
<https://doi.org/10.3389/fphar.2024.1294620>

- Xu Z, Cai K, Su SL, Zhu Y, Liu F, Duan JA (2024b): Salvianolic acid B and tanshinone IIA synergistically improve early diabetic nephropathy through regulating PI3K/Akt/NF- κ B signaling pathway. *J. Ethnopharmacol.* **319**, 117356
<https://doi.org/10.1016/j.jep.2023.117356>
- Xu Z, Jia K, Wang H, Gao F, Zhao S, Li F, Hao J (2021): METTL14-regulated PI3K/Akt signaling pathway via PTEN affects HDAC5-mediated epithelial-mesenchymal transition of renal tubular cells in diabetic kidney disease. *Cell. Death. Dis.* **12**, 32
<https://doi.org/10.1038/s41419-020-03312-0>
- Xue M, Cheng Y, Han F, Chang Y, Yang Y, Li X, Chen L, Lu Y, Sun B, Chen L (2018): Triptolide attenuates renal tubular epithelial-mesenchymal transition via the miR-188-5p-mediated PI3K/AKT pathway in diabetic kidney disease. *Int. J. Biol. Sci.* **14**, 1545-1557
<https://doi.org/10.7150/ijbs.24032>
- Yan L, Vaghari-Tabari M, Malakoti F, Moein S, Qujeq D, Yousefi B, Asemi Z (2023): Quercetin: an effective polyphenol in alleviating diabetes and diabetic complications. *Crit. Rev. Food. Sci. Nutr.* **63**, 9163-9186
<https://doi.org/10.1080/10408398.2022.2067825>
- Yang H, Xie T, Li D, Du X, Wang T, Li C, Song X, Xu L, Yi F, Liang X, et al (2019): Tim-3 aggravates podocyte injury in diabetic nephropathy by promoting macrophage activation via the NF- κ B/TNF- α pathway. *Mol. Metab.* **23**, 24-36
<https://doi.org/10.1016/j.molmet.2019.02.007>
- Yang J, Liu Z (2022): Mechanistic pathogenesis of endothelial dysfunction in diabetic nephropathy and retinopathy. *Front. Endocrinol. (Lausanne)* **13**, 816400
<https://doi.org/10.3389/fendo.2022.816400>
- Yao L, Liang X, Liu Y, Li B, Hong M, Wang X, Chen B, Liu Z, Wang P (2023): Non-steroidal mineralocorticoid receptor antagonist finerenone ameliorates mitochondrial dysfunction via PI3K/Akt/eNOS signaling pathway in diabetic tubulopathy. *Redox. Biol.* **68**, 102946
<https://doi.org/10.1016/j.redox.2023.102946>
- Yu J, Da J, Yu F, Yuan J, Zha Y (2024): HMGN1 down-regulation in the diabetic kidney attenuates tubular cells injury and protects against renal inflammation via suppressing MCP-1 and KIM-1 expression through TLR4. *J. Endocrinol. Invest.* **47**, 1015-1027
<https://doi.org/10.1007/s40618-023-02292-0>
- Zhang L, Miao R, Yu T, Wei R, Tian F, Huang Y, Tong X, Zhao L (2022): Comparative effectiveness of traditional Chinese medicine and angiotensin converting enzyme inhibitors, angiotensin receptor blockers, and sodium glucose cotransporter inhibitors in patients with diabetic kidney disease: A systematic review and network meta-analysis. *Pharmacol. Res.* **177**, 106111
<https://doi.org/10.1016/j.phrs.2022.106111>
- Zhang L, Yang F (2022): Tanshinone IIA improves diabetes-induced renal fibrosis by regulating the miR-34-5p/Notch1 axis. *Food. Sci. Nutr.* **10**, 4019-4040
<https://doi.org/10.1002/fsn3.2998>
- Zhang M, He L, Liu J, Zhou L (2021): Luteolin attenuates diabetic nephropathy through suppressing inflammatory response and oxidative stress by inhibiting STAT3 pathway. *Exp. Clin. Endocrinol. Diabetes.* **129**, 729-739
<https://doi.org/10.1055/a-0998-7985>

Received: January 21, 2025

Final version accepted: June 8, 2025