Diabetes, Metabolic Syndrome and Obesity

Dovepress
Taylor & Francis Group

Open Access Full Text Article

ORIGINAL RESEARCH

Yiqi Yangyin Tongluo Recipe Alleviates Diabetic Kidney Disease Through AGE-RAGE Signalling Axis

Rucui Yu[1], Ruiying Wu[1], Tingting Chen[1], Yan Zhang[2], Xiangqing Chen[3]

[1]Department of Traditional Chinese Medicine, The First Affiliated Hospital of USTC, Division of Life Sciences and Medicine, University of Science and Technology of China, Hefei, 230001, People's Republic of China; [2]Department of Anorectal, 901 Hospital of Joint Service Support Force of PLA, Hefei, 230031, People's Republic of China; [3]Department of Pharmacy, The First Affiliated Hospital of USTC, Division of Life Sciences and Medicine, University of Science and Technology of China, Hefei, 230001, People's Republic of China

Correspondence: Rucui Yu, Department of Traditional Chinese Medicine, The First Affiliated Hospital of USTC, Division of Life Sciences and Medicine, University of Science and Technology of China, No. 17 Lujiang Road, Luyang District, Hefei, Anhui Province, 230001, People's Republic of China, Email yurucui_169@163.com; Xiangqing Chen, Department of Pharmacy, The First Affiliated Hospital of USTC, Division of Life Sciences and Medicine, University of Science and Technology of China, No. 17 Lujiang Road, Luyang District, Hefei, Anhui Province, 230001, People's Republic of China, Email cxqah@163.com

Purpose: This study used network pharmacology to explore how Yiqi Yangyin Tongluo Formula (YQYTLF) alleviates diabetic nephropathy (DN), focusing on the AGE-RAGE signaling pathway.

Methods: Active compounds and targets of YQYTLF were identified via TCMSP, and a herb-compound-target network was constructed using Cytoscape. Differentially expressed genes from DN kidney tissues (GEO dataset GSE104948) were intersected with drug targets for KEGG and GO enrichment analysis. Protein-protein interaction (PPI) networks were analyzed in STRING and visualized in Cytoscape. A DN rat model was treated with YQYTLF, with weekly monitoring of body weight, food and water intake. Fasting blood glucose (FBG), insulin (FINS), serum creatinine (Scr), blood urea nitrogen (BUN), and urinary albumin excretion rate (UAER) were measured. HOMA-β and HOMA-IR assessed beta cell function and insulin resistance. Kidney pathology was evaluated by HE and Sirius Red staining. Kidney tissue levels of AGEs, oxidative stress markers (ROS, MDA, GSH, SOD), and RAGE expression (by WB) were analyzed. Molecular docking assessed binding between active compounds and core targets.

Results: Network pharmacology identified 13 core targets, 8 enriched in the AGE-RAGE pathway. YQYTLF significantly reduced FBG, FINS, Scr, BUN, UAER, HOMA-IR, renal index, and pruritus while improving HOMA-β in DN rats. Renal pathological changes including Bowman's capsule dilation, mesangial proliferation, and fibrosis were alleviated. YQYTLF inhibited kidney AGEs, oxidative stress, and RAGE expression. Molecular docking confirmed strong binding between active ingredients and core targets.

Conclusion: YQYTLF alleviates DN in rats by inhibiting AGE-RAGE pathway activation and reducing oxidative stress, providing a theoretical basis for DN therapy.

Keywords: diabetes nephropathy, Yiqi Yangyin Tongluo formula, AGE-RAGE, inflammation, network pharmacology

Introduction

Diabetic nephropathy (DN) is one of the most prevalent complications of diabetes mellitus all over the world and the major cause of the end-stage renal disease.[1,2] Approximately 50% of end-stage renal disease cases are attributed to DN.[3,4] The functional characteristics of DN include proteinuria and albuminuria, while its pathological features are characterized by glomerular hypertrophy, mesangial expansion, and tubulointerstitial fibrosis.[5–8] At present, the treatment of DN mainly involves nutritional intervention and the control of blood glucose and hypertension, including taking hypoglycemic drugs that directly affect renal function and drugs that inhibit the renin-angiotensin system.[1,9] However, the current treatment of DN is limited to delaying the onset and progression of the disease and cannot effectively reverse renal injury and dysfunction or prevent the development of end-stage renal disease.[9,10] Traditional Chinese medicine

Received: 19 February 2025
Accepted: 19 June 2025
Published: 28 June 2025

(TCM) has been proved to play a role in alleviating kidney diseases including DN.[11–14] Thus, investigating the molecular mechanisms through which TCM alleviates DN is crucial for its management.

TCM has long been an important resource for protecting human health, improving quality of life, and treating various diseases, including diabetes and its complications.[15,16] The herbal compound Yi Qi Yangyin Tong Luo Fang (YQYTLF) has been shown to have significant effects in improving DN.[17] However, the specific mechanisms of action of YQYTLF have not been fully elucidated. TCM formulas have shown promising prospects in improving chronic kidney diseases (CKD), including DN, through multi-target and multi-pathway mechanisms.[18,19] In recent years, studies have confirmed that both single herbs and compound TCM preparations exhibit beneficial effects in alleviating renal injury and metabolic dysfunction in DN models.[20,21] These findings provide a theoretical basis for further exploring the therapeutic mechanisms of Yiqi Yangyin Tongluo Formula (YQYTLF) in the treatment of DN.

Network pharmacology using big data has become a powerful tool for investigating the mechanisms of TCM formulas with the development of bioinformatics.[22] This method is different from previous research methods, as it provides a systematic framework to address various scientific issues.[23] It is particularly suitable for analyzing multi-target drugs[24] and has become one of the promising methods to accelerate drug development.[25] Therefore, analysing YQYTLF by network pharmacology can help to further understand the mechanism of YQYTLF in treating DN.

This study aims to utilize network pharmacology to investigate the potential mechanisms of YQYTLF in treating DN, identify its active ingredients, targets, and pathways of action, provide a theoretical foundation for further understanding its effects, and offer scientific evidence to support its clinical application.

Methods

Identification of Active Ingredients and Target Prediction of YQYTLF

YQYTLF consists of *Astragalus membranaceus, Codonopsis pilosula, Salvia miltiorrhiza, Ophiopogon japonicus, Schisandra chinensis, Trichosanthes kirilowii, Dioscorea opposita, Panax notoginseng, Adenophorae Radix, Glehnia littoralis, Earthworm, Rehmannia glutinosa, Plantago asiatica, Alisma orientalis*, and *Cornus officinalis*. We utilized the TCM Systems Pharmacology Database (TCMSP) platform (https://old.tcmsp-e.com/index.php) to identify active compounds, applying the criteria of oral bioavailability (OB) ≥ 30% and drug-likeness (DL) ≥ 0.18.[26] The potential targets of the active components of YQYTLF were obtained through the TCMSP target module. Additionally, we supplemented the active components of YQYTLF herbs using the Herb Database (http://herb.ac.cn/). To show the relationships between active chemicals and possible targets, the network was displayed using Cytoscape 3.10.2. The bioactive substances and targets are represented by nodes, and the molecular interactions between them are shown by edges.

Collection and Analysis of DN Target Genes

We intersected the differentially expressed genes (DEGs) between DN kidney tissues and normal tissues ($P < 0.05$) from the GEO dataset GSE104948 with the drug targets. This intersection was visualized using Cytoscape 3.10.2. The intersecting factors were subjected to Gene Ontology (GO) enrichment analysis via the DAVID platform (https://davidbioinformatics.nih.gov/).[27] The STRING platform (https://string-db.org/) was used to perform Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis, and pathways were ordered based on the quantity of enriched genes in each signaling pathway. The human gene expression datasets used in this study were obtained from publicly available and anonymized databases (GEO). No identifiable personal information was included, and no direct contact with human participants occurred. According to Items 1 and 2 of Article 32 of the Measures for Ethical Review of Life Science and Medical Research Involving Human Subjects (effective February 18, 2023, China), studies using legally obtained, publicly available data or anonymized information that do not involve harm to human subjects, sensitive personal information, or commercial interests may be exempt from ethical review. Therefore, no additional institutional ethical approval was required for this study.

PPI Network Analysis

The STRING platform was used to collect protein-protein interaction (PPI) data for all YQYTLF targets. Cytoscape software was then used to examine these targets' connection (degree).

Molecular Docking

The key components and targets were used in a molecular docking analysis. The primary active chemicals' three-dimensional structures were obtained from PubChem and transformed into Protein Data Bank (PDB) format using Python-enhanced Molecular Graphics Tool (PyMOL).[28] The core targets' structures were obtained from the PDB (https://www.rcsb.org/). PyMOL was used to identify active binding sites after water molecules, metal ions, and tiny ligands were eliminated. AutoDock Vina 1.1.2 was used to prepare Protein Data Bank, Partial Charge (Q), Torsion (T) format (PDBQT) files for docking simulations. The binding energy, which indicates the stability of the target-compound complex, was calculated, and docking poses with the lowest binding energy were visualized in PyMOL to examine specific binding interactions.

Animal Husbandry

This study has been approved by the Animal Ethics Committee of The First Affiliated Hospital of USTC (Anhui Provincial Hospital) (No. 2025-N(A)-023), and all procedures involving animals were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (8th edition, 2011). A total of 24 male Sprague–Dawley (SD) rats (6 weeks old, 220–250 g) were obtained from Shanghai Yilebo Biological Technology Co., Ltd. They were maintained in a controlled environment with a 12-hour light/dark cycle, a temperature of 22 ± 2°C, and a relative humidity of 50 ± 5%. The rats were given free access to food and water and were acclimatized for one week.

Animal Grouping and Modeling

Rats were split up into the following groups at random: Control, DN, Saline, YQYTLF. The rats in the Control group were given a normal diet (ND), while the other rats were provided with a high-fat diet (HFD). After three weeks of exposure to the respective diets, rats on the HFD were injected with streptozotocin (STZ) (30 mg/kg/day, STZ; HY-13753, MedChemExpress) dissolved in 0.1 M citrate buffer (HY-B1610N, MedChemExpress, New Jersey, USA) intraperitoneally for ten consecutive days after overnight fasting. The vehicle (0.1 M citrate buffer) was given in a comparable volume to rats who were fed a regular diet. Following a STZ injection, blood glucose levels were measured with a glucose meter. Rats were only considered diabetic and included in the study if their blood glucose levels were ≥ 15 mM for two consecutive morning fasting readings. Based on these criteria, the success rate of diabetes induction was approximately 85%, which confirms the reliability of the DN model used in this study.

Drug Treatment

The basic composition of YQYTLF consists of: *Astragalus membranaceus* (20 g, 2318050, TRT, Beijing, China), *Codonopsis pilosula* (15 g. 100155415539, TRT), *Salvia miltiorrhiza* (10 g,100013741708, TRT), *Ophiopogon japonicus* (10 g, 1026899, TRT), *Schisandra chinensis* (10 g,7507088, TRT), *Trichosanthes kirilowii* (10 g, 10135591022840, Beijing TRT, China), *Dioscorea opposita* (10 g, 100088412657, TRT), *Panax notoginseng* (3 g, 100016085336, TRT), *Adenophorae Radix* (10 g, 100119288650, Sichuan fuxitang pharmacy Co., Ltd, China), *Glehnia littoralis* (10 g, 100007552599, TRT), *Earthworm* (10 g, 10130606377112, BAOYUANTANG, Guangdong, China), *Rehmannia glutinosa* (10 g, 100051065215, TRT), *Plantago asiatica* (10 g, 100049097440, XIUNIANTANG, Guangdong, China), Alisma orientalis (10 g, 100114697979, Banshannong, Xiamen, China), and *Cornus officinalis* (10 g, 100101765358, TRT). The herbal formula was decocted in water, concentrated, and prepared into a 2 g/mL stock solution. The adult equivalent dosage was calculated based on the body surface area ratio between rats and a 60 kg adult (6.3). The equivalent dosage for rats was approximately 0.418 g/kg.[26] The formula used for dosage calculation is: Adult dosage (g/kg) = Total adult dosage (g) / 60 kg; Rat dosage (g/kg) = Adult dosage (g/kg) / 6.3.

Rat Physiological Parameters and Sample Collection

The body weight, food intake, and water intake of all rats were monitored weekly from week 0 to week 4 during the experiment. Urine samples were collected over a 24-hour period before the last day of the experiment using metabolic cages. On day 28, after an overnight fast, blood samples were collected from the tail vein. At the experiment's conclusion, rats were euthanized with an overdose of pentobarbital sodium (200 mg/kg, P3761, Sigma-Aldrich, Darmstadt, German). The kidneys were then removed, weighed, and stored at −80°C for subsequent analysis.

Biochemical Parameters and Kidney Index Measurement

Serum was extracted from blood samples by centrifuging them for 20 minutes at 4°C at 3000 rpm. A glucose meter and enzyme-linked immunosorbent assay (ELISA) kits (ERINS, Invitrogen, Waltham, MA, USA) were used to test the levels of fasting blood glucose (FBG) and fasting insulin (FINS). The Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) and β-cell function (HOMA-β) were computed using the following formulas:[29] HOMA-β = 20 × FINS / (FBG - 3.5); HOMA-IR = (FINS × FBG) / 22.5.

Serum creatinine (Scr) was assessed using an ELISA kit (E-EL-0058, Elabscience, Wuhan, China), blood urea nitrogen (BUN) was measured with a colorimetric assay kit (E-BC-K183-S, Elabscience), and urinary albumin excretion rate (UAER) was determined using an ELISA kit (E-EL-R0362, Elabscience). Calculate the kidney index by dividing the kidney weight (mg) by the body weight (g).

Pruritus Assessment

In order to evaluate the itching of diabetes rats, the scratching behavior was recorded and quantified. The Von Frey filaments (0.7 mN) were used to induce scratching responses on the dorsum of the neck, and the total number of scratches was recorded to determine the pruritus score.

Hematoxylin and Eosin (HE) and Sirius Red Staining

Store kidney tissue in 4% paraformaldehyde (158127, Sigma-Aldrich) for 24 hours. Then, dehydrate, dry, and embed in paraffin. Sections were deparaffinized by immersion in xylene (534056, Sigma-Aldrich) for 5 minutes, rehydrated using ethanol (E7023, Sigma-Aldrich), and stained with Hematoxylin and Eosin (HE) or Sirius Red. For HE staining, sections were stained with hematoxylin (HHS16, Sigma-Aldrich) for 5 minutes, differentiated in 1% hydrochloric acid alcohol, and counterstained in 0.2% ammonia water for 1 minute. Sections were then stained with eosin (HT110132, Sigma-Aldrich) for 1 minute and dehydrated in ethanol. For Sirius Red staining, sections were stained with Sirius Red solution (365548, Sigma-Aldrich) for 1 hour and differentiated in 1% hydrochloric acid alcohol. Finally, the sections were mounted with Organo/Limonene Mount™ (O8015, Sigma-Aldrich) and observed under a Nikon Eclipse E200 microscope (Nikon Corporation, Tokyo, Japan).

Biochemical Analysis

Following the manufacturer's instructions, ELISA kits were used to measure AGEs (abx150316, Abbexa, Cambridge, UK) and GSH (abx257145, Abbexa) levels. ROS levels in rat kidney tissues were measured using the DCFH-DA assay kit (EEA019, Invitrogen). MDA (EEA015, Invitrogen) and SOD (EIASODC, Invitrogen) levels were measured using colorimetric assay kits.

Western Blot (WB) Analysis

Trypsin was used to digest the kidney tissues, and they were subsequently lysed in RIPA buffer (R0278, Sigma-Aldrich) on ice for half an hour while being shaken intermittently every five minutes. The supernatant was obtained following centrifugation at 12,000 rpm for 10 minutes at 4 °C. The BCA Protein Assay Kit (23227, Thermo Fisher Scientific, Waltham, MA, USA) was used to measure the protein concentrations. SDS-PAGE was used to separate the proteins, which were then moved to a PVDF membrane (88518, Thermo Fisher Scientific) and blocked for an hour using 5% non-fat milk. Primary antibodies against RAGE (1:1000, PA1-075, Invitrogen) and GAPDH (1:50,000, 4A9L6, Invitrogen)

were added to membranes, which were then incubated for the entire night at 4 °C. Following washing, membranes were left at room temperature for an hour to incubate with HRP-conjugated secondary antibody (1:10,000, 31460, Invitrogen). Protein bands were detected using ECL chemiluminescence reagent (32106, Thermo Fisher Scientific), and relative protein density was analyzed using ImageJ software.

Statistical Analysis

Prism 9 software (GraphPad, USA) was used to analyze the data, and the results are shown as mean ± SD. For comparisons between two groups, a *t*-test was employed. Tukey's post hoc test was used after a one-way or two-way ANOVA for comparisons between three or more groups. Statistical significance was defined as a p-value of less than 0.05.

Results

Active Components and Target Prediction of YQYTLF

By searching the TCMSP database, the chemical components of 12 herbs in YQYTLF were identified, and a total of 192 compounds were obtained.[30] Among them, contains 20 compounds, Codonopsis 21, Salvia 65, Schisandra 8, Tacca 2, Chinese Yam 16, Panax notoginseng 8, South and North Ginseng 5 and 8, Plantago 9, Alisma 10, and Cornus 20. After eliminating redundancy, 170 compounds were confirmed. The target genes of these compounds were predicted: Astragalus had 462 targets, Codonopsis 185, Salvia 899, Schisandra 30, Tacca 5, Chinese Yam 144, Panax notoginseng 253, South Ginseng 45, North Ginseng 256, Plantago 195, Alisma 9, and Cornus 130. After removing duplicates, 143 target genes were retained. Using Cytoscape 3.10.2, a herb-compound-target network with 390 nodes and 1786 edges was created (Figure 1).

Enrichment Analysis of YQYTLF Targets in Treating DN

The potential therapeutic targets of YQYTLF for DN were identified by intersecting the disease targets with drug targets. A total of 99 potential targets was obtained for YQYTLF in treating DN (Figure 2A and B). GO analysis revealed that these core genes were mainly involved in biological processes such as cell transcriptional regulation, signal transduction, stress response and inflammation. These genes were localized to the plasma membrane and extracellular regions, playing roles in cell signaling, gene regulation, apoptosis, cell cycle regulation, and oxidative stress and metabolic regulation (Figure 2C). Significant enrichment in pathways such AGE-RAGE signaling, TNF signaling, and IL-17 signaling was found by KEGG analysis, underscoring their critical involvement in the development and progression of diabetes problems (Figure 2D).

Identification of Core Targets in YQYTLF Treatment for DN

To identify the core targets of YQYTLF in treating DN, an herb-compound-disease target network was constructed. Using the cytoHubba plugin in Cytoscape, the top three components based on network analysis were MOL000098 (quercetin), MOL000006 (luteolin), and MOL000422 (kaempferol) (Figure 3A). These core targets were further analyzed through the PPI network, constructed by intersecting the targets in the STRING database and visualized using Cytoscape 3.10.2 (Figure 3B). Degree and MCC rankings in cytoHubba were used to determine the top ten core targets (Figure 3C and D). After eliminating duplicates, 13 core targets were confirmed. Notably, these core targets were enriched in the top 10 KEGG pathways. Among them, 8 core targets (JUN, STAT3, IL6, TNF, CASP3, AKT1, BCL2, TP53) were specifically enriched in the AGE-RAGE signaling pathway (Figure 3E). In addition, we demonstrated through pathway mapping on the KEGG platform that the YQYTLF target is mainly enriched in the AGE-RAGE signaling pathway (Figure 3F). These findings suggest that YQYTLF primarily alleviates DN through the AGE-RAGE signaling pathway.

Effect of YQYTLF on Blood Glucose and Renal Protection in Diabetic Rats

Validate the alleviating effect of YQYTLF on DN by constructing DN rats. At the beginning of the treatment, there were no significant differences in body weight, food intake, or water consumption among the four experimental groups. However,

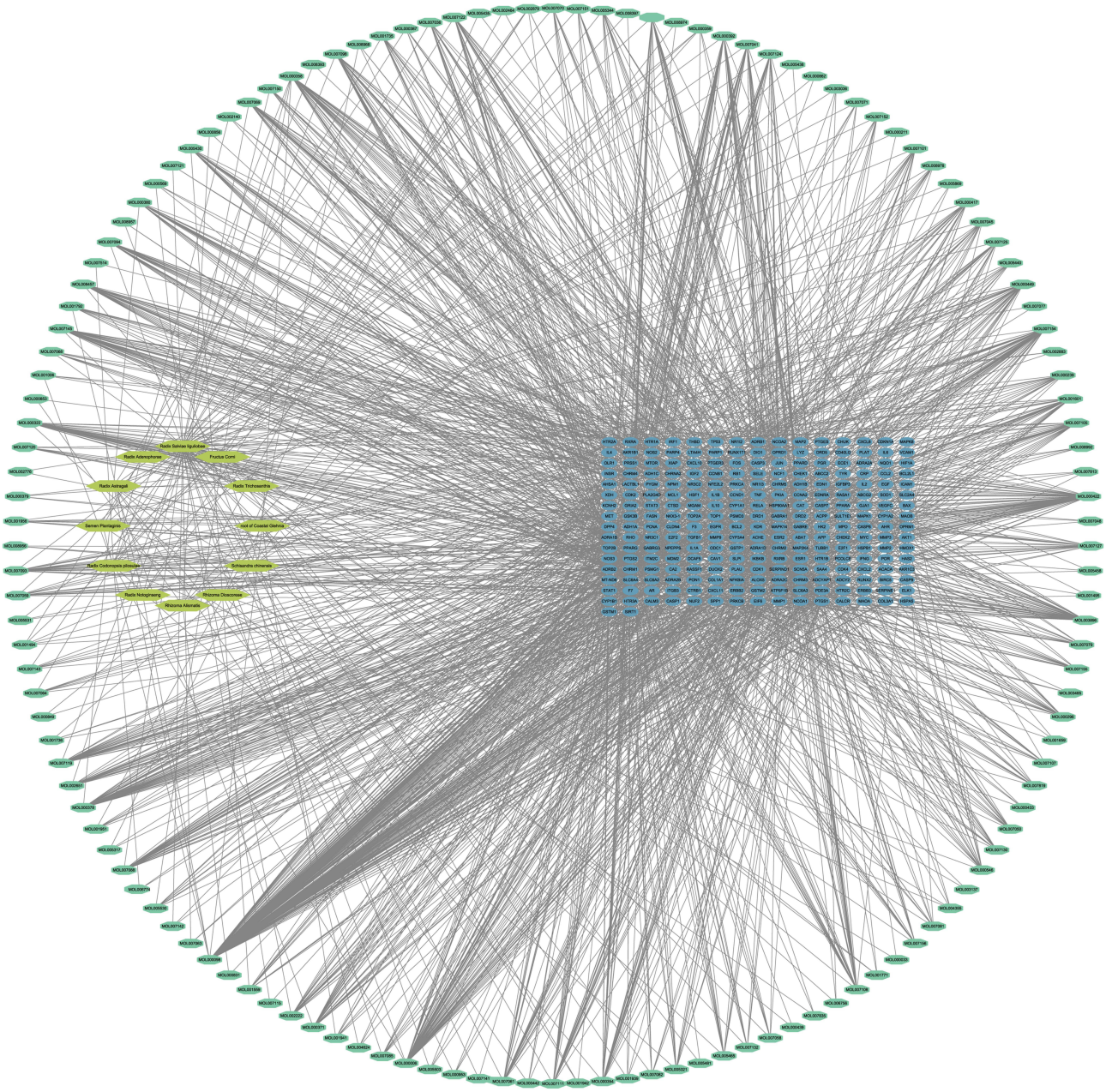

Figure 1 Herbal Compound Target Network Diagram.

from the second to the fourth week, the DN group showed a decrease in body weight and increased food and water intake compared to the control group. YQYTLF treatment effectively reversed these symptoms (Figure 4A), indirectly attesting to the DN model's effective establishment. As expected, the DN group had significantly elevated FBS and FINS levels compared to the control group, while YQYTLF treatment significantly reduced these levels (Figure 4B). Additionally, YQYTLF treatment improved the HOMA-β index and decreased the HOMA-IR index (Figure 4C). In the renal function assessment, the DN rats had markedly elevated Scr, BUN, and UAER, while YQYTLF treatment effectively suppressed the increase in Scr, BUN, and UAER induced by HFD/STZ (Figure 4D). Furthermore, DN rats exhibited itching behavior and increased kidney index, both of which were significantly improved after YQYTLF treatment (Figure 4E and F). HE staining results showed Bowman's capsule dilation and mesangial proliferation in the kidneys of the DN rats, which were markedly improved after YQYTLF treatment (Figure 4G). The diabetic rats had renal interstitial fibrosis, as seen by Sirius red staining,

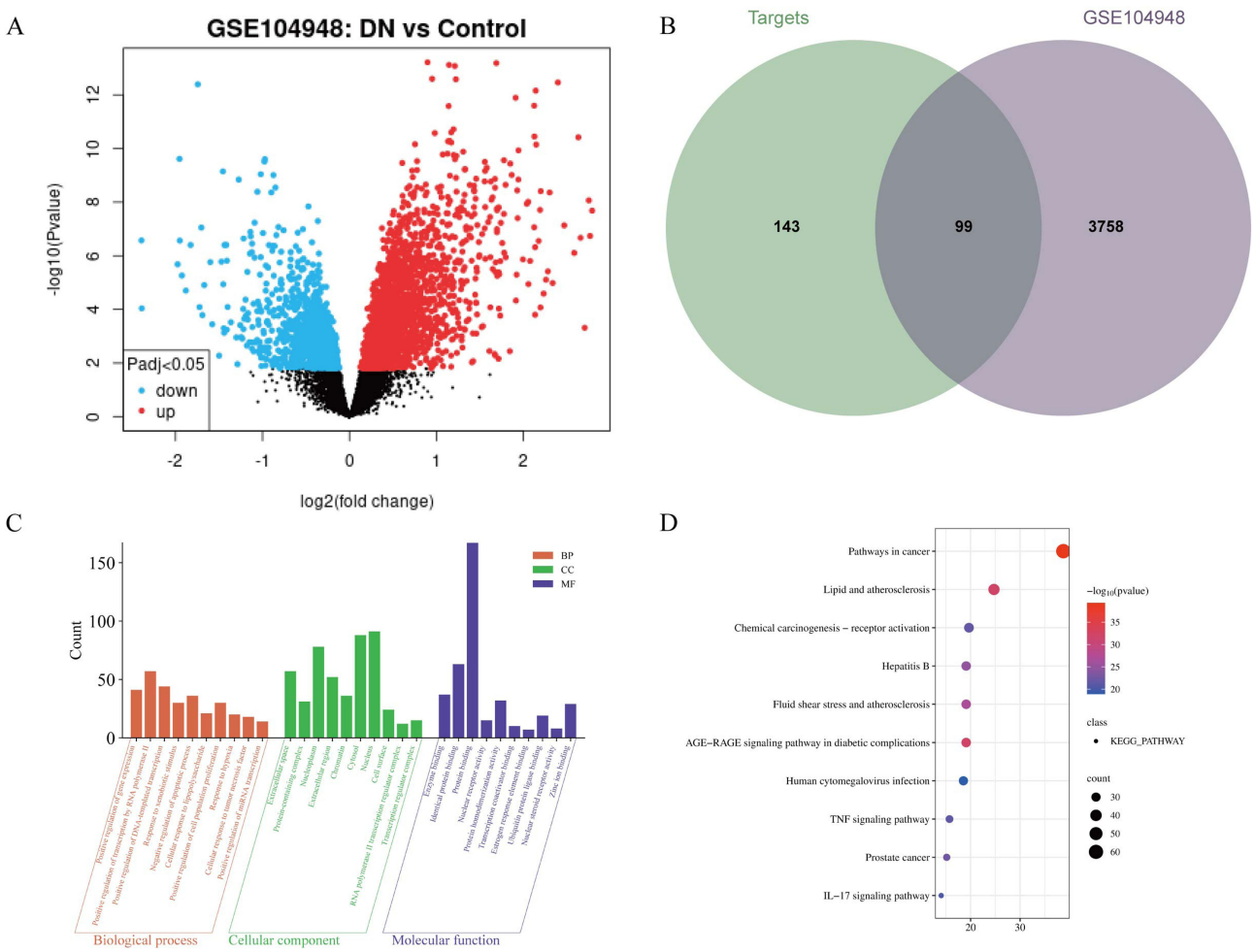

Figure 2 Enrichment Analysis of YQYTLF Targets in Treating DN. (**A**) Volcanic map of DEGs in DN screened by GSE10498. DN: Diabetic nephropathy. (**B**) Veen plot of intersection between drug targets and DEGs in GSE104948. (**C**) GO analysis bar chart. (**D**) KEGG enrichment analysis bubble plot.

while YQYTLF treatment effectively alleviated the fibrosis (Figure 4H). In conclusion, these data indicate that YQYTLF can effectively reduce blood glucose levels in diabetic rats and protect kidney function.

YQYTLF Alleviates Oxidative Stress in DN Through the AGE-RAGE Axis

There are studies indicating that the mechanism of high glucose induced cell damage involves oxidative stress.[31] Moreover, we have found through GO analysis that the core target genes of YQYTLF in treating DN are closely related to oxidative stress. The AGE/RAGE signaling pathway has been proven to exacerbate oxidative stress in diabetes,[32] with AGE stimulation promoting the expression of the AGE receptor RAGE.[33] ELISA results revealed that AGE levels were markedly elevated in the kidneys of diabetic rats, but YQYTLF treatment effectively reduced these levels (Figure 5A). Biochemical analysis showed increased ROS and MDA levels in the kidneys of DN rats, which were markedly decreased following YQYTLF treatment (Figure 5B and C), suggesting that YQYTLF mitigates oxidative stress. Furthermore, YQYTLF treatment significantly reversed the reduction in kidney GSH and SOD levels (Figure 5D–E), highlighting its potential in supporting the renal antioxidant defense system. Western blot analysis confirmed that RAGE expression in the kidneys of diabetic rats was markedly elevated compared to the control group, but YQYTLF treatment reversed this increase (Figure 5F). These findings indicate that YQYTLF inhibits the activation of the AGE-RAGE axis, alleviates oxidative stress, and protects kidney tissue in the progression of DN.

https://doi.org/10.2147/DMSO.S523784

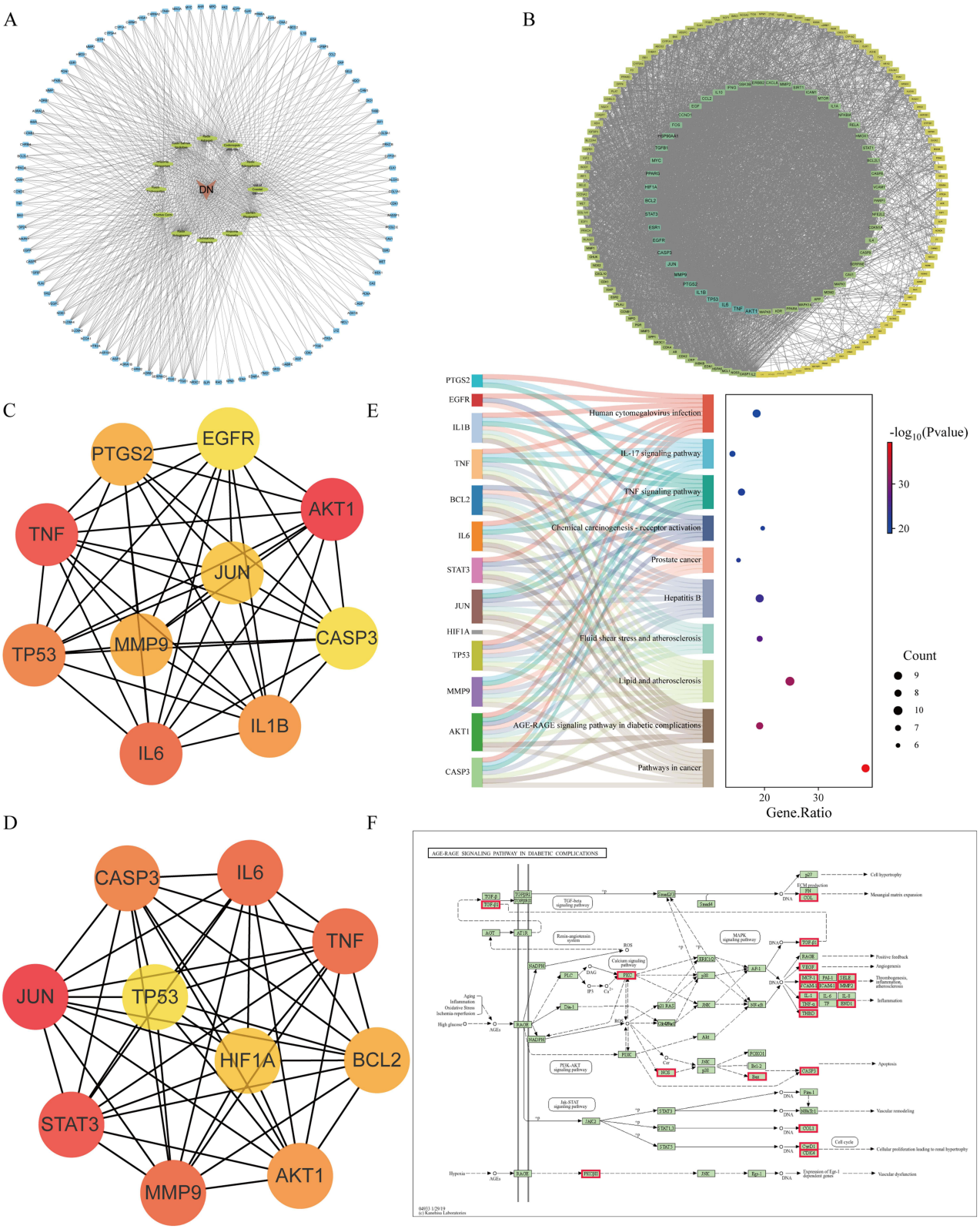

Figure 3 Identification of Core Targets in YQYTLF Treatment for DN. (**A**) Drug target disease network diagram. (**B**) Optimization diagram of PPI network for YQYTLF and DN intersection targets. (**C**) The network diagram of the top ten core targets ranked by degree value. (**D**) The network diagram of the top ten core targets with MCC values. (**E**) The Sankey plot of KEGG enrichment analysis shows the enrichment of core targets in the top 10 pathways. (**F**) The genes within the red rectangular box are the targets of YQYTLF, mainly enriched in the AGE-RAGE signaling pathway.

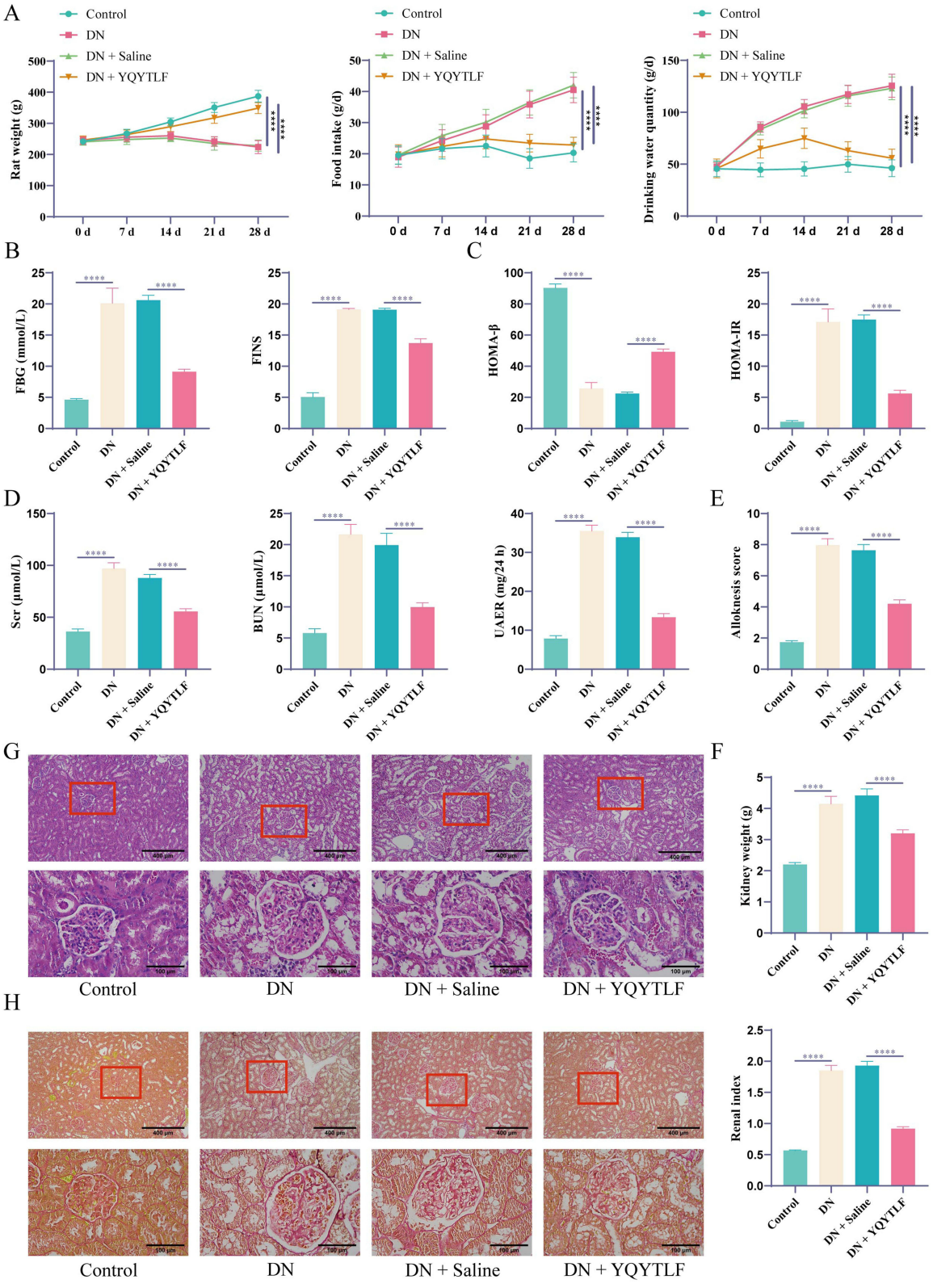

Figure 4 Effect of YQYTLF on Blood Glucose and Renal Protection in Diabetic Rats. (**A**) Comparison of body weight, food intake, and water intake of rats in each group within 4 weeks. (**B**) Detection of FBS and FINS levels in rats. (**C**) Detection of HOMA - β index and HOMA-IR index in rats. (**D**) Scr, BUN, and UAER detection in rats. (**E**) Record heterogeneity scores to evaluate mechanical itching behavior. (**F**) Measurement of kidney weight and kidney index in rats. (**G**) HE staining was used to detect Bowman's capsule dilation and mesangial proliferation in rat kidneys. (**H**) Sirius red staining was used to detect interstitial fibrosis in rat kidneys. n = 6. The image magnification is 100x and 400x respectively.The region within the red square represents the area shown at 400× magnification. **** P < 0.0001. Three or more sets of data will be analyzed using one-way or two-way ANOVA, and Tukey's will be used for post hoc testing.

https://doi.org/10.2147/DMSO.S523784

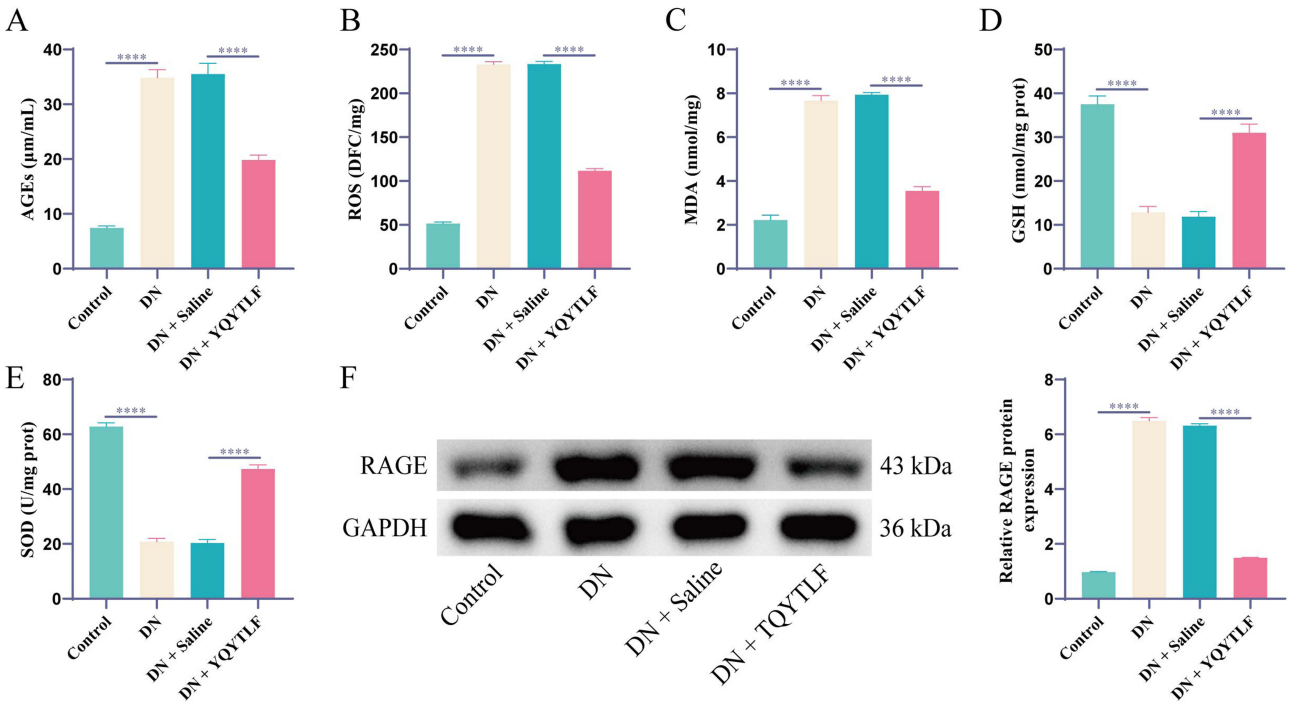

Figure 5 YQYTLF alleviates oxidative stress in DN through the AGE-RAGE axis. (**A**) ELISA detection of AGE levels in rat kidney tissue. (**B–E**) Biochemical analysis of ROS, MDA, GSH, and SOD levels in rat kidney tissue. (**F**) WB detection of RAGE expression in rat kidney tissue. n = 6. **** P < 0.0001. Three or more sets of data will be analyzed using one-way ANOVA, and Tukey's will be used for post hoc testing.

Molecular Docking

We performed molecular docking between the top three active compounds, quercetin, luteolin, and kaempferol, and the top 10 core targets (TNF, PTGS2, CASP3, IL1B, MMP2, TP53, TGFB1, ESR1, ICAM1, EGF) obtained from the intersection analysis (Figure 6A). The docking results were analyzed based on binding energy, with a binding energy of < −4.25 kcal/mol indicating moderate binding affinity, < −5.0 kcal/mol indicating high binding affinity, and < −7.0 kcal/mol indicating very strong binding affinity.[34] In this study, although we performed molecular docking between the target protein and small molecule compounds, in some cases, the distance between the compound and the protein was too large, preventing effective docking. Additionally, we conducted docking analysis between luteolin and PTGS2 and found some spatial compatibility between the two, but no stable binding was formed, primarily due to the lack of hydrogen bonding. As a result of the above factors, we ultimately obtained 15 valid molecular docking results. According to the molecular docking heatmap, all 15 docking results showed binding energies < −5.0 kcal/mol, and 13 of them (86.7%) had binding energies < −7.0 kcal/mol (Figure 6B). Additionally, we visualized the 15 docking results (Figure S1). Clearly, our results suggest that these active compounds have strong interactions with the target proteins in DN.

Discussion

Diabetic kidney disease (DKD) has increasing morbidity and mortality, especially from cardiovascular complications.[10,35] Diabetes involves a series of microvascular and macrovascular complications.[36] DN is one of the common microvascular complications of diabetes, which increases the incidence rate and mortality of diabetes patients.[37] The management of diabetes mainly focuses on strict blood glucose control, antihypertensive, and lipid-lowering treatments; however, these measures have not been effective in preventing the progression of DN in most patients.[38] TCM has shown significant efficacy in improving and preventing diabetes nephropathy.[20,39,40] Therefore, investigating the mechanisms by which TCM improves DN could help advance treatment options for DN.

To further understand the molecular mechanism of YQYTLF in treating DN, we screened the active compounds of the drug using the TCMSP database, a leading database of systems pharmacology for drug discovery from TCM and

Figure 6 Molecular Docking. (**A**) Venn diagrams showing the intersection of targets for quercetin, luteolin, and kaempferol with core genes. Colored boxes indicate overlapping target genes: yellow box for quercetin, red box for luteolin, and Orange box for kaempferol. (**B**) Molecular docking heatmap displaying binding energies (kcal/mol) of active compounds with core target proteins. The color scale represents binding affinity, with darker shades indicating stronger binding. Blank cells indicate no significant binding or docking was not performed.

https://doi.org/10.2147/DMSO.S523784

predicted their potential targets.[41,42] We then constructed a drug-compound-target network using Cytoscape. By performing an intersection analysis of drug targets and the DEGs from the DNGSE104948 dataset ($p < 0.05$), we identified 99 intersection factors. These core genes are mainly involved in cellular transcriptional regulation, signal transduction, stress responses, and inflammation, and are located on the plasma membrane and extracellular regions, demonstrating functions related to cellular signaling, gene regulation, apoptosis, cell cycle control, oxidative stress and metabolic regulation. Inflammatory and stress response pathways are closely associated with the development of CKD including DN,[43–46] suggesting that YQYTLF plays a crucial role in DN treatment. Additionally, AGE-RAGE signaling, TNF signaling, and IL-17 signaling pathways were significantly enriched in the genes studied. We also constructed an herbal-compound-disease target network and analyzed it using the Cytoscape plugin cytoHubba. Notably, the top three active compounds in YQYTLF quercetin, luteolin and kaempferol are all flavonoid compounds. Among numerous natural compounds, flavonoids are known for their prominent pharmacological activities, including antidiabetic, antioxidant, antihypertensive properties, and anti-inflammatory effects.[47–49] Specifically, quercetin exhibits antioxidant, anti-allergic, anti-inflammatory and anti-apoptotic activities.[50] Luteolin alleviates DN through its anti-inflammatory effects,[51] while kaempferol protects against DN and renal fibrosis by exerting antioxidant and anti-inflammatory actions.[52,53] These studies suggest that quercetin, luteolin, and kaempferol mainly exert their effects through anti-inflammatory and antioxidant pathways. Moreover, the 13 core targets selected based on Degree and MCC rankings in cytoHubba were enriched in the top 10 pathways from KEGG enrichment analysis, with eight core targets (JUN, STAT3, IL6, TNF, CASP3, AKT1, BCL2, and TP53) enriched in the AGE-RAGE signaling pathway. AGEs are markers of DN, and their binding with the AGE receptor (RAGE) mediates oxidative stress and chronic inflammation, leading to kidney damage.[54,55] AGE-RAGE signaling promotes NF-κB expression.[56] Clearly, the active compounds in YQYTLF may influence DN through the AGE-RAGE pathway.

We established a DN rat model and performed oral administration of YQYTLF. DN rats exhibited significant weight loss, increased food and water intake, which were reversed by YQYTLF treatment. Additionally, YQYTLF treatment significantly suppressed the increase in FBS, FINS and HOMA-IR index, as well as the decrease in HOMA-β index after modeling. Clearly, YQYTLF has a blood glucose-lowering effect. Furthermore, YQYTLF treatment inhibited the elevation of Scr, BUN, and UAER induced by HFD/STZ. YQYTLF also reduced the itching behavior and kidney index in DN rats. We further evaluated the structural and functional changes in the kidneys and found that YQYTLF treatment significantly inhibited Bowman's capsule expansion, mesangial proliferation, and renal interstitial fibrosis in DN rats. Clearly, we have confirmed that YQYTLF exerts therapeutic effects on DN.

Increasing evidence has indicated that high glucose-induced cellular damage involves oxidative stress.[31] In this investigation, we observed that the level of AGEs in the kidneys of diabetic rats was significantly elevated, and YQYTLF treatment significantly reduced AGEs levels. Moreover, YQYTLF treatment effectively suppressed oxidative stress in the kidney tissue of DN rats. This indicates that YQYTLF alleviates kidney oxidative stress, thereby protecting the kidney during the development of DN. Additionally, YQYTLF treatment reversed the increased expression of RAGE in DN rat kidney tissues. AGE stimulation promotes the expression of AGE receptor RAGE.[57] It has been demonstrated that AGE/RAGE signaling increases oxidative stress in diabetics.[32] Clearly, YQYTLF treatment inhibits the activation of the AGE-RAGE axis and alleviates oxidative stress in the kidney tissue of rats, exerting a protective effect. Recent studies have shown that TCM and their active components can improve renal oxidative damage and inflammation by modulating redox-sensitive signaling pathways and antioxidant enzyme systems. Several studies supported this mechanism: *Poria cocos* (Fuling) improved acute and chronic kidney injury and related diseases by regulating multiple signaling pathways;[58] natural products alleviated renal inflammation in DN by inhibiting NLRP3 inflammasome activation;[59] genipin gentiobioside mitigated chronic tubulointerstitial nephritis by regulating aryl hydrocarbon receptor-mediated NF-kB/Nrf2 pathway.[60] These findings are consistent with our results and further support the therapeutic potential of YQYTLF in DN through antioxidant and anti-inflammatory mechanisms.

In addition, the top three active compounds and ten core targets from molecular docking analysis revealed that all 15 compound-target complexes had binding energies below −5.0 kcal/mol,[34,61] with compound luteolin exhibiting the lowest binding energy with target TNF (−6.5 kcal/mol). This indicates that the key components of YQYTLF have strong binding affinities with core targets, providing a structural basis for modulating DN-related pathways such as AGE-

RAGE axis. This forms the basis of pharmacological effects of YQYTLF. Among 10 core targets, 8 are enriched in AGE-RAGE pathway. Therefore, we speculate that YQYTLF may alleviate DN by directly inhibiting AGE-RAGE pathway and affecting downstream targets of AGE-RAGE pathway.

Although our study demonstrates that YQYTLF exerts therapeutic effects on DN in rats by inhibiting AGE-RAGE axis and reducing renal oxidative stress, several important questions remain to be addressed in future research. Clinical translation will require rigorous evaluation of YQYTLF's efficacy, safety, and optimal dosing in human patients through well-designed clinical trials. Further mechanistic investigations should focus on identifying specific contributions of core active compounds within herbal formulation, as well as elucidating potential effects on downstream signaling pathways (NF-kB, MAPKs) and epigenetic regulation of RAGE expression. Given the chronic nature of DN, longer-term animal studies will be necessary to assess sustained renoprotective effects of YQYTLF. Additionally, while our findings are promising, future studies would benefit from formal power calculations to strengthen statistical reliability. These comprehensive investigations will be essential for fully characterizing YQYTLF's therapeutic potential and molecular mechanisms in DN.

Conclusions

Through network pharmacology analysis and studies using a DN rat model, we have confirmed that YQYTLF exerts a protective effect by inhibiting the activation of the AGE-RAGE axis and reducing oxidative stress in kidney tissues. This finding provides a potential new strategy for the subsequent treatment of DN.

Ethics Statement

This study has been approved by the Animal Ethics Committee of The First Affiliated Hospital of USTC (Anhui Provincial Hospital) (No. 2025-N(A)-023).

Funding

This study was supported by General Projects of National Natural Science Foundation of China (No. 82274426); Young Scientists Fund of the National Natural Science Foundation of China (No. 81904155); Anhui Province Traditional Chinese Medicine Inheritance and Innovation Research Project (No. 2024ZYYXH028).

Disclosure

The authors declare no competing conflicts of interest in this work.

References

1. Correa-Rotter R, Maple-Brown LJ, Sahay R, Tuttle KR, Ulasi II. New and emerging therapies for diabetic kidney disease. *Nat Rev Nephrol*. 2024;20(3):156–160. doi:10.1038/s41581-023-00782-1
2. Glastras SJ, Pollock CA. Targeted identification of risk and treatment of diabetic kidney disease. *Nat Rev Nephrol*. 2024;20(2):75–76. doi:10.1038/s41581-023-00796-9
3. Li S, Susztak K. Mitochondrial dysfunction has a central role in diabetic kidney disease. *Nat Rev Nephrol*. 2025;21(2):77–78. doi:10.1038/s41581-024-00919-w
4. Varatharajan S, Jain V, Pyati AK, et al. Neutrophil gelatinase-associated lipocalin, kidney injury molecule-1, and periostin: novel urinary biomarkers in diabetic nephropathy. *World J Nephrol*. 2024;13(4):98880. doi:10.5527/wjn.v13.i4.98880
5. Liu D, Chen X, He W, et al. Update on the pathogenesis, diagnosis, and treatment of diabetic tubulopathy. *Integr Med Nephrol Androl*. 2024;11(4):e23. doi:10.1097/IMNA-D-23-00029
6. Balakumar P. Unleashing the pathological role of epithelial-to-mesenchymal transition in diabetic nephropathy: the intricate connection with multifaceted mechanism. *World J Nephrol*. 2024;13(2):95410. doi:10.5527/wjn.v13.i2.95410
7. Ji JL, Shi HM, Li ZL, et al. Satellite cell-derived exosome-mediated delivery of microRNA-23a/27a/26a cluster ameliorates the renal tubulointerstitial fibrosis in mouse diabetic nephropathy. *Acta Pharmacol Sin*. 2023;44(12):2455–2468. doi:10.1038/s41401-023-01140-4
8. Raikou VD. Renoprotective strategies. *World J Nephrol*. 2024;13(1):89637. doi:10.5527/wjn.v13.i1.89637
9. DeFronzo RA, Reeves WB, Awad AS. Pathophysiology of diabetic kidney disease: impact of SGLT2 inhibitors. *Nat Rev Nephrol*. 2021;17(5):319–334. doi:10.1038/s41581-021-00393-8
10. Fioretto P, Pontremoli R. Expanding the therapy options for diabetic kidney disease. *Nat Rev Nephrol*. 2022;18(2):78–79. doi:10.1038/s41581-021-00522-3
11. Hu Q, Jiang L, Yan Q, Zeng J, Ma X, Zhao Y. A natural products solution to diabetic nephropathy therapy. *Pharmacol Ther*. 2023;241:108314. doi:10.1016/j.pharmthera.2022.108314

12. Wang YN, Zhang ZH, Liu HJ, et al. Integrative phosphatidylcholine metabolism through phospholipase A_2 in rats with chronic kidney disease. *Acta Pharmacol Sin*. 2023;44(2):393–405. doi:10.1038/s41401-022-00947-x
13. Zhong X, Jia J, Tan R, Wang L. Hederagenin improves Adriamycin-induced nephropathy by inhibiting the JAK/STAT signaling pathway. *Integr Med Nephrol Androl*. 2024;11(2):e22–00016. doi:10.1097/imna-d-22-00016
14. Kuang BC, Wang ZH, Hou SH, et al. Methyl eugenol protects the kidney from oxidative damage in mice by blocking the Nrf2 nuclear export signal through activation of the AMPK/GSK3β axis. *Acta Pharmacol Sin*. 2023;44(2):367–380. doi:10.1038/s41401-022-00942-2
15. Chen Y, Liu Y, Cao A. The potential of huangqi decoction for treating diabetic kidney disease. *Integr Med Nephrol Androl*. 2024;11(1):e00020. doi:10.1097/imna-d-23-00020
16. Zou TF, Liu ZG, Cao PC, et al. Fisetin treatment alleviates kidney injury in mice with diabetes-exacerbated atherosclerosis through inhibiting CD36/fibrosis pathway. *Acta Pharmacol Sin*. 2023;44(10):2065–2074. doi:10.1038/s41401-023-01106-6
17. Yurucui ZL, Jinhu L, Yan Z. Yiqi Yangyin Tongluo Recipe Regulates miR-485-5p by Targeting lncRNA UCA1 to Inhibit Renal Tubular Epithelial Cell Apoptosis and Inflammatory Response in diabetes Nephropathy Rats. *Shaanxi Tradit Chin Med*. 2023;44(08):1000–1004.
18. xx G, Lh C, Hx N, Zang ZY, Chang H. Chinese herbal medicine for the treatment of diabetic nephropathy: from clinical evidence to potential mechanisms. *J Ethnopharmacol*. 2024;330:118179. doi:10.1016/j.jep.2024.118179
19. Chen H, Cheng Q. Therapeutic effects of Jin Shui Bao capsules on kidney diseases. *Integr Med Nephrol Androl*. 2023;10(1):e00025. doi:10.1097/imna-d-22-00025
20. Geng Y, Dong Z, Wang Y, et al. Efficacy of Huangkui Capsules in the Treatment of Diabetic Kidney Disease: a Systematic Review and Using Network Pharmacology. *Integrat Med Nephrol Androl*. 2023;10(1):e00020. doi:10.1097/imna-d-22-00020
21. Gao Y, Li Z, Wang Y, et al. Analysis of clinical evidence on traditional Chinese medicine for the treatment of diabetic nephropathy: a comprehensive review with evidence mapping. *Front Endocrinol*. 2024;15:1324782. doi:10.3389/fendo.2024.1324782
22. Zhou S, Ai Z, Li W, et al. Deciphering the Pharmacological Mechanisms of Taohe-Chengqi Decoction Extract Against Renal Fibrosis Through Integrating Network Pharmacology and Experimental Validation In Vitro and In Vivo. *Front Pharmacol*. 2020;11:425. doi:10.3389/fphar.2020.00425
23. Wang C, Liu X, Guo S. Network pharmacology-based strategy to investigate the effect and mechanism of alpha-solanine against glioma. *BMC Complement Med Ther*. 2023;23(1):371. doi:10.1186/s12906-023-04215-1
24. Lee WY, Lee CY, Kim YS, Kim CE. The Methodological Trends of Traditional Herbal Medicine Employing Network Pharmacology. *Biomolecules*. 2019;9(8):362. doi:10.3390/biom9080362
25. Chen S, Li B, Chen L, Jiang H. Uncovering the mechanism of resveratrol in the treatment of diabetic kidney disease based on network pharmacology, molecular docking, and experimental validation. *J Transl Med*. 2023;21(1):380. doi:10.1186/s12967-023-04233-0
26. Xuan C, Ding W, Zhan L, et al. Potential Mechanisms of Yiqi Jiedu Huayu Decoction in the Treatment of Diabetic Microvascular Complications Based on Network Analysis, Molecular Docking, and Experimental Validation. *Evid Based Complement Alternat Med*. 2023;2023(1):5034687. doi:10.1155/2023/5034687
27. Sherman BT, Hao M, Qiu J, et al. DAVID: a web server for functional enrichment analysis and functional annotation of gene lists (2021 update). *Nucleic Acids Res*. 2022;50(W1):W216–W221. doi:10.1093/nar/gkac194
28. Berman HM, Westbrook J, Feng Z, et al. The Protein Data Bank. *Nucleic Acids Res*. 2000;28(1):235–242. doi:10.1093/nar/28.1.235
29. Tan H, Chen J, Li Y, et al. Glabridin, a bioactive component of licorice, ameliorates diabetic nephropathy by regulating ferroptosis and the VEGF/Akt/ERK pathways. *Mol Med*. 2022;28(1):58. doi:10.1186/s10020-022-00481-w
30. Tang L, Liu Y, Tao H, et al. Combination of Youhua Kuijie Prescription and sulfasalazine can alleviate experimental colitis via IL-6/JAK2/STAT3 pathway. *Front Pharmacol*. 2024;15:1437503. doi:10.3389/fphar.2024.1437503
31. Chen J, Ou Z, Gao T, et al. Ginkgolide B alleviates oxidative stress and ferroptosis by inhibiting GPX4 ubiquitination to improve diabetic nephropathy. *Biomed Pharmacother*. 2022;156:113953. doi:10.1016/j.biopha.2022.113953
32. Kay AM, Simpson CL, Stewart Jr JA. The Role of AGE/RAGE Signaling in Diabetes-Mediated Vascular Calcification. *J Diabetes Res*. 2016;2016:6809703. doi:10.1155/2016/6809703
33. Nonaka K, Bando M, Sakamoto E, et al. 6-Shogaol Inhibits Advanced Glycation End-Products-Induced IL-6 and ICAM-1 Expression by Regulating Oxidative Responses in Human Gingival Fibroblasts. *Molecules*. 2019;24(20):3705. doi:10.3390/molecules24203705
34. Yu H, Wei X, Ding H, et al. Exploring the potential mechanisms of Mahuang Fuzi Xixin decoction in treating elderly bronchial asthma through network pharmacology, molecular docking, and molecular dynamics simulations. *Medicine (Baltimore)*. 2024;103(41):e39921. doi:10.1097/MD.0000000000039921
35. Barrera-Chimal J, Lima-Posada I, Bakris GL, Jaisser F. Mineralocorticoid receptor antagonists in diabetic kidney disease - mechanistic and therapeutic effects. *Nat Rev Nephrol*. 2022;18(1):56–70. doi:10.1038/s41581-021-00490-8
36. Leung WK, Gao L, Siu PM, Lai CW. Diabetic nephropathy and endothelial dysfunction: current and future therapies, and emerging of vascular imaging for preclinical renal-kinetic study. *Life Sci*. 2016;166:121–130. doi:10.1016/j.lfs.2016.10.015
37. Yu JX, Chen X, Zang SG, et al. Gut microbiota microbial metabolites in diabetic nephropathy patients: far to go. *Front Cell Infect Microbiol*. 2024;14:1359432. doi:10.3389/fcimb.2024.1359432
38. Rayego-Mateos S, Morgado-Pascual JL, Opazo-Rios L, et al. Pathogenic Pathways and Therapeutic Approaches Targeting Inflammation in Diabetic Nephropathy. *Int J Mol Sci*. 2020;21(11). doi:10.3390/ijms21113798
39. Li H, Zhao X, Zheng L, et al. Bruceine A protects against diabetic kidney disease via inhibiting galectin-1. *Kidney Int*. 2022;102(3):521–535. doi:10.1016/j.kint.2022.04.020
40. Sun MY, Ye HJ, Zheng C, Jin ZJ, Yuan Y, Weng HB. Astragalin ameliorates renal injury in diabetic mice by modulating mitochondrial quality control via AMPK-dependent PGC1α pathway. *Acta Pharmacol Sin*. 2023;44(8):1676–1686. doi:10.1038/s41401-023-01064-z
41. Ru J, Li P, Wang J, et al. TCMSP: a database of systems pharmacology for drug discovery from herbal medicines. *J Cheminform*. 2014;6(1):13. doi:10.1186/1758-2946-6-13
42. Liu Z, Li Z, Sun Y, et al. Exploring the functional mechanism of action of Sanxiankaixin powder in preventing and treating reproductive aging based on network pharmacology and molecular docking. *Integr Med Nephrol Androl*. 2024;11(4):e24. doi:10.1097/IMNA-D-24-00022
43. Ren N, Wang WF, Zou L, Zhao YL, Miao H, Zhao YY. The nuclear factor kappa B signaling pathway is a master regulator of renal fibrosis. *Front Pharmacol*. 2023;14:1335094. doi:10.3389/fphar.2023.1335094

44. Wang YN, Miao H, Yu XY, et al. Oxidative stress and inflammation are mediated via aryl hydrocarbon receptor signalling in idiopathic membranous nephropathy. *Free Radic Biol Med*. 2023;207:89–106. doi:10.1016/j.freeradbiomed.2023.07.014
45. Ye HY, Song YL, Ye WT, et al. Serum granulosa cell-derived TNF-α promotes inflammation and apoptosis of renal tubular cells and PCOS-related kidney injury through NF-κB signaling. *Acta Pharmacol Sin*. 2023;44(12):2432–2444. doi:10.1038/s41401-023-01128-0
46. Zhao BR, Hu XR, Wang WD, Zhou Y. Cardiorenal syndrome: clinical diagnosis, molecular mechanisms and therapeutic strategies. *Acta Pharmacol Sin*. 2025;46(6):1539–1555. doi:10.1038/s41401-025-01476-z
47. Wang B, Yang LN, Yang LT, et al. Fisetin ameliorates fibrotic kidney disease in mice via inhibiting ACSL4-mediated tubular ferroptosis. *Acta Pharmacol Sin*. 2024;45(1):150–165. doi:10.1038/s41401-023-01156-w
48. Vagopoulou A, Theofilis P, Karasavvidou D, et al. Pilot study on the effect of flavonoids on arterial stiffness and oxidative stress in chronic kidney disease. *World J Nephrol*. 2024;13(3):95262. doi:10.5527/wjn.v13.i3.95262
49. Dong YP, Chen SZ, He HS, et al. Skullcapflavone II, a novel NQO1 inhibitor, alleviates aristolochic acid I-induced liver and kidney injury in mice. *Acta Pharmacol Sin*. 2023;44(7):1429–1441. doi:10.1038/s41401-023-01052-3
50. Liu Y, Li Y, Xu L, et al. Quercetin Attenuates Podocyte Apoptosis of Diabetic Nephropathy Through Targeting EGFR Signaling. *Front Pharmacol*. 2021;12:792777. doi:10.3389/fphar.2021.792777
51. Huang R, Zeng J, Yu X, et al. Luteolin Alleviates Diabetic Nephropathy Fibrosis Involving AMPK/NLRP3/TGF-beta Pathway. *Diabetes Metab Syndr Obes*. 2024;17:2855–2867. doi:10.2147/DMSO.S450094
52. Sharma D, Gondaliya P, Tiwari V, Kalia K. Kaempferol attenuates diabetic nephropathy by inhibiting RhoA/Rho-kinase mediated inflammatory signalling. *Biomed Pharmacother*. 2019;109:1610–1619. doi:10.1016/j.biopha.2018.10.195
53. Shao YF, Tang BB, Ding YH, et al. Kaempferide ameliorates cisplatin-induced nephrotoxicity via inhibiting oxidative stress and inducing autophagy. *Acta Pharmacol Sin*. 2023;44(7):1442–1454. doi:10.1038/s41401-023-01051-4
54. Cho CH, Yoo G, Kim M, Kurniawati UD, Choi IW, Lee S-H. Derived from the Edible Brown Algae Ecklonia cava, Attenuates Methylglyoxal-Associated Diabetic Nephropathy by Suppressing AGE-RAGE Interaction. *Antioxidants*. 2023;12(3). doi:10.3390/antiox12030593
55. Chang J, Li X, Wang J, Zhong Y. A network pharmacology approach to understand the action mechanisms of the tangshenkangping decoction for diabetic nephropathy treatment. *Integr Med Nephrol Androl*. 2023;10(4):e00006. doi:10.1097/IMNA-D-21-00006
56. Shu M, Cheng W, Jia X, et al. AGEs promote atherosclerosis by increasing LDL transcytosis across endothelial cells via RAGE/NF-kappaB/Caveolin-1 pathway. *Mol Med*. 2023;29(1):113. doi:10.1186/s10020-023-00715-5
57. Reynaert NL, Vanfleteren L, Perkins TN. The AGE-RAGE Axis and the Pathophysiology of Multimorbidity in COPD. *J Clin Med*. 2023;12(10). doi:10.3390/jcm12103366
58. Guo ZY, Wu X, Zhang SJ, Yang JH, Miao H, Zhao YY. *Poria cocos*: traditional uses, triterpenoid components and their renoprotective pharmacology. *Acta Pharmacol Sin*. 2024;46(4):836–851. doi:10.1038/s41401-024-01404-7
59. Wang Y, Sui Z, Wang M, Liu P. Natural products in attenuating renal inflammation via inhibiting the NLRP3 inflammasome in diabetic kidney disease. *Front Immunol*. 2023;14:1196016. doi:10.3389/fimmu.2023.1196016
60. Wang YN, Li XJ, Wang WF, Zou L, Miao H, Zhao YY. Geniposidic Acid Attenuates Chronic Tubulointerstitial Nephropathy Through Regulation of the NF-kB/Nrf2 Pathway Via Aryl Hydrocarbon Receptor Signaling. *Phytother Res*. 2024;38(11):5441–5457. doi:10.1002/ptr.8324
61. Zhou D, Zhou T, Tang S, et al. Network pharmacology combined with Mendelian randomization analysis to identify the key targets of renin-angiotensin-aldosterone system inhibitors in the treatment of diabetic nephropathy. *Front Endocrinol*. 2024;15:1354950. doi:10.3389/fendo.2024.1354950