



Exploring the mechanisms underlying the therapeutic effect of the drug pair Rhubarb-Coptis in diabetic nephropathy using network pharmacology and molecular docking analysis

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Background: To use network pharmacology to explore the mechanism of the drug pair Rhubarb-Coptis in the treatment of diabetic nephropathy (DN).

Methods: The Traditional Chinese Medicine Systems Pharmacology (TCMSP) database was used to screen active ingredients of drug pair Rhubarb-Coptis. Targets were obtained using the TCMSP and SwissTargetPrediction databases. DN disease targets were extracted from the Online Mendelian Inheritance in Man (OMIM), GeneCards, and Therapeutic Target database (TTD) databases. A “drug-compound-target” network and protein-protein interaction (PPI) network were constructed and analyzed through the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database and Cytoscape software. Gene Ontology (GO) functional and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were performed in the Database for Annotation, Visualization, and Integrated Discovery (DAVID) database. Molecular docking was performed using AutoDock Vina and PyMOL software.

Results: A total of 30 active components and 609 targets of Rhubarb-Coptis were screened out, and 98 common targets of DN and Rhubarb-Coptis were obtained. Quercetin, berberine, epiruberine, epautin, and moupinamide were the main active components in the treatment of DN. The *STAT3*, *CTNNB1*, *PIK3R1*, *PIK3CA*, and *TP53* genes were identified as the potential 5 key targets. The GO enrichment analysis showed that these 5 key targets mainly involved in inflammation, oxidative stress, and apoptosis. KEGG enrichment analysis showed that the pathways were mainly enriched in the AGE-RAGE and HIF-1 signaling pathways. Molecular docking revealed that the 5 key targets could combine well with their corresponding active compounds.

Conclusions: This study expounds the therapeutic effect of Rhubarb-Coptis on DN from a holistic perspective, and provides a valuable basis for clinical application and academic research.

Keywords: Network pharmacology; Rhubarb-Coptis; diabetic nephropathy (DR)

Submitted Oct 21, 2022. Accepted for publication Nov 30, 2022.

doi: 10.21037/atm-22-5550

View this article at: <https://dx.doi.org/10.21037/atm-22-5550>

Introduction

Diabetic nephropathy (DN) is a serious microvascular complication of diabetes (1). On a global scale, the incidence of DN is on the rise in some regions (2), and it has become one of the main causes of renal failure. About 40% of patients with DN eventually progress to end-stage renal disease (ESRD) (3). The main clinical manifestations of DN are proteinuria, edema, and high blood pressure, and the histopathological manifestations are glomerular dilatation, glomerular basement membrane thickening, and podocyte injury, which eventually lead to glomerular sclerosis and tubular interstitial fibrosis, affecting renal function (4,5). The pathogenesis of DN involves many factors such as abnormal glucose metabolism, oxidative stress, inflammation, and genetic factors, but the specific molecular mechanism is still unclear, and there is no effective treatment.

In long-term clinical practice, traditional Chinese medicine (TCM) plays a role in treating DN through multiple approaches, targets, and mechanisms based on the principle of syndrome differentiation and combination of local and global factors. In particular, TCM is effective in regulating blood glucose and lipid metabolism, reducing kidney injury, and other aspects, with less adverse reactions. The medicinal pairing of Rhubarb and Coptis was recorded in the classic TCM text the Treatise on Cold Damage

Diseases (Shanghan Lun), and has been used to treat diabetes and DN for more than 1,000 years. Modern pharmacological research displays that Coptis could protect the cardio-cerebrovascular system, regulate blood sugar level, and exert anti-inflammatory and anti-tumor effects (6). Rhubarb can regulate glucose and lipid metabolism, improve insulin resistance (7), inhibit the proliferation of mesangial cells (8), delay renal fibrosis (9), and improve oxidative stress, so as to reduce renal injury (10). However, the mechanism of action of Rhubarb-Coptis in the treatment of DN remains unclear.

Network pharmacology is a new discipline developed on the basis of systems biology theory, it constructs an interaction network of drug, target, pathway, and disease by abstracting the interaction relationship between drug and target into a network model. We could analyze the internal relationship between them by screening the key nodes. It is consistent with the overarching concept of TCM to study disease from a holistic perspective (11). In this paper, the effective active components of Rhubarb-Coptis and related targets of DN were screened and predicted by network pharmacology, and we used the molecular docking technology to verify the binding of the selected active substances with the target sites, which provided a basis for the subsequent study of Rhubarb-Coptis and DN. We present the following article in accordance with the STREGA reporting checklist (available at <https://atm.amegroups.com/article/view/10.21037/atm-22-5550/rc>).

Highlight box

Key findings

- We found that quercetin, berberine, eupatin, epiberberine, and moupinamide can be defined as 5 crucial compounds. *STAT3*, *CTNNB1*, *PIK3R1*, *PIK3CA*, and *TP53* may be the 5 most important therapeutic targets.

What is known and what is new?

- The pathogenesis of DN involves many factors such as abnormal glucose metabolism, oxidative stress, inflammation, and genetic factors, but the specific molecular mechanism is still unclear, and there is no effective treatment.
- The results of this study provide a reference for further exploring the mechanism of Rhubarb-Coptis in the treatment of DN.

What is the implication, and what should change now?

- The effective active components of Rhubarb-Coptis and related targets of DN were screened and predicted by network pharmacology, and we used the molecular docking technology to verify the binding of the selected active substances with the target sites, which provided a basis for the subsequent study of Rhubarb-Coptis and DN.

Methods

Screening of active components of Rhubarb-Coptis

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

The Traditional Chinese Medicine System Pharmacology Database (TCMSP; <https://www.tcmsp-e.com/tcmsp.php>) is a database of systems pharmacology for drug discovery from herbal medicines (12). Based on the pharmacokinetic parameters: absorption, distribution, metabolism, excretion (ADME), oral bioavailability (OB) $\geq 30\%$, and drug-likeness (DL) ≥ 0.18 , compounds were selected. Active compounds with no information on potential targets were excluded.

Screening of drug targets

Targets of potential active components Rhubarb-Coptis were obtained from TCMSP and SwissTargetPrediction databases (<http://www.swisstargetprediction.ch/>). Then, the

UniProtKB search function in the UniProt protein database (<https://www.uniprot.org/>) was used to standardize genes for related targets with species set as “human” and status set as “reviewed” (13). Compound targets retrieved by TCMSP and SwissTargetPrediction were combined and duplicated genes were deleted to obtain drug target genes.

Determination of DN targets

In order to obtain relevant targets for DN, “diabetic nephropathy” and “diabetic kidney disease” were set as keywords to search 3 disease databases: Online Mendelian Inheritance in Man (OMIM; <https://omim.org/>) (14), GeneCards (<https://www.genecards.org/>) (15), and Therapeutic Target Database (TTD) (<http://db.idrblab.net/ttd/>) (16). The target genes were combined, and duplicated data were removed to obtain candidate targets for the treatment of DN.

Determination of DN targets of the active components

Screened targets of the active components of Rhubarb-Coptis and DN targets were imported into a Venn diagram webtool (<http://bioinformatics.psb.ugent.be/webtools/Venn/>) for analysis, and the intersection was taken to obtain the potential targets of Rhubarb-Coptis in the treatment of DN.

Construction of a network of drug-components-target-disease

In order to construct the network of drug-components-target-disease, we used Cytoscape 3.8.2 software (<https://cytoscape.org/>) to ligate active components and their associated targets. And then, we used Network Analyzer for topological analysis to highlight the key active components and targets of Rhubarb-Coptis in the treatment of DN.

Construction of a protein-protein interaction network

For the purpose of studying the interactions between the active components of Rhubarb-Coptis and their target proteins, we used the interaction database platform Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) v.11.0 (<https://string-db.org/>) (17) to search for drug-disease intersection target genes, and a protein-protein interaction (PPI) network was constructed. STRING is a multifunctional data platform that can provide PPI evaluation and integration (18). In the database search, the

protein species was set as “Homo sapiens”, the minimum interaction threshold was 0.9, and isolated nodes were removed to construct a PPI network. After that, Cytoscape 3.8.2 software was used to visualize the interaction network.

Statistical analysis

Gene Ontology (GO) biological process enrichment analysis

The Database for Annotation, Visualization, and Integrated Discovery (DAVID; <https://david.ncifcrf.gov/>) is a comprehensive functional annotation tool that can be used not only for gene differential analysis, but also for pathway enrichment (19,20). The common targets of Rhubarb-Coptis and DN were analyzed by GO enrichment analysis through the DAVID database, and the main biological functions of Rhubarb-Coptis in the treatment of DN were analyzed. The enrichment results were visualized by R language, and the results were expressed as bubble charts.

Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis

The common targets of Rhubarb-Coptis and DN were imported into R language (The R Foundation for Statistical Computing, Vienna, Austria) to obtain the enrichment results of KEGG pathways, and the key pathways were analyzed with the help of R language.

Molecular docking

(I) Design and processing of small molecules: the 3-dimensional (3D) structure of small molecule MOL 2 format was downloaded from TCMSP database according to the MOL ID number of small molecule, and ChemBio3D Ultra 14.0 (PerkinElmer Informatics, Waltham, MA, USA) was imported for energy minimization. The Minimum RMS Gradient was set to 0.001, and the small molecule was saved as MOL 2 format. The optimized small molecules were imported into AutoDockTools-1.5.6 for hydrogenation, charge calculation, charge distribution, and rotatable bond setting, and then saved as “pdbqt” format. (II) Preparation and handling of proteins: The protein structure of the target was downloaded from the Protein Data Bank (PDB) database. Pymol 2.3.0 (<https://pymol.org/2/>) was used to remove the protein crystal water and original ligand, and the protein structure was imported into AutoDockTools-1.5.6 for hydrogenation, charge calculation, charge distribution, atom type designation, and was saved as “pdbqt” format.

(III) Autodock Vina V1.1.2 software (<https://vina.scripps.edu/>) was used for molecular docking to evaluate the affinity of the compounds with the target proteins. Further, PyMOL2.3.0 and LIGPLOT V 2.2.4 were used to analyze the interaction mode of the docking results.

Results

Screening of active components of Rhubarb-Coptis

“Rhubarb” and “Coptis” were entered into TCMSP database for retrieval. A total of 140 active components were retrieved, including 92 components of Rhubarb and 48 components of Coptis. Based on ADME, with OB $\geq 30\%$ and DL ≥ 0.18 as screening conditions, 30 active ingredients were screened, including 16 of Rhubarb and 14 of Coptis (Table 1).

Determination of targets of Rhubarb-Coptis active components and active component-target network construction

The TCMSP database and SwissTargetPrediction database were used to obtain 806 targets of active components of Rhubarb and Coptis including 312 targets of Rhubarb and 494 targets of Coptis, and 609 potential targets of the drug were finally obtained by deleting repetitive targets and eliminating inactive targets. This was imported into The UniProt database for gene name standardization, and then Cytoscape 3.8.2 software was used to construct the “Rhubarb, Coptis - active ingredient-target” network diagram (Figure 1).

Prediction results of targets related to DN

We used “Diabetic kidney disease” and “Diabetic nephropathy” as the key words. The OMIM, GeneCards, and TTD databases were searched, and 1,107 disease targets were obtained after the deduplication.

Drug-disease intersection targets

Venn analysis was performed on 609 targets of active components of Rhubarb-Coptis and 1,107 targets related to diabetic nephropathy. After intersection of the two, 98 drug-disease cross targets were obtained, and Venn diagram was drawn (Figure 2).

Construction and analysis of drug-component-target-disease network

A total of 30 potential active components of Rhubarb-Coptis and 98 common drug-disease targets were input into Cytoscape software, and isolated components that had no intersection with the targets were deleted to draw the “drug-ingredient-target-disease” network diagram (Figure 3). In the figure, yellow represents drugs, purple represents 28 active components in Rhubarb-Coptis, 2 active component targets have no intersection with disease targets and are deleted, blue represents 98 common targets, and red represents disease.

PPI network construction and topology analysis

A total of 98 common drug-disease targets were entered into the STRING database for retrieval, and a PPI network of protein interaction was constructed. The network had 98 nodes and 326 edges, and the average node degree was 6.65, with the PPI enrichment P value of $<1.0e-1.6$ (Figure 4A). The results of STRING analysis were imported into Cytoscape 3.8.2 software, and Network Analyzer was used for topology analysis. The node size was positively correlated with the degree value, the greater the degree of nodes, the more biological functions the node had in the network. The network was constructed as shown in Figure 4B. The dark and large nodes in the figure are the core targets. The first 20 targets were selected as the key targets of Rhubarb-Coptis compatibility in the treatment of DN, as shown in Table 2 and Figure 5. The 10 most-connected targets were signal transducer and activator of transcription 3 (STAT3), Catenin beta-1 (CTNBN1), phosphatidylinositol 3-kinase regulatory subunit alpha (PIK3R1), Phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit alpha isoform (PIK3CA), tumor protein p53 (TP53), AKT serine/threonine kinase 1 (AKT1), epidermal growth factor receptor (EGFR), mitogen-activated protein kinase 1 (MAPK1), histone acetyltransferase p300 (EP300), and vascular endothelial growth factor A (VEGFA), indicating their importance in the network.

GO functional analysis

A total of 860 GO items were obtained by GO enrichment analysis of 98 common targets using DAVID database and R language, including 673 biological processes (BP), 84

Table 1 Basic information on the main active ingredients of Rhubarb-Coptis

Mol ID	Compound	Drug	OB (%)	DL
MOL002235	EUPATIN	Rhubarb	50.8	0.41
MOL002251	Mutatochrome	Rhubarb	48.64	0.61
MOL002259	Physciondiglucoside	Rhubarb	41.65	0.63
MOL002260	Procyanidin B-5,3'-O-gallate	Rhubarb	31.99	0.32
MOL002268	Rhein	Rhubarb	47.07	0.28
MOL002276	Sennoside E_qt	Rhubarb	50.69	0.61
MOL002280	Torachryson-8-O-beta-D-(6'-oxayl)-glucoside	Rhubarb	43.02	0.74
MOL002281	Toralactone	Rhubarb	46.46	0.24
MOL002288	Emodin-1-O-beta-D-glucopyranoside	Rhubarb	44.81	0.8
MOL002293	Sennoside D_qt	Rhubarb	61.06	0.61
MOL002297	Daucosterol_qt	Rhubarb	35.89	0.7
MOL002303	Palmidin A	Rhubarb	32.45	0.65
MOL000358	Beta-sitosterol	Rhubarb	36.91	0.75
MOL000471	Aloe-emodin	Rhubarb	83.38	0.24
MOL000554	gallic acid-3-O-(6'-O-galloyl)-glucoside	Rhubarb	30.25	0.67
MOL000096	(-)-catechin	Rhubarb	49.68	0.24
MOL001454	Berberine	Coptis	36.86	0.78
MOL013352	Obacunone	Coptis	43.29	0.77
MOL002894	Berberrubine	Coptis	35.74	0.73
MOL002897	Epiberberine	Coptis	43.09	0.78
MOL002903	(R)-Canadine	Coptis	55.37	0.77
MOL002904	Berlambine	Coptis	36.68	0.82
MOL002907	Corchoroside A_qt	Coptis	104.95	0.78
MOL000622	Magnograndiolide	Coptis	63.71	0.19
MOL000762	Palmidin A	Coptis	35.36	0.65
MOL000785	Palmatine	Coptis	64.6	0.65
MOL000098	Quercetin	Coptis	46.43	0.28
MOL001458	Coptisine	Coptis	30.67	0.86
MOL002668	Worenine	Coptis	45.83	0.87
MOL008647	Moupinamide	Coptis	86.71	0.26

OB, oral bioavailability; DL, drug-likeness.

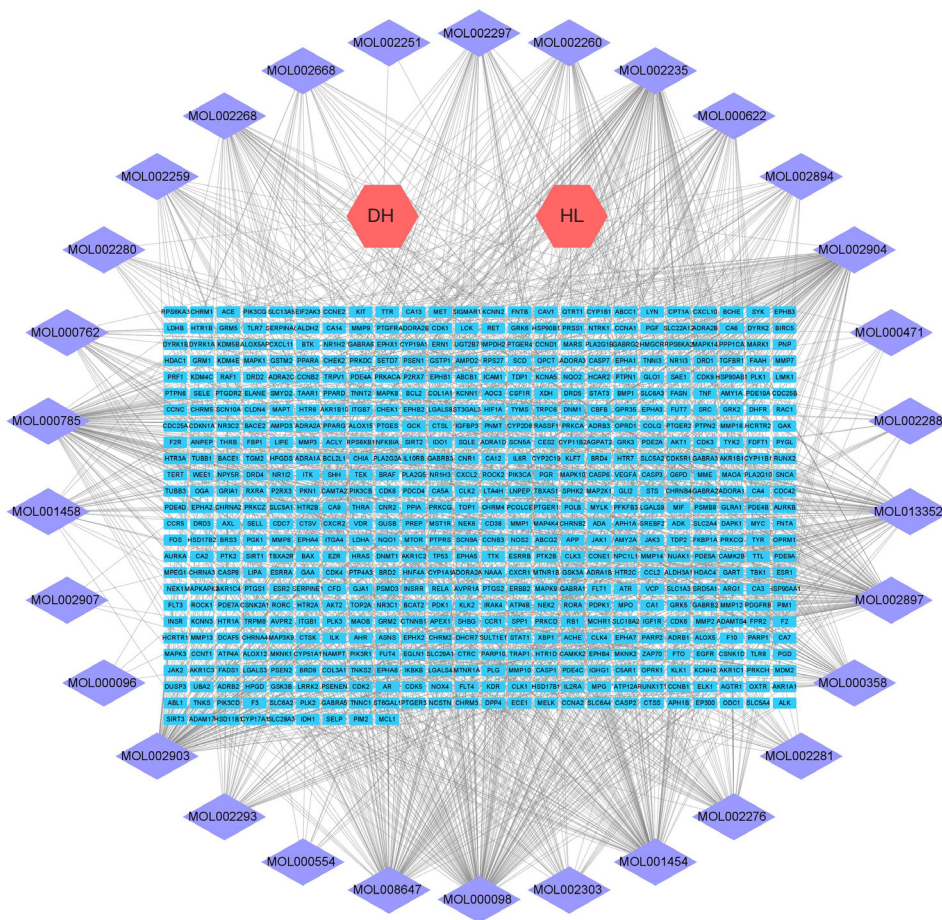


Figure 1 ‘Ingredients-targets’ network construction. The DH and HL represent Rhubarb and Coptis, respectively, and the light purple represents the components of Rhubarb-Coptis, the blue rectangle represents the target.

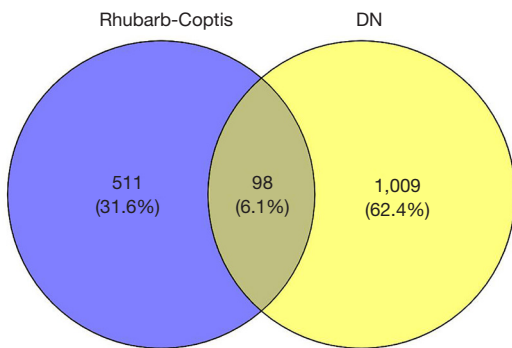


Figure 2 Rhubarb-Coptis/DN common target genes. DN, diabetic nephropathy.

cellular components (CC), and 103 molecular functions (MF). In order to understand the gene function more intuitively, the P value was used as the reference for sorting, and the top 10 items were selected for each part to generate a bubble chart (Figure 6).

The BP terms mainly involved in the regulation of gene expression, the positive regulation of MAPK cascade, the positive regulation of cell migration, the positive regulation of cell migration, response to xenobiotic stimulus, positive regulation of MAP kinase activity, and response to drugs. The CC terms were mainly enriched in membrane raft, plasma membrane, macromolecular complex, receptor

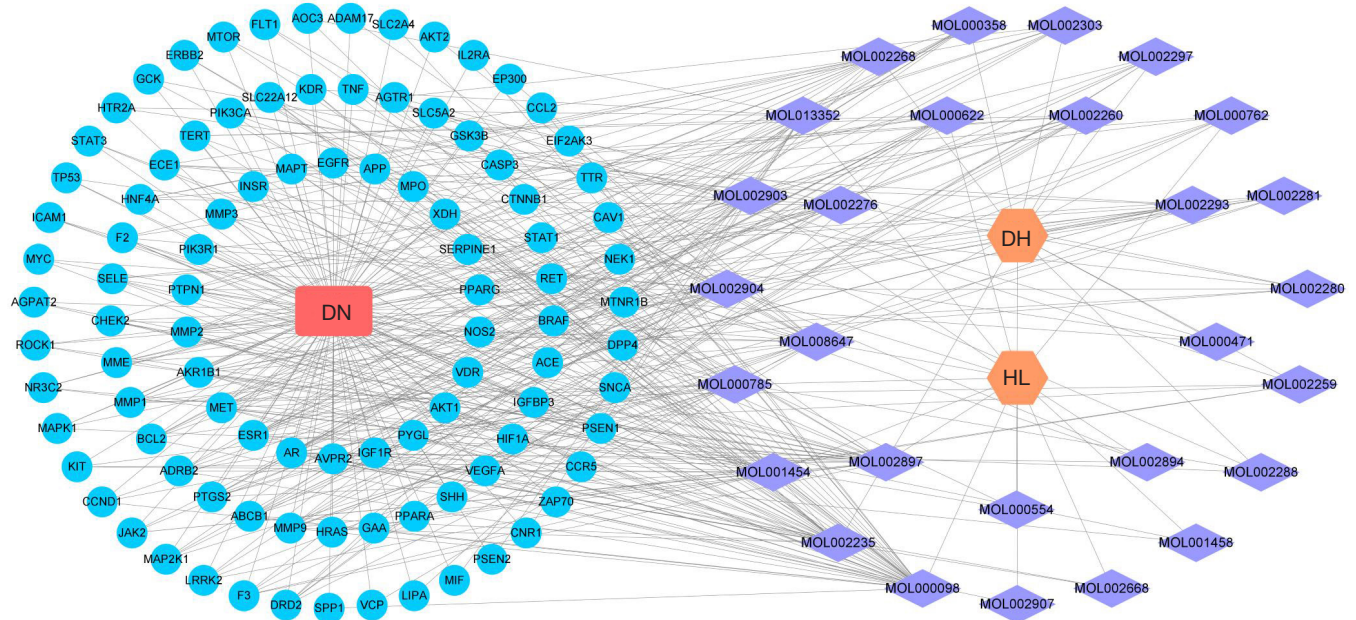


Figure 3 Network structure of ‘drug-component-target-DN’. The rectangle represents DN, hexagons represent Rhubarb and Coptis, diamonds represent components, and circle represents the target. DN, diabetic nephropathy.

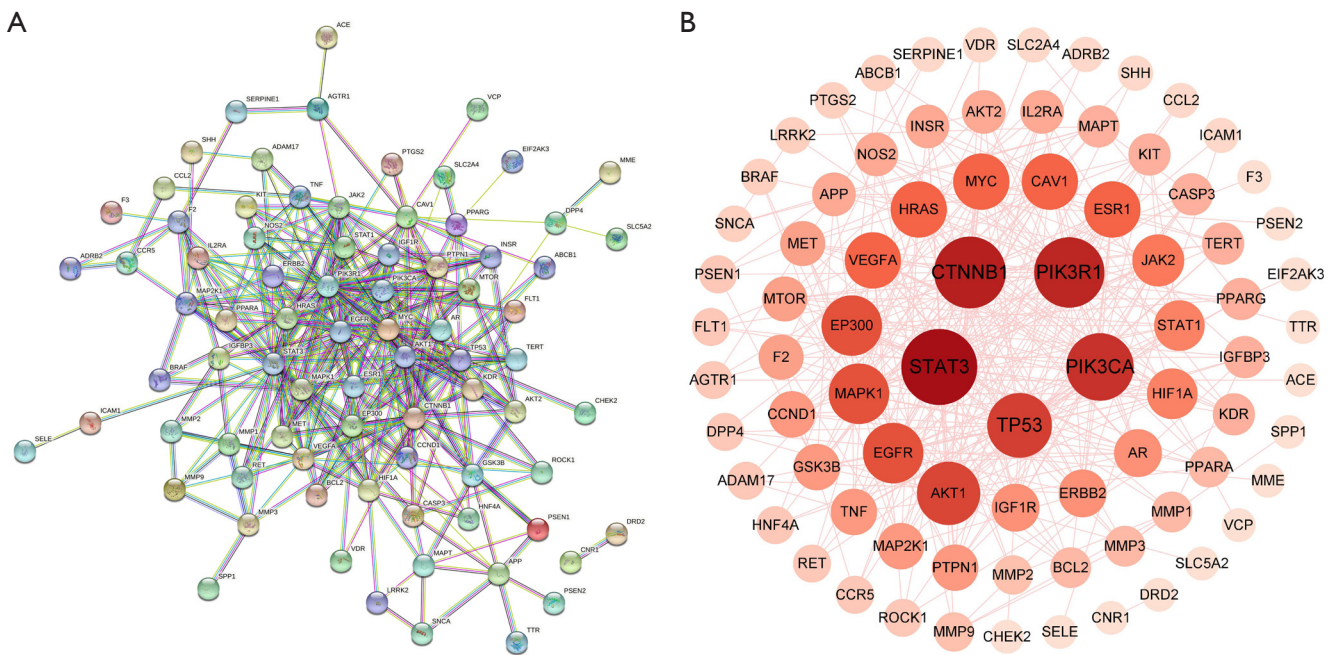


Figure 4 PPI network analysis. (A) PPI network of targets generated using STRING 11.0. Nodes represent targets, and edges represent associations between targets. (B) Potential targets are arranged clockwise from the inside out according to the degree value from large to small. PPI, protein-protein interaction; STRING, Search Tool for the Retrieval of Interacting Genes/Proteins.

Table 2 Main target gene network node characteristic parameters

Gene	Degree	Closeness	Betweenness
<i>STAT3</i>	31	0.558	0.177
<i>CTNNB1</i>	28	0.527	0.114
<i>PIK3R1</i>	27	0.524	0.048
<i>PIK3CA</i>	25	0.517	0.040
<i>TP53</i>	23	0.510	0.113
<i>AKT1</i>	22	0.513	0.035
<i>EP300</i>	20	0.524	0.093
<i>EGFR</i>	20	0.510	0.033
<i>MAPK1</i>	20	0.524	0.084
<i>CAV1</i>	17	0.484	0.100
<i>ESR1</i>	17	0.510	0.012
<i>MYC</i>	17	0.503	0.036
<i>HRAS</i>	17	0.470	0.034
<i>VEGFA</i>	17	0.472	0.036
<i>JAK2</i>	14	0.456	0.029
<i>STAT1</i>	13	0.484	0.010
<i>HIF1A</i>	13	0.490	0.030
<i>ERBB2</i>	11	0.453	0.008
<i>AR</i>	11	0.481	0.006
<i>IGF1R</i>	11	0.470	0.005

complex, and perinuclear region of cytoplasm. The MF terms mainly involved identical protein binding, protein tyrosine kinase activity, protein kinase activity, transmembrane receptor protein tyrosine kinase activity, and endopeptidase activity.

KEGG pathway enrichment analysis

In order to reveal the potential mechanism of Rhubarb-Coptis in the treatment of DN, we used the DAVID database and R language to conduct KEGG enrichment analysis on 98 targets of drug-diabetic nephropathy, and a total of 151 KEGG pathways were obtained. The top

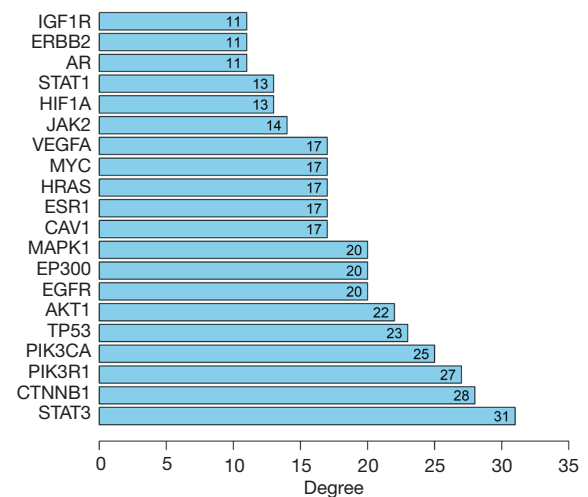


Figure 5 Core target ranking based on PPI topology analysis. PPI, protein-protein interaction.

20 pathways ranked by P value were selected to draw a bar bubble chart of KEGG enrichment (Figure 7). The $-\log_{10}(P\text{-value})$ represents the significance of enrichment, and the redder the color, the higher the significance.

The results showed that the anterior pathways involved in the treatment of DN by Rhubarb-Coptis were mainly involved in tumor signaling pathway and AGE-RAGE signaling pathway, HIF-1 signaling pathway, and endocrine resistance signaling pathway in diabetes and diabetic complications.

Molecular docking

In order to validate the study results of network analysis, the top 5 active components and the top 5 potential targets of Rhubarb-Coptis were selected. The molecular docking was performed by AutoDock software, and the visualization was performed by PyMOL software. Generally, the lower the binding energy of small molecule ligands and protein receptors, the more stable the binding molecules are, indicating the greater possibility of interaction between molecules. The binding energy < -5 kcal/mol indicates that the small molecule binds well to the large molecule, and the smaller the value, the more likely it is to bind spontaneously (21). Through docking simulations, 25 pairs of docking results were yielded (Table 3). The results showed that quercetion, epiberberine, berberine, moupinamide, and eupatin were associated with *CTNNB1*, *PIK3CA*, *PIK3R1*, *STAT3*, and *TP53* combined energies were all < -5 kcal/mol, indicating good molecular docking. The docking results

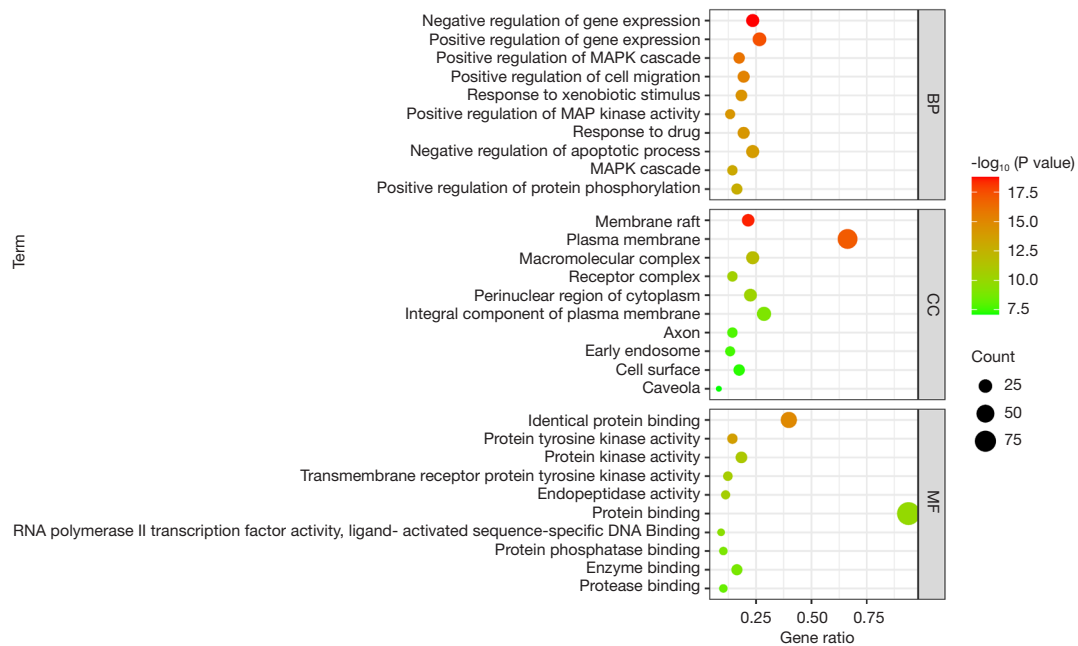


Figure 6 GO enrichment analysis of Rhubarb-Coptis in the treatment of DN. GO, Gene Ontology; DN, diabetic nephropathy; BP, biological processes; CC, cellular components; MF, molecular functions.

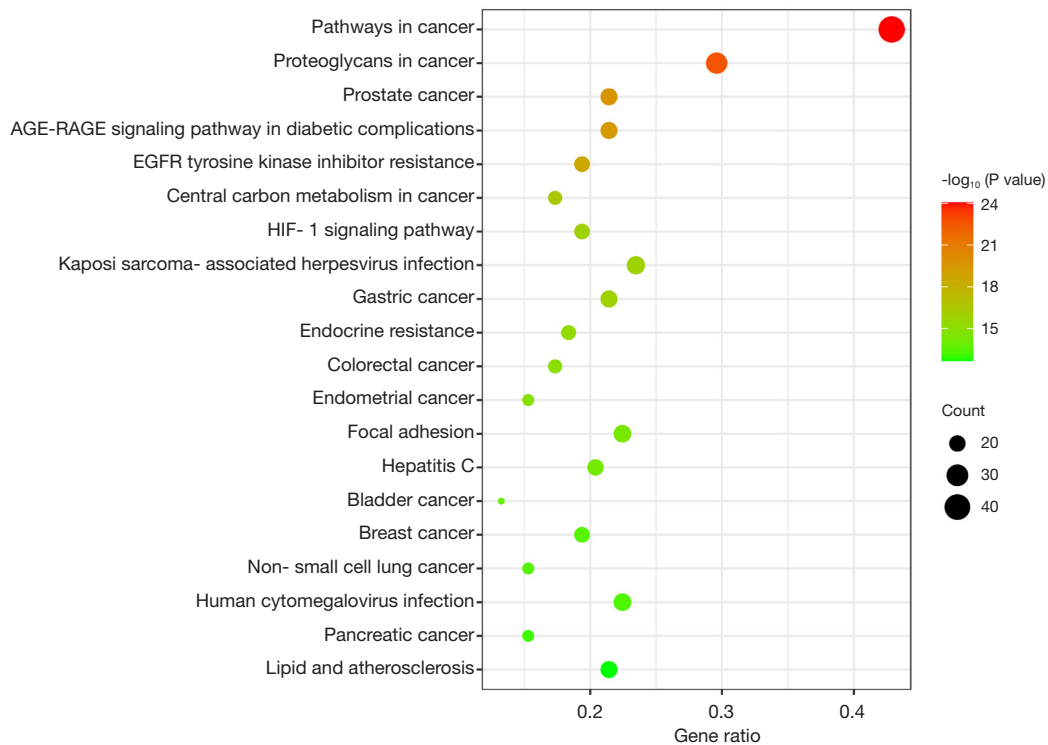


Figure 7 Bubble diagram of analysis of KEGG enrichment in top 20 pathways. KEGG, Kyoto Encyclopedia of Genes and Genomes.

Table 3 Molecular docking results of 5 key genes with their corresponding compounds in Rhubarb-Coptis

PDB ID	Key targets	Compounds	Binding energy (kcal/mol)
4JPS	PIK3CA	Quercetin	-9.2
4JPS	PIK3CA	Berberine	-8.9
4JPS	PIK3CA	Epiberberine	-8.5
4JPS	PIK3CA	Moupinamide	-8.5
4JPS	PIK3CA	EUPATIN	-8.0
6NJS	STAT3	Berberine	-7.5
1UOL	TP53	Berberine	-7.4
6NJS	STAT3	Epiberberine	-7.4
1UOL	TP53	Moupinamide	-6.9
6NJS	STAT3	EUPATIN	-6.8
1UOL	TP53	Epiberberine	-6.8
1JDH	CTNNB1	Quercetin	-6.6
1JDH	CTNNB1	Berberine	-6.6
1JDH	CTNNB1	EUPATIN	-6.6
1UOL	TP53	Quercetin	-6.5
1JDH	CTNNB1	Epiberberine	-6.5
6NJS	STAT3	Moupinamide	-6.4
1JDH	CTNNB1	Moupinamide	-6.3
1UOL	TP53	EUPATIN	-6.3
6NJS	STAT3	Quercetin	-6.2
5M6U	PIK3R1	Berberine	-6.1
5M6U	PIK3R1	Epiberberine	-6.1
5M6U	PIK3R1	Moupinamide	-6.1
5M6U	PIK3R1	Quercetin	-5.5
5M6U	PIK3R1	EUPATIN	-5.2

PDB, Protein Data Bank.

of *PIK3CA* with quercetin, berberine, epiberberine, and moupinamide with the strongest binding force were selected and displayed (Figure 8). The binding energy of quercetin with *PIK3CA* was the lowest (-9.2 kcal/mol), the binding energy of quercetin with berberine was -8.9 kcal/mol, and the binding energy of quercetin with epiberberine and moupinamide was -8.5 kcal/mol. The above molecular docking results indicate that their combination may have an essential role in treating DN with Rhubarb-Coptis.

Discussion

DN is considered one of the 4 major fatal chronic diseases in the world (22), which seriously threatens the health and life of diabetic patients. However, because the pathogenesis of DN is still unclear, most patients with DN cannot get achieve a good treatment effect. A growing body of evidence shows that TCM has sufficient advantages in the treatment of diabetes and its complications. However, due to the multi-approach, multi-target, and multi-mechanism characteristics of TCM, its specific mechanism of action is still unclear. Network pharmacology can systematically combine network analysis with pharmacology to illustrate the interactions between diseases, drugs, and targets. For example, in the treatment of COVID-19, aiming at ACE/AngII/AT1R pathway, the network pharmacology method was used to explore effective traditional Chinese medicine and its active compounds, providing reference for further exploring effective traditional Chinese medicine and its mechanism of action in the treatment of COVID-19 (23). Therefore, the use of network pharmacology can combine the holistic view of traditional Chinese medicine and network information, and can more directly clarify the mechanism of Rhubarb-Coptis in treating DN.

In the present study, quercetin, berberine, eupatin, epiberberine, and moupinamide were defined as the crucial compounds of Rhubarb-Coptis in treating DN. It is reported that quercetin can fight inflammation, reduce oxidative stress injury of the kidneys, improve renal morphology, reduce renal pathological injury, and delay the progression of DN (24,25). Berberine can accelerate the secretion of antioxidant molecule Nrf2 and promote the synthesis of superoxide dismutase (SOD) by upregulating the KEAP1-NRF2/ARE signaling pathway, so as to reduce the levels of oxidative stress, inflammation, and fibrosis in the kidneys of DN rats (26). Epiberberine can reduce the expression of TGF- β 1 and Smad2, alleviate diabetes-related metabolic disorders, improve renal function, and alleviate renal tissue fibrosis (27). Eupatin has a good anti-inflammatory effect (28). Moupinamide is closely related to α -glucosidase inhibitory activity and free radical scavenging activity (29). These major compounds of Rhubarb-Coptis have the functions of regulating glucose and lipid metabolism, antioxidant, anti-inflammatory, and anti-fibrosis, which comprise the pharmacological basis of Rhubarb-Coptis in the treatment of DN.

Through the PPI network analysis, *STAT3*, *CTNNB1*,

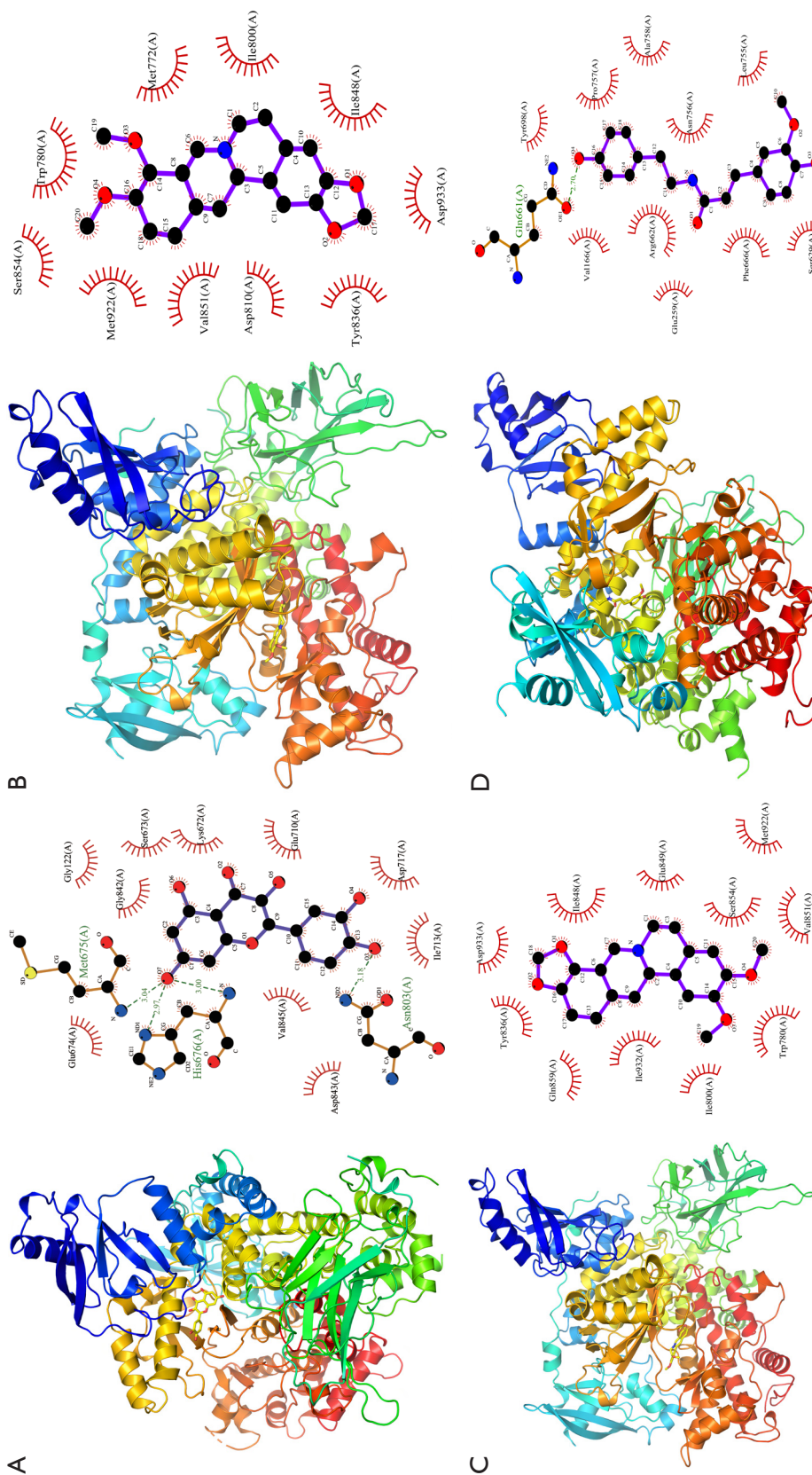


Figure 8 The docking results of *PIK3CA* with quercetin, berberine, epiberberine, and moupinamide with the strongest binding force were selected and displayed. (A) Quercetin action on *PIK3CA* (affinity = -9.2 kcal/mol); (B) berberine action on *PIK3CA* (affinity = -8.9 kcal/mol); (C) epiberberine act on *PIK3CA* (affinity = -8.5 kcal/mol); (D) moupinamide act on *PIK3CA* (affinity = -8.5 kcal/mol).

PIK3R1, *PIK3CA*, *TP53*, *AKT1*, *EGFR*, *MAPK1*, *EP300*, and *VEGFA* were the key targets of Rhubarb-Coptis in treating DN. These targets are mainly related to inflammation, oxidative stress, blood lipids, and vascular permeability, which are in line with the pathogenesis of DN to a certain extent. Study has shown that (30) *STAT3* plays an important role in the occurrence and development of DN, and is widely involved in cell proliferation, differentiation, inflammation, and other biological effects. Under chronic hyperglycemia, the expression of *STAT3* is increased, which promotes inflammation and apoptosis, leading to DN. The rapid accumulation of *CTNNB1* in renal tubular epithelial cells promotes the formation of renal mesenchymal fibroblasts and benign proliferation of other renal mesenchymal epithelial cells, aggravating renal mesenchymal fibrosis (31). *PIK3R1* and *PIK3CA* are members of the phosphatidylinositol-3-kinase (PI3K) protein family, including PI3K regulatory subunit and catalytic subunit, respectively. Studies have shown that the activation of PI3K pathway can promote the proliferation of mesangial cells, mediate podocyte injury, and accelerate the progression of DN (32,33). *TP53* is a tumor suppressor that induces TGF- β 1 signaling, and renal cells produce fibrotic extracellular matrix proteins, leading to glomerulosclerosis and tubulointerstitial fibrosis (34).

The GO functional enrichment analysis of 98 common targets related to Rhubarb-Coptis active ingredients in the treatment of DN was conducted. As shown in *Figure 6*, the top 10 most meaningful BP terms were mainly related to oxidative stress, apoptosis, and gene regulation. Studies have shown that the occurrence and development of DN are closely related to oxidative stress, apoptosis, and gene regulation (35,36). The MF terms were mainly included identical protein binding, protein kinase activity, endopeptidase activity, transcription factor binding, and protein kinase binding, and the main targets involved include *STAT3*, *PIK3R1*, *VEGFA*, *TP53*, and *MAPK1*, among others, which are mainly involved in inflammation, oxidative stress, and apoptosis. Both play an important role in the development of DN. In addition, CC terms were mainly enriched in membrane rafts, plasma membrane, macromolecular complex, receptor complex, and the perinuclear region of cytoplasm; the key targets *STAT3*, *PIK3R1*, *CTNNB1*, and *VEGFA* are included in these terms. In conclusion, the pathological mechanisms of DN are complex and diverse. Rhubarb-Coptis can treat DN by regulating various molecular functions and cellular components.

For the purpose of exploring the potential mechanism of Rhubarb-Coptis in the treatment of DN, we conducted KEGG enrichment analysis on 98 potential targets of Rhubarb-Coptis in DN. As shown in *Figure 7*, we found that the main pathways of Rhubarb-Coptis in the treatment of DN may be the AGE-RAGE signaling pathway and HIF-1 signaling pathway, which mainly involve 2 aspects: (I) accumulation of advanced glycation end-products: The metabolism of AGEs is accelerated in high glucose environment, and its accumulation in the kidney is the main cause of progressive renal damage and the key factor leading to DN. The toxic effects of AGEs, on the one hand, can directly destroy renal tissues and cells, and on the other hand, they can specifically bind to RAGE and activate a series of downstream signaling pathways, which can cause inflammation, oxidative stress, and apoptosis leading to renal injury. Studies have shown that the AGE-RAGE signaling pathway can activate NADPH oxidase to cause a large amount of reactive oxygen species (ROS) production, induce persistent oxidative stress in vascular tissues (37), and further activate the JAK/STAT, AKT, mTOR, MAPK, and other signaling pathways to phosphorylate and activate NF- κ B. This causes the expression and release of a large number of adhesion factors, transforming growth factors, proinflammatory cytokines, increase vascular permeability, such as MCP-1, TGF- β 1, VEGF, and TNF- α , increases proteinuria, and promotes renal fibrosis, leading to the occurrence and development of DN (38,39). (II) Dysregulation of oxygen metabolism: studies have shown that chronic hypoxia is an important pathogenic mechanism and potential initiating factor of the occurrence and development of DN (40). The stability of HIF-1 α plays a key role in the activity of HIF-1, it can participate in renal inflammation and fibrosis by regulating different targets (41). Other studies have shown that HIF-1 α mediates renal hypoxic metabolic response by regulating the expression of VEGF, inhibits HIF-1 α and VEGF, and increases the expression of podocyte related proteins, which can alleviate podocyte injury and slow down the process of glomerular fibrosis in diabetic patients (42). HIF-1 α may play a protective role in diabetic renal tubular injury by regulating mitochondrial dynamics through up-regulating HO-1 (43).

To further explore the potential molecular mechanism of Rhubarb-Coptis in treatment of DN, we conducted molecular docking studies of the first 5 key targets closely related to DN. The results showed that *STAT3*, *CTNNB1*, *PIK3R1*, *PIK3CA*, and *TP53* had good ability to bind relevant active compounds in Rhubarb-Coptis.

In the field of traditional Chinese medicine in the treatment of DN, in addition to rhubarb and coptis, there are many traditional Chinese medicines that have significant effects in the treatment of DN. For example, the isoflavone compound puerarin extracted from kudzu root can significantly improve the disorder of lipid metabolism and insulin resistance in diabetic rats, and inhibit podocyte autophagy to reduce the progress of DN damage (44). Tanshinone, the active ingredient in *Salvia miltiorrhiza*, can change TGF in kidney- β /Smad and NF- κ B pathway expression to inhibit inflammation and renal fibrosis (45). Astragaloside A can inhibit endoplasmic reticulum stress and mitochondrial mediated apoptosis (46). *Sophora Qi Huang* can reduce podocyte apoptosis by regulating endoplasmic reticulum stress protein, maintaining mitochondrial calcium homeostasis (47). Therefore, traditional Chinese medicine has significant advantages in treating DN, which can be treated from multiple angles, channels and targets.

There were still some limitations to this study, which need to be continuously improved. For example, we only discussed the mechanism of Rhubarb-Coptis in the treatment of DN from the perspective of network pharmacology. However, in view of the current information technology and data accuracy, the real-time effects need to be improved, it is necessary to conduct further mechanism experiments to clarify the synergistic effects of multi-components, multi-targets, and multi-pathways involved in the treatment process of DN by Rhubarb-Coptis.

In the future drug research and development, we can use network pharmacology to study the active components and mechanism of action of drugs to treat diseases, and to screen the active components, action targets and pathways of known drugs, so as to find new indications and related mechanisms of action of drugs. However, there are still some problems to be solved in the application of network pharmacology in studying the treatment of DN with traditional Chinese medicine; the data of each database platform is different, which makes the prediction result biased. Therefore, in the application of network pharmacology to the study of the mechanism of action of traditional Chinese medicine in treating DN, we should take into account its informatization, networking and the application of the holistic view, further explore the TCM syndromes of DN, take syndrome differentiation as the program, integrate the basic experimental data and TCM clinical syndromes, and build a network model of disease syndrome integration.

Conclusions

The present study analyzed the mechanisms of Rhubarb-Coptis in the treatment DN by constructing Rhubarb-Coptis-target-DN-related pathway network using network pharmacology. We found that quercetin, berberine, eupatin, epiberberine, and moupinamide can be defined as 5 crucial compounds. *STAT3*, *CTNNB1*, *PIK3R1*, *PIK3CA*, and *TP53* may be the 5 most important therapeutic targets. The AGE-RAGE and HIF-1 are 2 key signaling pathways of Rhubarb-Coptis for the treatment of DN. The underlying mechanisms are mainly related to oxidative stress, inflammatory response, and apoptosis. The results of this study provide a reference for further exploring the mechanism of Rhubarb-Coptis in the treatment of DN.

Acknowledgments

Funding: This work was supported by Jilin Provincial Natural Science Foundation, based on miR-200a, discusses the mechanism of detoxification, unblocking collaterals and expelling wind therapy for diabetic nephropathy (No. YDZJ202201ZYTS192).

Footnote

Reporting Checklist: The authors have completed the STREGA reporting checklist. Available at <https://atm.amegroups.com/article/view/10.21037/atm-22-5550/rc>

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at <https://atm.amegroups.com/article/view/10.21037/atm-22-5550/coif>). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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- (English Language Editor: J. Jones)

Cite this article as: Wu C, Cao L, Liu M, Zhang W, Chen H, Wang R, Liu C, He Z. Exploring the mechanisms underlying the therapeutic effect of the drug pair Rhubarb-Coptis in diabetic nephropathy using network pharmacology and molecular docking analysis. *Ann Transl Med* 2022;10(24):1343. doi: 10.21037/atm-22-5550