Research Article

Cheng Li, Hongli Jiang, Jingchun Yao, Xulong Chen*, PuXun Tian*

* Corresponding author: Xulong Chen, School of Clinical Medical, Jiujiang University, Jiujiang, Jiangxi, China, e-mail: cxl_0517@163.com
* Corresponding author: PuXun Tian, Department of Kidney Transplantation, Nephropathy Hospital, The First Affiliated Hospital of Xi’an Jiaotong University, Xi’an, Shanxi, China; Institute of Organ Transplantation, Xi’an Jiaotong University, Xi’an, Shanxi, China, e-mail: yuantian@mail.xjtu.edu.cn

Radix puerariae in the treatment of diabetic nephropathy: A network pharmacology analysis and experimental validation

https://doi.org/10.1515/chem-2022-0311
received February 13, 2023; accepted March 20, 2023

Abstract: Radix puerariae has become the most commonly used medicine for diabetic nephropathy (DN). However, the mechanism of Radix puerariae in the treatment of DN is not completely clear. This study is to determine the active ingredients, targets, and signaling pathways of Radix puerariae for the treatment of DN using network pharmacology analysis and animal experiments to confirm its possible mechanism of action. A total of 12 potential effective components and 10 key therapeutic targets were obtained. The Kyoto Encyclopedia of Genes and Genomes enrichment analysis indicated that the use of Radix puerariae in DN treatment mainly involves HIF-1 signaling pathway, TNF signaling pathway, mTOR signaling pathway, PI3K-Akt signaling pathway, Foxo signaling, and VEGF signaling pathway. Molecular docking proved that the combined activity of the components with potential key targets were excellent. Animal experiments showed that Radix puerariae could improve the renal pathological structure in DN rats. Radix puerariae could decrease the content of AKT1, IL-6, INS, and reduce the expression levels of p-Akt/Akt and p-FoxO1/FoxO1 in renal tissue of DN rats. This study provides insight into the therapeutic potential and molecular mechanisms of Radix puerariae for treating DN.

Keywords: Radix puerariae, diabetic nephropathy, network pharmacology, molecular docking, Foxo1 signaling pathway

1 Introduction

Diabetic nephropathy (DN) is a chronic non-communicable condition that adversely affects the health and quality of life of diabetes patients. DN has now emerged as the most common cause of end-stage renal disease (ESRD) in Europe, the United States, and Japan. In addition, as the life expectancy of the general population increases, the obesity rate increases, and the sedentary lifestyle leads to an increase in the incidence of diabetes and its chronic complications, the number of patients affected in some developing countries is rapidly increasing. Patients and the healthcare system are facing significant challenges [1,2]. According to the latest US Renal Data System, from 1990 to 2013, the annual mortality rate of DN has increased by more than two-fold (from 1.4 to 2.9 per 100,000 populations) [3]. Modern medical molecular biology found that the pathogenesis of DN is undoubtedly multi-factorial. During the development of DN, various cellular events and signaling pathways are activated, and the activated signaling pathways are regulated by a large number of molecules, which regulate each other’s biological activities [4]. At present, there are no targeted specific drugs for the treatment of DN, which mainly delay the progression of DN by improving lifestyle, controlling blood sugar, controlling blood pressure, and correcting lipid metabolism disorders [5]. Studies [6,7] have confirmed that the efficacy of combined traditional Chinese and western medicine in the treatment of DN is better than that of western medicine alone. Therefore, exploring the research of traditional
Chinese medicine in the treatment of DN has important socio-economic significance.

*Radix puerariae*, a traditional Chinese medicine, was first published in the “Shen Nong’s Materia Medica,” “mainly diminishes thirst” and enters the spleen and stomach meridian. Because it promotes body fluid and quenches thirst, it has become the most commonly used medicine for diabetes in the category of “diminishing thirst” in Chinese medicine. Puerarin is the main effective constituent separated and extracted from the dried roots of *Radix puerariae*, and it is also an effective natural, free radical scavenger. The chemical name for this compound is 4,7-dihydroxy-8-β-d-glucosyl isoflavones [8]. Modern pharmacological studies have demonstrated that puerarin reduces blood sugar levels, improves insulin resistance, protects islets, inhibits inflammation, reduces oxidative stress, prevents Maillard reactions, and prevents advanced glycation end products in diabetes patients [9]. In addition, puerarin plays an important role in clinical treatment by delaying and improving a series of complications of diabetes. It inhibits renal oxidative stress, improves vascular endothelial dysfunction, protects glomerular podocytes, and reduces renal tubular interstitial damage [10]. However, its targets and mechanisms are not yet clear.

Network pharmacology has evolved from rapid developments in computer science and systems biology. This process makes use of a number of technologies, such as omics, high-throughput screening, network visualization, and network analysis. As a result of the complex network analysis, drugs are systematically predicted to affect the disease network in accordance with the multi-pathway and multi-target characteristics of traditional Chinese medicine [11]. In this study, network pharmacology is used to predict and analyze the effective components, therapeutic targets, and signal pathways of *Radix puerariae* for DN treatment. The above molecules were verified in animal studies to provide evidence for further technical research and the application of *Radix puerariae* as a treatment for DN (Figure 1).

2 Materials and methods

2.1 Methods

2.1.1 Network pharmacology study

2.1.1.1 Identification and screening of the main constituents and targets of *Radix puerariae*

The active ingredients of *Radix puerariae* were screened using the TCMSP platform (http://lsp.nwu.edu.cn/TCMSP.php) [12] of the Chinese Medicine System Pharmacology Database and related literature from CNKI (https://www.cnki.net/) and PubMed databases (https://pubmed.ncbi.nlm.nih.gov/). Oral bioavailability (OB) ≥30% and drug-likeness (DL) ≥0.18 served as the basis for initial screening [13]. According to the selected constituents, the target protein name was obtained from the “Targets Information” in the TCMSP platform, and then the active components that did not correspond to the target protein were deleted. Additionally, the targets from the “homo sapiens” source have been converted into the corresponding genes by using the UniProt database (http://www.uniprot.org) for further analysis. A network diagram of the active ingredient-target relationship was drawn using Cytoscape 3.8.

2.1.2 Obtaining the key targets of *Radix puerariae* against DN

“Nephropathies, Diabetic” as the keyword, and the DN-related target information were retrieved from GeneCards (https://www.genecards.org/) and DisGeNET databases (https://www.disgenet.org/). Finally, Venn2.1.0 software (https://bioinfogp.cnb.csic.es/tools/venny/index.html) was applied to map the potential targets of *Radix puerariae* and DN-related targets to obtain the common targets of *Radix puerariae*-DN.

“Drug-disease” targets were imported into the STRING database (https://string-db.org/), and the maximum number of interactions were designed to not exceed ten interactors in order to structure PPI (protein–protein interaction) diagram. The results saved in TSV format were imported into Cytoscape 3.8.0, and algorithms of cytoHubba plug-in with Degree values were used to determine the core targets of *Radix puerariae* against DN.

2.1.3 Gene ontology (GO) analysis, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis, and construction of the “component-target-pathway” network

In order to understand the function of the selected core genes and their roles in the signaling pathway, the core targets were imported into the DAVID 6.8 (https://david.ncifcrf.gov/) database for GO and KEGG enrichment analysis. The “component-target-pathway” interaction network was formed using Cytoscape 3.8.0.

2.1.4 Molecular docking study

Molecular docking was performed on the top three effective components and key genes related to DN to
determine whether the ingredients were related to the targets. The Protein Data Bank (http://www.rcsb.org/pdb) and PubChem databases (https://pubchem.ncbi.nlm.nih.gov/) were utilized to obtain three-dimensional chemical structures of the targets and components. The final docking conformation was determined using AutoDock Vina software in order to determine the best conformation for affinity.

2.2 Experimental verification

2.2.1 Materials and reagents

*Radix puerariae* was provided by Jiangxi Xun Fengtang Traditional Chinese Medicine Co., Ltd, identified by Professor Gao Chunhua of Jiujiang University as a genuine product. In addition, herbs were morphologically authenticated by a
Chinese herbalist and chemically tested by thin layer chromatography, as per the Chinese Pharmacopoeia 2020 [14].

Preparation of Radix puerariae extract: Radix puerariae 30 g was decocted twice with water (300 mL and then 225 mL) for 2 h and the extracted solution was concentrated to 30 mL to obtain Radix puerariae extract [15].

2.2.2 Experimental animals

Sprague Dawley (SD) rats (5 weeks old, 120 ± 10 g) were purchased from SPF Biotechnology Co., Ltd (Beijing, China) and raised in a quiet room with a temperature of 20–28°C, relative humidity of 60%, and alternating time of light and dark 12:12 h for 1 week before treatment. These animals had sufficient standard feed and ensured freedom of drinking water. This study was approved by the Medical Ethics Committee of Jiujiang University Affiliated Hospital, Jiujiang, China (protocol approval number: jjuhera-2022-02; approval date: 15 April 2022).

2.2.3 Experimental designs

A week after adapting to the diet, the control group rats received common food and the model group rats received high-fat food for 4 weeks. After fasting for 12 h, rats in the model group were given an intraperitoneal injection of streptozotocin (STZ; 30 mg/kg) once a day for 72 h, while the control group was treated with citrate buffer of equal volume as placebo. After 72 h of STZ injection, if blood glucose was more than 16.6 mmol/L for three consecutive days, urine glucose was strongly positive, and urine protein content at 24 h was more than 30 mg, the DN model was confirmed to be successful.

Rats with successful modeling were assigned randomly to either the DN (n = 10) or the DN + Radix puerariae (n = 10) group. Radix puerariae treatment began on the first day after successful modeling and continued for 5 weeks. Radix puerariae was suspended in distilled water and given at a dose of 3.2 g/kg/day. Rats in the DN group received the same volume of distilled water.

2.2.4 Quantitative real-time PCR

Total RNA was extracted from renal cortical tissue with TransZol Up (TransGen Biotech, Beijing, China) according to the manufacturer’s protocols, then reversely transcribed into cDNA using EasyScript One-Step gDNA Removal and cDNA Synthesis Supermix (TransGen Biotech, Beijing, China). PCR primers were designed with the Primer Express 5.0 software and synthesized by Sangon Biotech Co., Ltd (Shanghai, China). The ratio of specific mRNA:GAPDH mRNA was calculated using the 2^(-ΔΔCT) method. Primers used to amplify IL-6, INS, TNF, AKT1, VEGFA, IGF1, and GAPDH are shown in Table S1.

2.2.5 Western blotting analysis

The total protein of each rat’s kidney tissue was extracted by RIPA lysate (Solarbio, Beijing, China), the protein concentration was determined by BCA method (Thermo Fisher, 23223; or Micro BCA, Thermo Fisher, 23223). Briefly, protein quantification involves the following steps: adding appropriate volume of 5× Protein Loading Buffer, denaturing in boiling water bath, 10% SDS-PAGE gel for electrophoresis separation, PVDF membrane (Millipore, Billerica, MA, USA) transfer and sealing, and adding the corresponding primary antibodies. The membranes were incubated overnight at 4°C with primary antibodies including anti-AKT antibody (1:1,000; Proteintech, Chicago, IL, USA), anti-p-AKT antibody (1:1,000; Proteintech, Chicago, IL, USA), anti-FoxO1 antibody (1:1,000; Proteintech, Chicago, IL, USA), anti-FOXO1 antibody (1:1,000; Abcam, Cambridge, MA, USA), and anti-β-actin antibody (1:50,000; Proteintech, Chicago, IL, USA), followed by incubation with a secondary antibody (1:5,000; Proteintech, Chicago, IL, USA) for 1–2 h. Bound antibodies were visualized using chemiluminescence detection on autoradiographic film. The protein bands were quantified by densitometry using ImageJ software. The levels of the detected proteins were normalized against β-actin.

2.3 Statistical analysis

SPSS 26.0 statistical software was used for data analysis. The measurement data conform to the normal distribution and the variance was homogeneous, one-way ANOVA was used for inter-group comparison. All data were expressed with mean values ± SD, P < 0.05 was considered statistically significant.
Table 1: Basic information about the active components of *Radix puerariae*

<table>
<thead>
<tr>
<th>Mol ID</th>
<th>Molecule name</th>
<th>Structure</th>
<th>OB (%)</th>
<th>DL</th>
</tr>
</thead>
<tbody>
<tr>
<td>MOL000392</td>
<td>Formononetin</td>
<td><img src="image1" alt="Formononetin" /></td>
<td>69.67</td>
<td>0.21</td>
</tr>
<tr>
<td>MOL000358</td>
<td>Beta-sitosterol</td>
<td><img src="image2" alt="Beta-sitosterol" /></td>
<td>36.91</td>
<td>0.75</td>
</tr>
<tr>
<td>MOL002959</td>
<td>3′-Methoxydaidzein</td>
<td><img src="image3" alt="3′-Methoxydaidzein" /></td>
<td>48.57</td>
<td>0.24</td>
</tr>
<tr>
<td>MOL003629</td>
<td>Daidzein-4,7-diglucoside</td>
<td><img src="image4" alt="Daidzein-4,7-diglucoside" /></td>
<td>47.27</td>
<td>0.67</td>
</tr>
<tr>
<td>MOL12297</td>
<td>Puerarin</td>
<td><img src="image5" alt="Puerarin" /></td>
<td>24.03</td>
<td>0.69</td>
</tr>
<tr>
<td>MOL000390</td>
<td>Daidzein</td>
<td><img src="image6" alt="Daidzein" /></td>
<td>19.44</td>
<td>0.19</td>
</tr>
<tr>
<td>MOL009720</td>
<td>Daidzin</td>
<td><img src="image7" alt="Daidzin" /></td>
<td>14.32</td>
<td>0.73</td>
</tr>
<tr>
<td>MOL000481</td>
<td>Genistein</td>
<td><img src="image8" alt="Genistein" /></td>
<td>17.93</td>
<td>0.21</td>
</tr>
<tr>
<td>MOL000357</td>
<td>Sitogluside</td>
<td><img src="image9" alt="Sitogluside" /></td>
<td>20.63</td>
<td>0.62</td>
</tr>
<tr>
<td>MOL000391</td>
<td>Ononin</td>
<td><img src="image10" alt="Ononin" /></td>
<td>11.52</td>
<td>0.78</td>
</tr>
</tbody>
</table>

(Continued)
3 Results

3.1 Active ingredients and target prediction of *Radix puerariae*

Through TCMPSP platform and literature search, the main chemical components and corresponding targets of *Radix puerariae* were obtained, a total of five active components were screened according to their DL and OB values. Puerarin, daidzein, daidzin, genistein, sitoglucoside, ononin, and 7,8,4′-trihydroxyisoavone were in conformity with the standard, but according to some reports [16–22], these ingredients have high pharmacological activity, so this study also took these ingredients as bioactive compounds for further analysis. The TCMPSP number, molecular name, and related parameters for each active ingredient are displayed in Table 1. After Uniprot screening and correction, a total of 209 targets of compounds were screened, and combined with 12 bioactive compounds, establishing the ingredient-target network (Figure 2).

3.2 Intersection of disease targets and drug-disease targets

The GeneCards and DisGeNET databases provided 440 DN-related target genes (Figure 3). A Venn diagram analysis revealed 53 intersecting targets between *Radix puerariae* and DN (Figure 4).

3.3 Construction and screening of *Radix puerariae*-DN protein interaction network

The 53 intersection targets of “drug-disease” were uploaded to the STRING database to obtain the PPI network, then imported these data into Cytoscape software for visualization (Figure 5a and b). The network contained 53 nodes and 692 protein interaction relationships. The topological analysis indicated that the median values of Betweenness Centrality, Closeness Centrality, and Degree were 0.093, 0.729, and 26.1, respectively. Finally, ten key targets were screened from the Cytoscape software via the “CytoHubba” plug-in as shown in Figure 5c, including IL-6, INS, TNF, VEGFR, PTGS2, MMP9, AKT1, MAPK1, IGF1, and FN1.

3.4 Enrichment results of GO and KEGG pathways

We conducted GO function enrichment analysis on the top ten key targets using David 6.8 by setting the index to \( P < 0.05 \). A total of 114 GO items were obtained, including 99 items of biological processes, 6 items of cellular component, and 9 items of molecular function. The biological process items were the most important. Among them, the positive regulation of cell proliferation, positive regulation of glycogen biosynthetic process, negative regulation of the apoptotic process, and the positive regulation of glucose import were ranked high and were closely related to DN (Figure 6a–c).

73 signal pathways were screened by KEGG enrichment pathway analysis \( (P < 0.05) \) using the DAVID 6.8 database, and the top 20 pathways were shown in Table 2 and Figure 7. Six of these pathways were related to DN’s occurrence and development, and *Radix puerariae* might be effective in treating DN by acting on the HIF-1 pathway, TNF pathway, mTOR pathway, PI3K-Akt pathway, FoxO pathway, and VEGF pathway. The FoxO pathway (Figure 8) was one of the top 20 signalling pathways that plays an important role in anti-apoptotic mechanisms. Among the FoxO signaling
pathways, Akt and FOXO were the key proteins that mediate apoptotic processes.

### 3.5 Composition-target-pathway network

Component-target-pathway network (Figure 9) demonstrated the relationship between effective components, related protein targets, and action pathways. The network comprised 50 nodes and 106 edges, including 11 core active components, 223 targets, and 20 KEGG pathways. Calculated by Degree value (the connectivity between nodes), the top three main components were genistein (Degree = 96), daidzein (Degree = 70), and puerarin (Degree = 55).

### 3.6 Molecular docking results

Molecular docking was employed to verify the correlation between *Radix puerariae* and DN. According to the Degree value, the top three active ingredients were selected, including genistein, daidzein, and puerarin. Then, Molecular docking was performed between the selected components and six core targets. All the molecular docking binding energy
between receptors and ligands were drawn into heatmap as shown in Figure 10(a). The result showed that the binding energies were less than 5 kcal/mol, indicating that the molecular components of *Radix puerariae* had a strong affinity for the six hub targets. The 2D structure display was screened as shown in Figure 10(b), in which hydrogen bonds were connected by green dashed lines between atoms, and the hydrophobic interaction was represented by red arcs.

### 3.7 *Radix puerariae* effected on renal histopathology of DN rats

Renal tissue sections were stained with HE, PASM, and Masson, respectively. The renal structure of normal rats was clean and morphologically fine, the glomerular mesangial cells and matrix appeared normal. Also, glomerular basement membrane (GBM) appeared normal, and there was no proliferation of fibrous tissue. In the DN group, the renal cortex exhibited various stages of glomerulosclerosis and tubular damage, including glomerular inherent cell hypertrophy, GBM thickening, mesangial matrix deposition, and vacuolar degeneration of tubular epithelial cells. *Radix puerariae*-treated kidney sections demonstrated intact glomerulus and Bowman’s space in comparison to the DN group. Several lumens were observed within the thin glomerulus loops. Also, mesangial expansion and tubulointerstitial injury were partially ameliorated (Figure 11(a)).

### 3.8 *Radix puerariae* alleviated oxidative stress in DN rats

The oxidative stress factor levels were measured to establish whether *Radix puerariae* could attenuate the oxidative stress response in DN rats. RT-PCR showed that, compared with the control group, the levels of AKT1, IL-6, and INS in the DN group were higher; the transcriptional levels of AKT1, IL-6, and INS were lower in the group that was given *Radix puerariae* than in the DN group (Figure 11(b)).

### 3.9 *Radix puerariae* suppressed the FoxO1 signal pathway

The FoxO1 pathways in kidney tissues were studied in order to discover possible mechanisms by which *Radix
*Pueraia* protects the renal system. Western blotting was performed in order to determine the protein expression of p-AKT, AKT, p-FoxO1, and FoxO1. The ratios of p-Akt/Akt and p-FoxO1/FoxO1 were utilized to show the phosphorylation of Akt and FoxO1, respectively. AKT and FoxO1 phosphorylation ratios were higher in the DN group than in the control group, as shown in Figure 11(c). Compared with those in the DN group, decreased phosphorylated AKT and FoxO1 ratios were detected in the *Radix puerariae*-treated group.

### 4 Discussion

DN poses a significant risk to the health and well-being of diabetic patients. Although ACEI/ARB drugs have been widely used, there is an increase in the number of people developing ESRD [23]. Consequently, new drugs are necessary for the treatment of DN. In recent years, many researchers have found that *Radix puerariae* has anti-DN efficacy and is mainly used to reduce oxidative stress, change kidney autophagy, and anti-cell apoptosis. However, most studies explored a single component or single pathway [16,24,25]. Traditional Chinese medicine has a multi-component, multi-target mechanism and regulates multiple signaling pathways. It is difficult to clarify the systematic and synergistic effect of *Radix puerariae* against DN from a single aspect of research. Therefore, with the help of a network pharmacological analysis platform, our study systematically and comprehensively reveals the relationship between the effective main constituents, targets, and pathways.

Our study focuses on the anti-DN mechanism of *Radix puerariae*, the effective active ingredients and its corresponding targets. Previous studies have found that puerarin, the main active ingredient of *Radix puerariae*, can significantly improve blood glucose, proteinuria, renal function, hemodynamic status, blood lipids, blood pressure, and other indicators in patients with DN [26]. In addition to puerarin, this study found that genistein and...
Figure 6: Analysis of functional enrichment. (a) Top 20 biological processes. (b) Top 20 cellular components. (c) Top 20 molecular functions.
daidzein were also related to multiple key targets of DN, revealing that these components also play a key role in treating DN, which needs to be further verified in the follow-up studies.

Comprehensive analysis of key targets, GO, KEGG enrichment, and molecular docking results have shown that *Radix puerariae* may regulate the HIF-1 signaling pathway, TNF signaling pathway, mTOR signaling pathway, PI3K-Akt

**Table 2:** Analysis of the KEGG pathway of *Radix puerariae* in the treatment of DN

<table>
<thead>
<tr>
<th>ID</th>
<th>Term</th>
<th>P value</th>
<th>Genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>hsa05205</td>
<td>Proteoglycans in cancer</td>
<td>$4.38 \times 10^{-8}$</td>
<td>FN1, MAPK1, AKT1, IGF1, TNF, MMP9, VEGFA</td>
</tr>
<tr>
<td>hsa04066</td>
<td>HIF-1 signaling pathway</td>
<td>$5.75 \times 10^{-8}$</td>
<td>IL6, MAPK1, AKT1, IGF1, INS, VEGFA</td>
</tr>
<tr>
<td>hsa05200</td>
<td>Pathways in cancer</td>
<td>$6.15 \times 10^{-8}$</td>
<td>IL6, FN1, MAPK1, AKT1, IGF1, PTGS2, MMP9, VEGFA</td>
</tr>
<tr>
<td>hsa04668</td>
<td>TNF signaling pathway</td>
<td>$9.94 \times 10^{-8}$</td>
<td>IL6, MAPK1, AKT1, PTGS2, TNF, MMP9</td>
</tr>
<tr>
<td>hsa04150</td>
<td>mTOR signaling pathway</td>
<td>$5.56 \times 10^{-7}$</td>
<td>MAPK1, AKT1, IGF1, TNF, INS</td>
</tr>
<tr>
<td>hsa04151</td>
<td>PI3K-Akt signaling pathway</td>
<td>$1.13 \times 10^{-6}$</td>
<td>IL6, FN1, MAPK1, AKT1, IGF1, INS, VEGFA</td>
</tr>
<tr>
<td>hsa04068</td>
<td>FoxO signaling pathway</td>
<td>$1.61 \times 10^{-5}$</td>
<td>IL6, MAPK1, AKT1, IGF1, INS</td>
</tr>
<tr>
<td>hsa05161</td>
<td>Hepatitis B</td>
<td>$2.20 \times 10^{-5}$</td>
<td>IL6, MAPK1, AKT1, TNF, MMP9</td>
</tr>
<tr>
<td>hsa04370</td>
<td>VEGF signaling pathway</td>
<td>$5.37 \times 10^{-5}$</td>
<td>MAPK1, AKT1, PTGS2, VEGFA</td>
</tr>
<tr>
<td>hsa04510</td>
<td>Focal adhesion</td>
<td>$8.75 \times 10^{-5}$</td>
<td>FN1, MAPK1, AKT1, IGF1, VEGFA</td>
</tr>
<tr>
<td>hsa04015</td>
<td>Rap1 signaling pathway</td>
<td>$9.43 \times 10^{-5}$</td>
<td>MAPK1, AKT1, IGF1, INS, VEGFA</td>
</tr>
<tr>
<td>hsa04014</td>
<td>Ras signaling pathway</td>
<td>$1.26 \times 10^{-4}$</td>
<td>MAPK1, AKT1, IGF1, INS, VEGFA</td>
</tr>
<tr>
<td>hsa04914</td>
<td>Progesterone-mediated oocyte maturation</td>
<td>$1.55 \times 10^{-4}$</td>
<td>MAPK1, AKT1, IGF1, INS</td>
</tr>
<tr>
<td>hsa05215</td>
<td>Prostate cancer</td>
<td>$1.61 \times 10^{-4}$</td>
<td>MAPK1, AKT1, IGF1, INS</td>
</tr>
<tr>
<td>hsa05142</td>
<td>Chagas disease (American trypanosomiasis)</td>
<td>$2.64 \times 10^{-4}$</td>
<td>IL6, MAPK1, AKT1, TNF</td>
</tr>
<tr>
<td>hsa04620</td>
<td>Toll-like receptor signaling pathway</td>
<td>$2.79 \times 10^{-4}$</td>
<td>IL6, MAPK1, AKT1, TNF</td>
</tr>
<tr>
<td>hsa04931</td>
<td>Insulin resistance</td>
<td>$2.95 \times 10^{-4}$</td>
<td>IL6, AKT1, TNF, INS</td>
</tr>
<tr>
<td>hsa04932</td>
<td>Non-alcoholic fatty liver disease</td>
<td>$7.90 \times 10^{-4}$</td>
<td>IL6, AKT1, TNF, INS</td>
</tr>
<tr>
<td>hsa04960</td>
<td>Aldosterone-regulated sodium reabsorption</td>
<td>$1.10 \times 10^{-3}$</td>
<td>MAPK1, IGF1, INS</td>
</tr>
<tr>
<td>hsa05164</td>
<td>Influenza A</td>
<td>$1.19 \times 10^{-3}$</td>
<td>IL6, MAPK1, AKT1, TNF</td>
</tr>
</tbody>
</table>
Figure 7: Bubble diagram of KEGG pathway enrichment analysis.

Figure 8: Metabolic pathway map of FoxO signaling pathway.
signaling pathway, FoxO signaling pathway, and VEGF signaling pathway by affecting IL-6, INS, TNF, AKT1, VEGFA, and IGF1, regulates renal microcirculation and anti-podocyte apoptosis, protecting vascular endothelial cells, changing renal autophagy status, and reducing oxidative stress, thus playing a role in the treatment of DN.

According to several studies, IL-6 levels are closely related to the development of DN [27–29]. As a multifunctional cytokine, it activates the PI3K-AKT signal pathway. In vitro investigations have demonstrated that IL-6 trans-signaling triggers the release of monocyte chemoattractant protein-1 from human vascular endothelial cells. Furthermore, this process requires simultaneous activation of PI3K/AKT pathways. With the decrease in IL-6 level, the down-regulation of AKT expression and vascular endothelial injury lessened. It is confirmed that inhibition of the PI3K-AKT signal pathway can protect blood vessels and prevent vascular complications of DN [30]. Wu et al. [27] established a rat model of DN with the aim to evaluate the role of IL-6 receptor antibodies in DN. In the treatment group, albuminuria and glomerular mesangial matrix accumulation were reduced, proving that IL-6 is associated with inflammatory reaction and oxidative stress of DN. The above research works support that IL-6 is a key target for anti-DN.

Islet β cells secrete the only hypoglycemic hormone in the body. INS mutation/variation affects insulin secretion, which contributes to the occurrence and development of diabetes [31]. In addition, INS is a key target in the PI3K-Akt-mTOR signaling pathway. The PI3K-Akt-mTOR signaling pathway plays a key role in mediating cell apoptosis, promoting cell differentiation and survival, and contributes to the onset and progression of DN by inhibiting autophagy, promoting inflammation, and aggravating oxidative stress [32,33]. Therefore, the effective active components of Radix puerariae may inhibit the above signal pathways, promoting mTOR-mediated autophagy, resisting podocyte apoptosis, and reducing renal interstitial fibrosis, thus exerting the efficacy of treating DN.

Akt is one of the most important proteins in the downstream signal pathway of the PI3K/Akt/FoxO1 pathway, which is a key insulin signal molecule and has a significant regulatory role in glucose and lipid metabolism [34]. The activated form of AKT can regulate and control glucose and lipid metabolism by regulating GLUT-4, GSK-3β, Fox O1, and mTOR activities [35], thus participating in the occurrence and development of DN and drug therapy.

Fox transcription factors can be found in organisms from yeast to humans, as well as in kidneys, where they are widely expressed. Among them, Fox O1 is widely expressed in a variety of renal parenchyma cells, such as podocytes and mesangial cells, and plays an essential role in the pathogenesis of various renal injuries [36]. Several studies [37–39] have shown that PI3K/Akt can directly phosphorylate Fox O1, hence inhibiting Fox O1 activity. The decrease in the expression level of Fox O1 will lead to the decrease in antioxidant enzymes, the reduction in autophagy level, the increase in blood
Figure 10: Validation of molecular docking. (a) Molecule docking heat map of the key pharmacodynamic molecule-key target. (b) Molecular docking diagram of key ingredient and key target.
Figure 11: Radix puerariae alleviates the progress of diabetes nephropathy. (a) HE, Masson, and PASM stained at 200× magnification, respectively; (b) the levels of AKT1, IGF1, IL-6, INS, TNF, and VEGFA by RT-PCR in kidney tissues; (c) western blot expressions of p-AKT, AKT, p-FoxO1, and FoxO1 and the ratio of p-AKT to AKT and p-FoxO1 to FoxO1. Data are presented as the mean value ± standard deviation. **P < 0.01 compared with the control group; ##P < 0.01 compared with the DN group. DN, diabetic nephropathy; RP, Radix puerariae.
glucose level, and the disorder of lipid metabolism, thereby accelerating the progress of DN. Relevant studies [40,41] suggest that in the diabetes model, the reduction in FoxO1 phosphorylation contribute to the improvement of renal function, while the high expression of FoxO1 can reduce the apoptosis induced by high glucose through activating PTEN-Induced putative kinase (PINK1), improve the autophagy level and cell vitality, effectively alleviate renal injury, and inhibit the process of renal fibrosis.

In this study, we established a DN rat model and preliminarily explored the mechanism of *Radix puerariae* in treating DN by observing the pathological changes in kidney tissue, renal tissue cytokines, and the key proteins in the FoxO1 signal pathway. Histopathological characteristics of DN rats’ kidneys included glomerular inherent cell hypertrophy, GBM thickening, mesangial matrix deposition, and vacuolar degeneration of tubular epithelial cells. *Radix puerariae* treatment partially improved glomerular hypertrophy, mesangial matrix expansion, and tubulointerstitial injury. PASM and Masson’s trichrome showed a moderate expansion of the mesangial matrix, and extracellular matrix deposition in both glomeruli and tubulointerstitium in the DN group, treatment with *Radix puerariae* significantly inhibited these histologic injuries. AKT1, IL-6, and INS were the core targets screened by network pharmacology. This study has shown that the expression of AKT1, IL-6, and INS mRNA in the DN group was significantly higher than those in the control group. *Radix puerariae* down-regulated the expression level of AKT1, IL-6, and INS and showed a significant effect on alleviating the progress of DN by reducing oxidative stress injury. Furthermore, FoxO1 has been associated with the development of DN and has been shown to play a role in its development. In our study, we found that the FoxO1 pathway was upregulated in the DN group, as we found overexpression of p-Akt and p-FoxO1 in the kidney, suggesting that high glucose may increase the phosphorylation of FoxO1 through activating the FoxO1 pathway. However, the FoxO1 pathway was down-regulated after *Radix puerariae* treatment. The activity of FoxO1 increased, thus slowing down the development of DN.

5 Conclusion

In conclusion, we used the network pharmacological approach to investigate the main target proteins of *Radix puerariae* compounds that displayed anti-DN activity by constructing a “component-target-disease” network and validated our findings in animal models. The results of our study indicated that 12 compounds, including genistein, daidzein, puerarin, and so on, had the potential to be used as therapeutic agents in the treatment of DN. The interaction of these bioactive compounds with AKT1, IL-6, INS, and other enzymes was predicted. Additionally, biological process and pathway enrichment analysis of the target proteins of *Radix puerariae* strengthened our understanding of the mechanism of action of the active ingredients. This study provides valuable insight into the mechanism by which *Radix puerariae* inhibits the progression of DN by interfering with the FOX1 signaling pathway. Further studies will be conducted to investigate the possible therapeutic benefits and mechanisms of *Radix puerariae* and combination therapies for the treatment of DN.

**Funding information:** This study was supported by the Educational Department in Jiangxi Province under the Grants [GJJ201819 and GJJ211805], Health Commission of Jiangxi Provincial under the Grant [202212007], Jiangxi Provincial Administration of Traditional Chinese Medicine under the grant [2022A137], Beijing Medical and Health Foundation under the Grant [TYU046B], and Beijing Medical Award Foundation under the Grant [YXJL-2022-0734-0294].

**Author contributions:** Cheng Li: data curation, formal analysis, development or design of methodology, validation, and writing – original draft. Hongli Jiang: project administration. Jingchun Yao: creation of models, data/evidence collection, and visualization. Xulong Chen: funding acquisition, resources, writing – review and editing. PuXun Tian: conceptualization and supervision.

**Conflict of interest:** The authors declare that the research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

**Ethical approval:** This study was approved by the Medical Ethics Committee of Jiujiang University Affiliated Hospital, Jiujiang, China (protocol approval number: jjuhmer-a-2022-02; approval date: 15 April 2022).

**Data availability statement:** The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

**References**


