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Research on Predicting the Nephrotoxicity Mechanism of Lianqiao-4 Based on Network Pharmacology and Molecular Docking

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Abstract: *Objective:* To predict the nephrotoxicity mechanism of Lianqiao-4 through network pharmacology and molecular docking methods. *Methods:* The main chemical components of Lianqiao (Forsythia suspensa), Bistortae rhizoma, Ophiopogonis radix, and Clematidis radix et rhizoma, as well as nephrotoxicity-related targets, were screened through databases such as TCMSP, Swiss Target Prediction, GeneCards, and ETCM. Venny 2.1.0 was used to identify the main components of Lianqiao-4 and nephrotoxicity targets. The STRING platform and David database were utilized to construct a protein-protein interaction (PPI) network diagram, while gene function (GO) enrichment analysis and KEGG pathway analysis were conducted. The "Lianqiao-4 active ingredients-nephrotoxicity targets-signaling pathways" network model was constructed using Cytoscape 3.9.1 software. *Results:* Network pharmacology and molecular docking analysis revealed that the core active ingredients responsible for the nephrotoxicity mechanism of Mongolian medicine Lianqiao-4 include steroidal saponins such as ophiopogonin A, flavonoids like kaempferol and quercetin, steroidal compounds such as β-sitosterol and sitosterol, and other key regulatory targets including STAT3, ABCG2, HSP90AA1, MMP9, PTGS2, and EGFR. Major pathways involved include lipid and atherosclerosis, chemical carcinogenesis - DNA adducts, and arachidonic acid metabolism. *Conclusion:* Mongolian medicine Lianqiao-4 exerts its therapeutic effect on nephrotoxicity through multiple components, targets, and pathways, pending experimental verification.

Keywords: Network pharmacology; Molecular docking; Lianqiao-4; Nephrotoxicity

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1. Introduction

Lianqiao-4, a commonly used Mongolian medicine compound, is also known as Yindarixi Tang or Yindari-4 Wei Tang, and is recorded in the "Four Tantras of Medicine." This compound consists of four herbal ingredients:

Lianqiao (*Forsythia suspensa*), *Bistortae rhizoma*, *Ophiopogonis radix*, and *Clematidis radix et rhizoma*. Classified as a cooling formula, it exhibits antidiarrheal and heat-clearing properties, primarily used to treat intestinal heat dysentery, abdominal pain, diarrhea, and other ailments. Clinically, Lianqiao-4 is often employed in the treatment of infantile diarrhea [1]. Studies have demonstrated its significant protective effects against CCL4-induced acute liver injury and its hepatoprotective and enzyme-lowering effects in models of pylorus ligation-induced liver injury [2,3]. Additionally, research has revealed its remarkable gastroprotective effects against gastric and duodenal ulcers [4].

The kidney is a crucial organ in the human body, and drugs and their metabolites can potentially cause harm to it [5]. Due to its abundant blood flow, high oxygen consumption, elevated enzymatic activity, and high tissue metabolic rate, the kidney is susceptible to the influx of drugs and metabolites, which can affect its normal functioning and, in severe cases, be life-threatening [6]. Therefore, this study aims to investigate the preliminary effects of Lianqiao-4 on nephrotoxicity using network pharmacology methods.

2. Experimental methods

2.1. Collection of active ingredients and target prediction for Mongolian medicine Lianqiao-4

The main active ingredients of Lianqiao, *Ophiopogonis radix*, *Bistortae rhizoma*, and *Clematidis radix et rhizoma* were collected through literature searches and databases such as TCMSP and HERB. These ingredients were screened based on oral bioavailability (OB ≥ 30%) and drug-likeness (DL ≥ 0.18). The two-dimensional molecular structures of the active ingredients were searched using the PubChem database and then imported into the Swiss Target Prediction database for target prediction. The target names were corrected using the Uniprot database.

2.2. Acquisition of disease targets

Using “nephrotoxicity” as the keyword, disease targets for nephrotoxicity were screened from the GeneCards and DisGeNET databases. After removing duplicates, the targets were corrected using the UniProt database.

2.3. Construction of PPI network for potential nephrotoxicity targets of Mongolian medicine Lianqiao-4

Venny 2.1.0 software was used to find the intersection of drug active targets and disease targets, and a Venn diagram was drawn. The intersecting targets were then imported into the STRING database, with the protein species set to “Homo sapiens” for protein-protein interaction analysis. The analysis results were imported into Cytoscape 3.9.1 software to construct a PPI network diagram and screen for core targets.

2.4. GO enrichment and KEGG pathway analysis

The intersecting targets were imported into the DAVID database for GO enrichment and KEGG pathway analysis, respectively. GO covers three aspects: biological process (BP), cellular component (CC), and molecular function (MF). Subsequently, the Microbiome Online Platform was used to sort the top 10 rankings based on *P*-value from smallest to largest and create bar charts and bubble charts.

2.5. Construction of "Active ingredient-target-signal pathway" network diagram

The active ingredients of Mongolian medicine Lianqiao-4, renal toxicity-related targets, and related pathways were imported into Cytoscape 3.9.1 to construct an "Active ingredient-target-signal pathway" network diagram, further exploring the mechanism of action of Mongolian medicine Lianqiao-4 on renal toxicity.

2.6. Molecular docking

Using AutoDockTools 1.5.6 software, molecular docking was performed on the key active ingredients and core protein targets of Lianqiao-4 for treating renal toxicity based on the Degree value. Visualization analysis was assisted by PYMOL 3.11 software.

3. Experimental results

3.1. Analysis of screening results for main components of Lianqiao-4

Using the TCMSP and HERB platforms, all chemical components contained in the four Mongolian medicines were evaluated for ADEM properties. There were 19 chemical components in Lianqiao, 31 in *Ophiopogon japonicus*, 3 in *Clematis armandii*, and 6 in *Bistortae rhizoma*. The two-dimensional molecular structural formulas of the active ingredients were searched using the PubChem database, and then imported into the Swiss Target Prediction database for target prediction of the active ingredients (226 targets). The names of the drug active ingredient targets were then corrected using the Uniprot database.

3.2. Acquisition of disease targets

Using "nephrotoxicity" as a keyword, renal toxicity targets were screened from the GeneCards and DisGeNET databases. After merging and removing duplicates, 612 renal toxicity-related targets were obtained. The disease target names were then corrected using the UniProt database.

3.3. Construction of PPI network for potential targets of Mongolian medicine Lianqiao-4 in treating renal toxicity

There were 184 main component targets and 612 disease targets. Using Venny 2.1.0 software, 39 intersecting targets of Lianqiao-4 for treating renal toxicity were obtained (**Figure 1**).

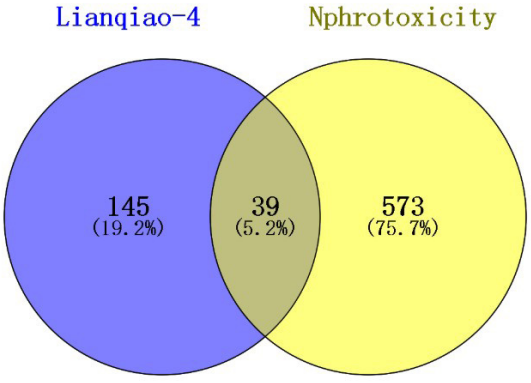


Figure 1. Venn diagram of targets for Forsythia-4 in treating renal toxicity.

After importing the intersecting targets into the STRING database to obtain protein-protein interaction information, there were 39 nodes and 232 edges, with an average node degree of 11.9. The PPI network diagram was drawn using Cytoscape 3.9.1 software, as shown in **Figure 2**. In the PPI protein interaction network diagram, the top 7 target proteins ranked by Degree value are PTGS2 (Prostaglandin-Endoperoxide Synthase 2, COX-2), BCL2 (B-cell lymphoma 2 protein), ABCG2 (ATP-binding cassette transporter G2), HSP90AA1, MMP9 (Matrix Metallopeptidase 9), STAT3 (Signal Transducer and Activator of Transcription 3), and EGFR (Epidermal Growth Factor Receptor). These are all core targets in the PPI protein interaction network diagram.

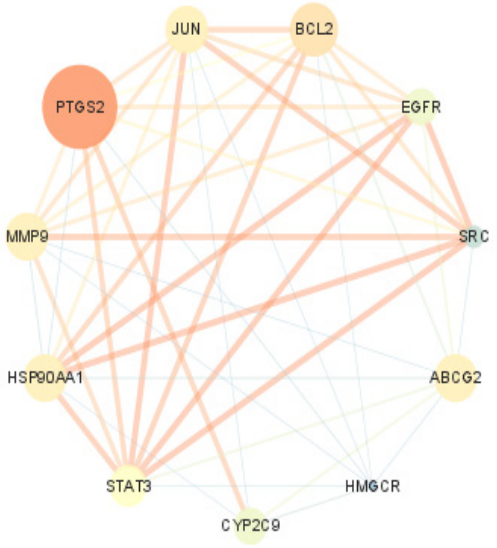


Figure 2. Intersection target PPI network diagram.

3.4. GO enrichment and KEGG pathway analysis

The intersecting targets were imported into the DAVID database for GO enrichment and KEGG pathway analysis. The analysis results were screened with a *P*-value < 0.05 as the criterion, resulting in 115 biological processes (BP), 21 cellular components (CC), and 48 molecular functions (MF). The top 10 results were plotted in a bar chart, as shown in **Figure 3**. BP mainly includes the cytochrome P450 epoxygenase pathway, xenobiotic metabolic process, steroid metabolic process, etc. CC mainly includes endoplasmic reticulum membrane, membrane-bounded intracellular organelles, caveolae, cytoplasm, etc. MF mainly includes heme binding, aromatase activity, etc. KEGG pathway analysis yielded 87 pathways, and the top 10 were plotted in a bar chart (**Figure 4**). Pathway analysis suggests that Forsythia-4 may exert its effects through pathways such as lipid and atherosclerosis, chemical carcinogenesis - DNA adducts, and arachidonic acid metabolism. These results indicate that Forsythia-4 regulates and treats renal toxicity through multiple biological processes.

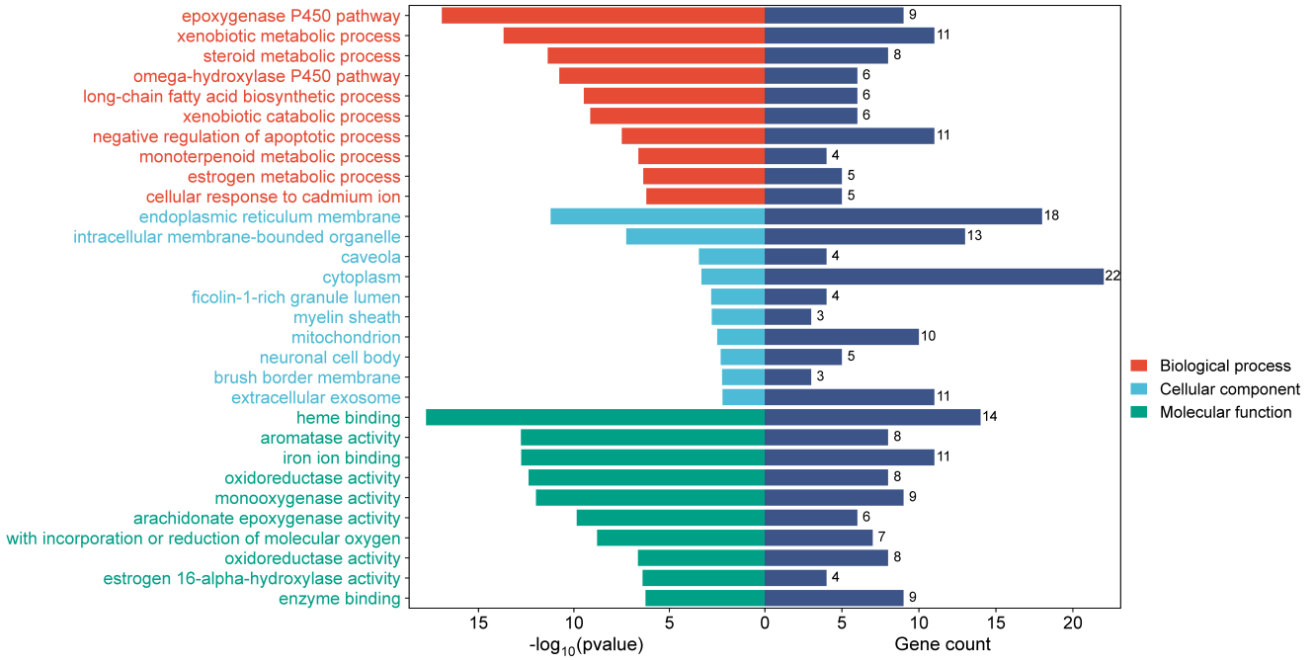


Figure 3. GO enrichment analysis diagram.

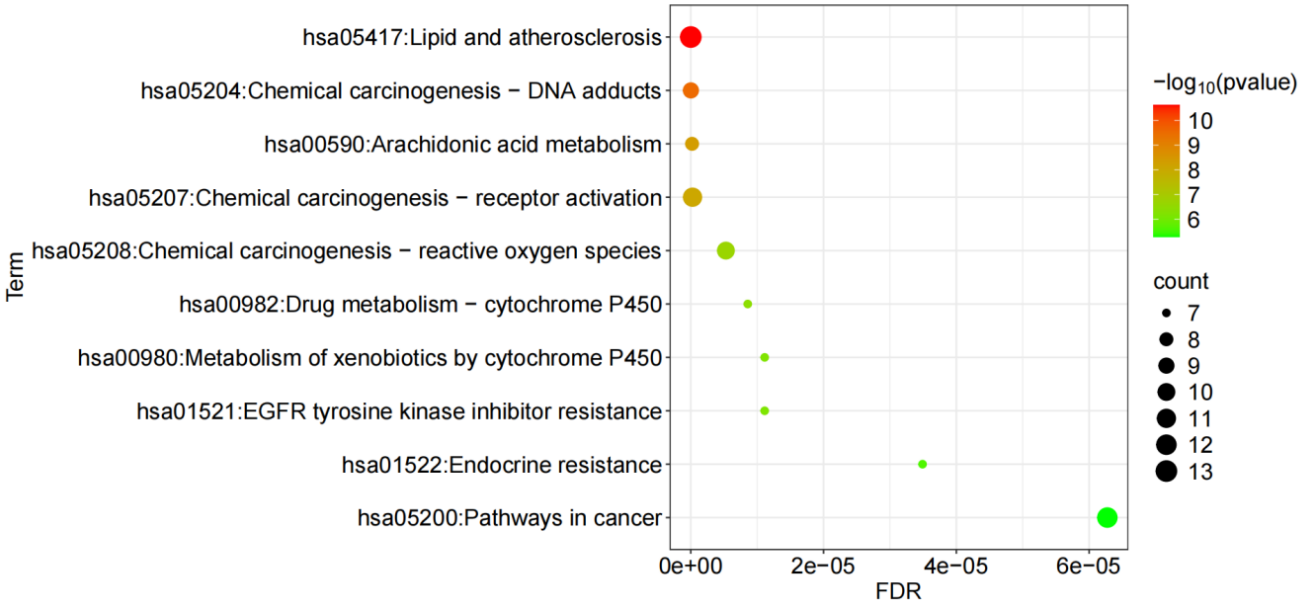


Figure 4. KEGG pathway enrichment analysis.

3.5. Construction of the "Active ingredient-target-signal pathway" network diagram

The "Active ingredient-target-signal pathway" network diagram was constructed using Cytoscape 3.9.1 software. With the Degree value as a reference, the core components of Forsythia-4 include kaempferol, beta-sitosterol, ophiopogonin A, quercetin, and β-sitosterol. The core target proteins for the treatment of renal toxicity include Signal Transducer and Activator of Transcription 3, ATP-binding cassette transporter G2, HSP90AA1, Matrix Metallopeptidase 9, Prostaglandin-Endoperoxide Synthase 2, and Epidermal Growth Factor Receptor. The core pathways include drug metabolism - cytochrome P450, metabolism of xenobiotics by cytochrome P450, EGFR

tyrosine kinase inhibitor resistance, and arachidonic acid metabolism (**Figure 5**).

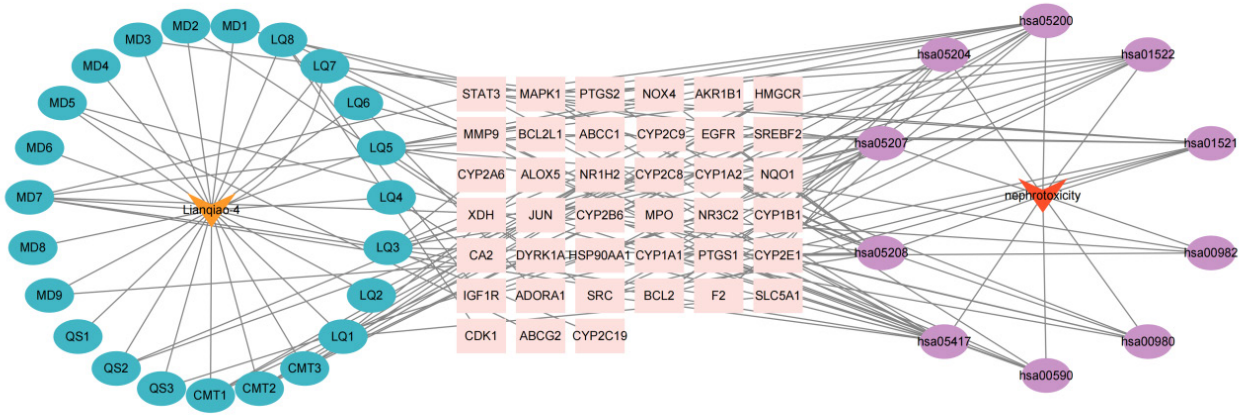


Figure 5. Active ingredient-target-signal pathway diagram.Note: Green circles represent drug active ingredients, blue circles represent pathways, and light squares represent targets.

3.6. Molecular docking

Using AutoDockTools 1.5.6 software, molecular docking was performed between the main active ingredients of Forsythia-4 (kaempferol, beta-sitosterol, ophiopogonin A, quercetin, β-sitosterol) and the core protein targets (Signal Transducer and Activator of Transcription 3, ATP-binding cassette transporter G2, HSP90AA1, Matrix Metallopeptidase 9, Prostaglandin-Endoperoxide Synthase 2, Epidermal Growth Factor Receptor) based on their Degree values. Visualization analysis of the high binding energy results was carried out using PYMOL 3.11 software (**Figure 6** and **Figure 7**).

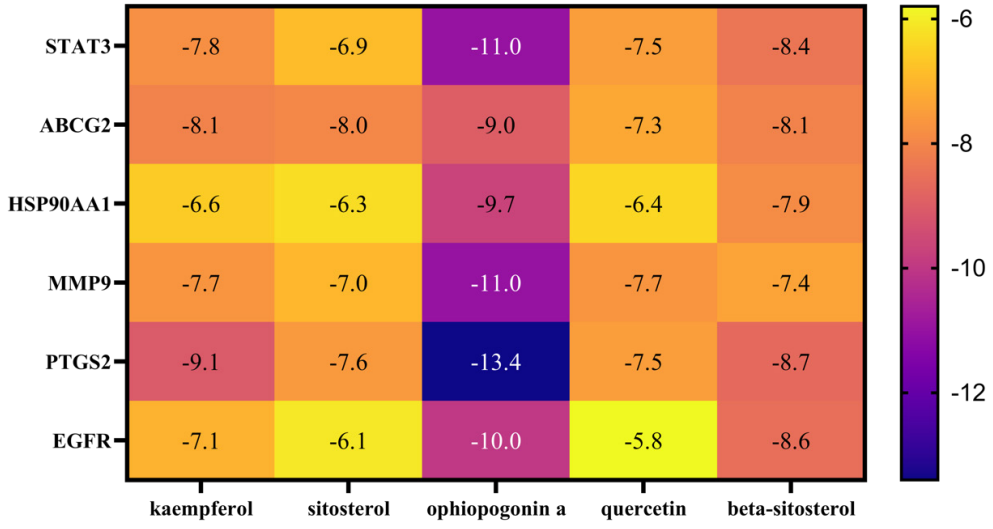
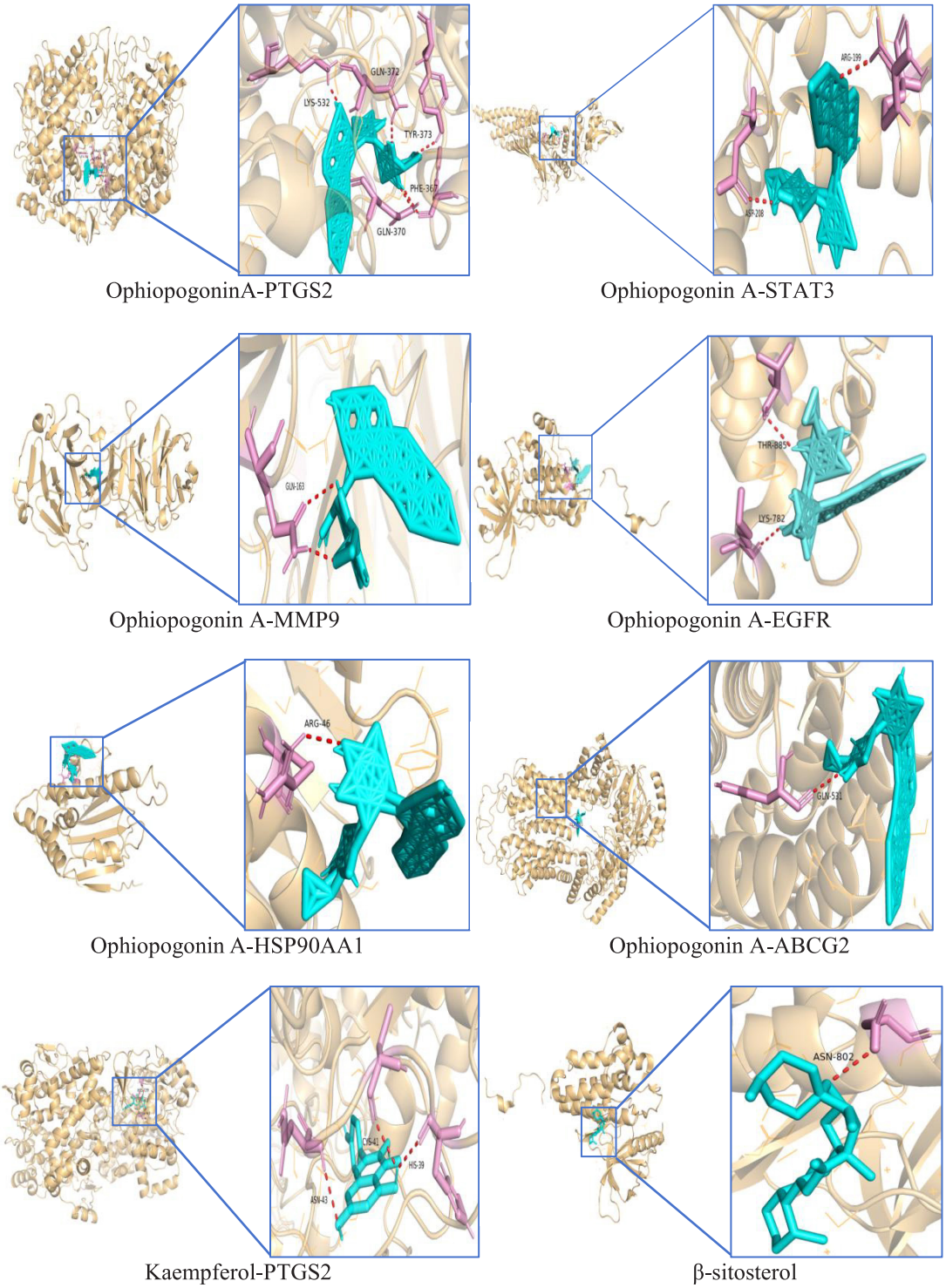


Figure 6. Molecular docking heatmap.



OphiopogoninA-PTGS2 Ophiopogonin A-STAT3

Ophiopogonin A-MMP9 Ophiopogonin A-EGFR

Ophiopogonin A-HSP90AA1 Ophiopogonin A-ABCG2

Kaempferol-PTGS2 β-sitosterol

Figure 7. Molecular docking diagram.

4. Discussion

Through analysis of database screening results, it was found that Mongolian medicine Lianqiao-4 has 39 potential targets for the treatment of renal toxicity. The main active ingredients of Lianqiao-4 include ophiopogonin A,

kaempferol, β-sitosterol, quercetin, and other components. Core targets such as PTGS2, ABCG2, HSP90AA1, MMP9, STAT3, and EGFR may play a role in the treatment of renal toxicity.

Mongolian medicine Lianqiao-4 is composed of four herbal medicines: Forsythia, *Polygonum bistorta*, *Clematis armandii*, and *Ophiopogon japonicus*. According to literature reports [7–10], kaempferol and quercetin belong to flavonoids and are present in all four herbal medicines. Beta-sitosterol and sitosterol, which belong to steroid compounds, are also found in all four herbs of Lianqiao-4. Ophiopogon japonicus contains various steroidal saponins, flavonoids, polysaccharides, and other components, among which ophiopogonin A is one of the steroidal saponin compounds [10].

According to the literature, ophiopogonin A, as one of the key active ingredients in Ophiopogon japonicus, not only exhibits antioxidant and anti-inflammatory effects but also effectively reduces the expression levels of inflammatory markers such as TNF-α, IL-1β, and IL-6 in renal tissue. This significantly alleviates hypoxia-induced apoptosis of renal tubular epithelial cells [11].

Both kaempferol and quercetin, flavonoid compounds, possess antioxidant and anti-inflammatory properties. Studies have revealed that kaempferol can reduce the activity of enzymes like MP-9 and COX-2, exerting an anti-tumor effect [12]. Kaempferol activates the AMPK/Nrf2 signaling pathway, effectively suppressing the oxidative stress state and the release of inflammatory factors in diabetic nephropathy rats, thereby promoting the recovery of renal function [13]. On the other hand, quercetin mitigates renal function damage and renal fibrosis by inhibiting the PI3K/Akt/NF-κB signaling pathway [14].

Research indicates that prostaglandin-endoperoxide synthase 2 (PTGS2), also known as cyclooxygenase 2 (COX2), is widely distributed in the body [15]. Under normal physiological conditions, the expression level of PTGS2 is very low. However, it can be secreted in large amounts when activated in pathological states, promoting the synthesis of prostaglandins and leading to inflammatory responses [16, 17]. Yang *et al.* [18] found that renal inflammatory responses can be reduced by inhibiting the expression of inflammatory cytokines such as cyclooxygenase 2 (COX2). Additionally, Guo *et al.* [19] discovered that parecoxib sodium, a specific inhibitor of COX-2, can effectively block the expression of COX-2 and thus inhibit inflammatory responses caused by acute kidney injury. Furthermore, COX2 plays a key role in inflammation and tumor development [20]. Signal transducer and activator of transcription 3 (STAT3) is a core member of the JAK/STAT signaling pathway and is closely associated with kidney diseases [21]. Sun *et al.* [22] found that polysulfides and hydrogen sulfide can provide protective effects against nephrotoxicity by inhibiting NF-kB-mediated renal inflammatory responses through sulfuration modification of STAT3 and IKKβ. Lee *et al.* [23] showed that inhibiting the activity of STAT3 can reduce renal tubular epithelial cell apoptosis and oxidative stress, thereby improving renal function. Epidermal growth factor receptor (EGFR) is a member of the ErbB family of receptor tyrosine kinases. Research has found that EGFR protein expression is a key factor in kidney diseases in various animal models, including glomerulonephritis, hypertensive nephropathy, and diabetic nephropathy [24]. According to Singh *et al.* [25], DAM17-mediated EGFR ligand shedding can activate EGFR signaling, promote the release of proinflammatory cytokines, and exacerbate renal inflammation. Studies have demonstrated that matrix metalloproteinase 9 (MMP9) releases inflammatory factors through the NF-kB pathway, causing glomerular injury [26], and thus inhibiting MMP9 can reduce renal injury [27].

KEGG pathway enrichment analysis revealed that the main pathways for Forsythia suspensa-4 to alleviate nephrotoxicity include lipid and atherosclerosis, chemical carcinogenesis - DNA adducts, and arachidonic acid metabolism. Among them, the metabolic process of the arachidonic acid pathway is mainly achieved through three

enzymes: cyclooxygenase, lipoxygenase, and cytochrome P450 enzymes [28]. Research has found that regulating the arachidonic acid metabolism pathway by inhibiting the activity of cyclooxygenase 2 (COX-2) and 5-LOX can effectively alleviate renal inflammation [29].

Molecular docking verified the core targets and components associated with nephrotoxicity in Forsythia suspensa-4. Active ingredients such as ophiopogonin A, kaempferol, β-sitosterol, and quercetin were found to tightly bind to core targets such as PTGS2, ABCG2, HSP90AA1, MMP9, STAT3, and EGFR. Studies have shown that ophiopogonin A can reduce the expression of inflammatory factors in renal tissue [11], and PTGS2 protein plays a key role in the treatment of nephrotoxicity [16–20]. Therefore, Forsythia suspensa-4 may have a protective effect on renal toxicity by inhibiting the expression of proteins such as PTGS2, STAT3, and MMP9, and its pathway may be the arachidonic acid metabolism pathway.

5. Conclusion

In summary, through network pharmacology and molecular docking studies, it is initially predicted that Forsythia suspensa-4 has a multi-component, multi-target, and multi-pathway mechanism of action in the treatment of nephrotoxicity. This study provides new targets and pathways for the experimental treatment of nephrotoxicity with Forsythia suspensa-4, but the exact mechanism of action still needs further validation in cellular and animal experiments.

Disclosure statement

The authors declare no conflict of interest.

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