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*Original Article*

# Qigui-Yishen decoction delays renal fibrosis in mice with chronic kidney disease by regulating TM and PAI-1

Xun Lu, Xiao-Wen Wan

*Department of Traditional Chinese Medicine, The Affiliated Suzhou Hospital of Nanjing Medical University, Suzhou 215008, Jiangsu, China*

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**Abstract:** Objective: To explore the mechanism of Qigui-Yishen decoction in delaying renal fibrosis in mice by regulating thrombin regulatory protein (Thrombomodulin, TM) and plasminogen activator inhibitor-1 (PAI-1) based on network pharmacology. Methods: The active ingredients of Qigui Yishen decoction and their target molecules associated with chronic kidney disease (CKD) were retrieved from websites and databases, sorted out, and screened, and the possible targets of Qigui Yishen decoction for reducing CKD renal fibrosis were predicted and analyzed. Forty Institute of Cancer research (ICR) rats were used to establish a unilateral ureteral obstruction (UUO) model, and divided into several groups: sham operation group, model group, high concentration decoction group (1 g/mL), low concentration decoction group (0.46 g/mL), and benazepril group (0.1 g/mL). At the end of the experiment, the levels of serum creatinine (Scr) and blood urea nitrogen (BUN) were detected. Masson staining was used to observe changes in the renal interstitial fibrosis index. Immunohistochemistry and western blot were used to detect the expressions of TM, PAI-1, transforming growth factor-β1 (TGF-β1) and collagen I (Col I) in kidney tissues, and the differences between groups were compared. Results: Qigui Yishen decoction contains 42 effective ingredients such as sitosterol, mannitol, and quercetin, with 662 drug targets and 16154 disease targets. Analysis revealed 570 potential targets, including TM4SF19, PAIP1, TGF-β1, and Col I-AI. Compared to the sham operation group, all treatment groups exhibited increased Scr and BUN levels ($P<0.05$) and enhanced renal interstitial fibrosis ($P<0.05$) after UUO model establishment. Moreover, immunohistochemical results showed significant increases in PAI-1, TGF-β1, and Col I (all $P<0.05$), and a significant decrease in TM expression ($P<0.05$). Compared to the model group, the high concentration decoction group, low concentration decoction group and benazepril group had no significant difference in Scr and BUN values ($P>0.05$), but the renal interstitial fibrosis index was lower ($P<0.05$). Also, the relative expressions of PAI-1, TGF-β1 and Col I in the kidney tissue of mice were decreased, while the relative expression of TM was increased ($P<0.05$). Conclusion: Qigi Yishen decoction has the characteristics of multiple components and multiple targets, and can play a role in delaying renal fibrosis by regulating the expression of PAI-1, TGF-β1, Col I, and TM.

**Keywords:** Renal fibrosis, thrombin regulatory protein, plasminogen activator inhibitor type-1, Qigui Yishen decoction

## Introduction

Chronic kidney disease (CKD) poses a significant public health challenge globally due to its high prevalence and increasing incidence [1]. Renal fibrosis is a central pathologic issue of CKD, primarily characterized by glomerular sclerosis and renal tubulointerstitial fibrosis. This fibrotic transformation is often associated with immune abnormalities and tissue inflammation, which promote the excessive accumulation of extracellular matrix (ECM) components [2, 3]. Therefore, addressing renal tissue inflammation and preventing renal fibrosis are critical to managing CKD.

Qigui Yishen decoction is a traditional Chinese medicine compound preparation that stops renal fibrosis, mainly composed of Angelica sinensis, Astragalus membranaceus, Euryale seed, Ginkgo biloba, Radix pseudorrhaeosa, and Atractyloides atractyloides. This decoction is recognized for its abilities to invigorate the kidney and spleen, enhance qi, and stimulate blood circulation with a national patentee (Patent No. ZL201310069692.0) [4]. Previous

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studies have found that Qigui Yishen decoction and its main components can regulate the transforming growth factor-β1 (TGF-β1) signaling pathway, reduce the abnormal accumulation of ECM components such as collagen I (Col I) and fibronectin (FN), reduce the apoptosis of renal tubular epithelial cells, and effectively delay the process of renal fibrosis [5]. Modern medicine has confirmed that the occurrence and development of kidney disease is related to coagulation and fibrinolysis abnormalities. TCM suggests that enhancing blood circulation could positively influence coagulation and fibrinolytic processes, thereby affecting renal fibrosis [6]. However, the active compounds and their mechanisms in Qigui Yishen decoction have not been fully identified. To bridge this knowledge gap, network pharmacology offers a promising approach. It uses computer software and biologic databases to explore the biological network relationships between disease-related genes and drug targets. It is especially suitable for the study of the mechanism of traditional Chinese medicine and compounds [7, 8]. Based on this, the present study used network pharmacology to analyze the active components and targets of Qigi-Yishen Decoction for anti-CKD renal fibrosis and its effects on renal pathology and fibrosis in mice by animal experiments.

## Materials and methods

### *Acquisition and analysis of the target of Qigui Yishen decoction in improving CKD*

The TCMSP database (https://old.tcmsp-e.com/tcmsp.php) was searched to query all the active ingredients of Angelica sinensis, Astragalus membranaceus, Euryale uryale, Cerasus chinensis, Radix pseudorrhoeae, Atractylodes atractylodes and other drugs. According to the ADME (Absorption, Distribution, Metabolism, and Excretion) parameters, the screening criteria were as follows: oral bioavailability (OB) ≥30%, drug-likeness (DL) ≥0.18. The molecules of the active ingredients were verified for molecular weight and structural formula using PubChem (https://pub-chem.ncbi.nlm.nih.gov/) database. Effective components were input as SMILES (Simplified Molecular Input Line Entry System) formulas into the SwissTargetPrediction database to identify potential drug targets. Only targets with a prediction probability greater than 0.1 were considered for further analysis. GeneCards database (https://www.genecards.org/) was searched with "chronic kidney disease", as a keyword to retrieve CKD related targets. A Venn diagram was generated using the Venn mapping web site (https://bioinfogp.cnb.csic.es/tools/venny/) to obtain a common target between the compounds and disease. Subsequent GO (Gene Ontology) annotation and KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway analyses were performed to understand the biological functions and pathways involved.

### *Animal experiments*

*Experimental animals:* Forty male Institute of Cancer Research (ICR) rats, weighing (21±2) g were provided by the Medical Laboratory Animal Center of Suzhou University, with the qualification number of SYXK - (su): 2022-0016. The animals were housed under controlled environmental conditions with a room temperature maintained at 22±2°C, and humidity between 55 to 65%. Adequate lighting and ventilation were ensured. The rats were acclimatized to these conditions with 3 days of prefeeding before the commencement of the experiment.

*Experimental drug and its preparation:* Qigui Yishen decoction: The ingredients Astragalus membranaceus 30 g, Radix pseudotumor 15 g, Eurylonia uralensis 30 g, cherry fructus chinensis 30 g, Angelica sinensis 15 g, Achyranthes bidentata 15 g, Ligusticum chuanxiong 15 g, fried Atractylodes atractylodes 15 g, hedyotis diffusa 30 g, cicatricae exuviae 12 g, licorice 6 g were decocted with distilled water to create two concentrations of the TCM solution: 1 g/ml of crude drugs (high concentration) and 0.46 g/ml (low concentration). The herbs were purchased from the Traditional Chinese Medicine Pharmacy of Suzhou Municipal Hospital North District, and decocted by Suzhou Tianling Decoction Piece Co., LTD.

*Western medicine:* Benazepril hydrochloride tablets (10 mg/tablet, Novartis Pharmaceuticals, batch number: H20030514) were dissolved in 100 ml distilled water to prepare a solution of 0.1 g/mL benazepril solution.

*Experimental reagents and instruments:* Main reagents: Immunohistochemical kit and Goat anti-mouse secondary antibodies for immunohistochemistry were supplied by Suzhou Yingfei

Trusted Biotechnology Co., LTD. (Batch numbers: G1216 for the kit and G1214 for the goat anti-mouse secondary antibody). Broad-spectrum secondary antibody was sourced from Shanghai Changdao Biotechnology Co., Ltd., Bathc number: D-3004; PAI-1 antibody, TM antibody, Col I antibody, and TGF-β1 antibody were provided by Wuhan Dr. De Biological Engineering Co., Ltd. (Lot numbers: BP4873 for PAI-1, EK1119 for TM, BA0533 for Col I, and BA0290 for TGF-β1). Masson Trichrome staining solution was obtained from Suzhou Yingfei Trusted Biotechnology Co., LTD., Batch number: G1006.

*Main instruments:* An upright optical microscope from OLYMPUS (CX41), a Mini-PROTEAN electrophoresis system and Mini Trans-Blot transfer system (Bio-Rad), SUNRISE absorbance microplate reader (TECAN, Switzerland), a centrifuge (Beijing Jingli Centrifuge Co., Ltd.) and an electronic analysis balance (Shanghai Jingke Balance Factory).

*Molding method:* Mice were anesthetized with 2% pentobarbital sodium. Under aseptic conditions, a midline abdominal incision was made to expose the peritoneum, which was then carefully incised to reveal the left kidney and ureter. The ureter was ligated with 5/0 silk suture, and the abdominal layers were subsequently closed in sequence. For the sham operation group, the abdomen was opened similarly, and the left ureter was exposed but not ligated. To clear the systemic circulation, 4°C normal saline was injected into the left ventricle while the right atrial appendage was incised to allow blood outflow. Immediately following the operation, the left kidney was dissected. Part of the kidney tissue was fixed in 4% paraformaldehyde for subsequent tissue staining and immunodetection, while the remainder was snap-frozen in liquid nitrogen and stored at -80°C for western blot analysis.

*Group and administration:* Forty mice were randomly assigned into a sham operation group (normal saline), a model group (normal saline), a high concentration decoction group (1 g/mL), a low concentration decoction group (0.46 g/mL), and a benazepril group (0.1 g/mL) with eight mice in each group. Starting from the day after surgery, treatments were administered by gastric lavage for 10 consecutive days. On day 11, the mice were sacrificed by cervical dislocation, and the left kidney was quickly dissected, the capsule was removed and rinsed with normal saline. Each kidney was longitudinally bisected; one half was stored in liquid nitrogen for molecular biologic assays, and the other half was fixed in 4% paraformaldehyde for histologic and immunohistochemical analysis.

*Observation indicators and detection methods:* 1) Serum creatinine (Scr) and blood urea nitrogen (BUN) were detected by ELISA kit. 2) Masson staining: The kidney tissues from each group were processed for routine paraffin embedding, sectioning, and deparaffinization. The sections were stained with various Masson's trichrome solutions, dehydrated, cleared, and mounted with neutral resin. This staining differentiates collagen fibers (stained blue) from muscle fibers, cellulose, and red blood cells (stained red). Morphology was assessed in 8-10 randomly selected glomeruli and associated tubulointerstitium per section, with the renal interstitial fibrosis index quantified using a semiquantitative score ranging from 0 to 3. 3) Immunohistochemistry: Renal tissues were fixed in paraformaldehyde, embedded in paraffin, and sectioned. The expression levels of TM, PAI-1, TGF-β1, and ColI were detected using the streptavidin-peroxidase (SP) method. Staining intensity was classified as follows: no staining (negative), clear brown-yellow staining (<10% of cells positive as "+", 10-50% of cells positive as "++", and >50% of cells positive as "+++"). Analysis was performed using pathologic graphic analysis software. 4) Western blot: The total proteins were extracted, quantified using the BCA method, and subjected to SDS-PAGE followed by transfer to a PVDF membrane. The membranes were incubated overnight at 4°C with the primary antibodies against TM, PAI-1, TGF-β1, and Col I, followed by 1-hour incubation at room temperature with HRP-labeled secondary antibodies. Protein bands were visualized using ECL exposure. The relative expression of target protein was calculated by the gray ratio of target protein to internal reference Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and the relative expression of target protein was = {target protein (optical density value)/internal reference (optical density value)} × 10n.

*Statistical processing*

SPSS27.0 was used to process the data. The measured data were expressed as mean ± SD. One-way analysis of variance, followed by the

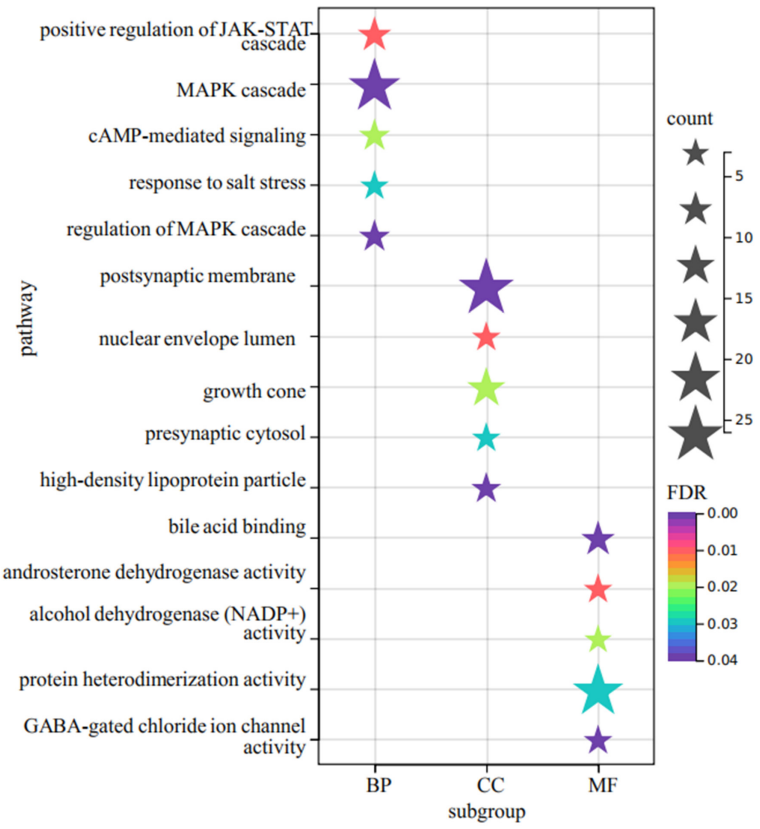


**Figure 1.** Bioinformatic analysis chart of Qigui-Yishen decoction for improving CKD - GO annotation.

LSD-t test was applied for the comparison among the groups. *P*<0.05 was considered significant.

## Results

*Bioinformatic analysis of the common targets of CKD and drugs*

Analysis of the data from the databases revealed 42 active drug ingredients, 662 drug targets, and 16154 disease targets, including sitosterol, stigmasterol, mannitol and quercetin. Intersection of these data yielded 570 targets. Gene symbols associated with these targets underwent GO and KEGG analyses to visualize the results (**Figures 1**, **2**).

*Comparison of Scr and BUN levels in the blood of mice among groups*

After modeling, the Scr and BUN levels of the model group, high concentration decoction group, low concentration decoction group and benazepril group were significantly higher than those of the sham group (all *P*<0.05); however, there was no significant difference among the four groups (*P*>0.05) (**Table 1**).

*Comparison of renal interstitial fibrosis index of mice among groups*

Compared to the sham operation group, the renal tissue of other four groups exhibited a significant increase in hematoxylin staining and renal interstitial fibrosis index (*P*< 0.05). Compared to the model group, the renal interstitial fibrosis indexes of the high concentration decoction group, low concentration decoction group and benazepril group was lower (*P*<0.05) (**Figure 3**).

*Comparison of the expression of Thrombomodulin, (TM), PAI-1, TGF-β1, and Col I in kidney tissues of mice among groups*

Immunohistochemical staining showed that compared to the sham operation group, the other four groups showed brownish yellow granules in the kidney tissues after model establishment (**Figure 4**). Notably, the high and low concentration decoction and benazepril groups exhibited lower expression of PAI-1, TGF-β1, and Col I and higher expression of TM compared to the model group, with significant differences (P<0.05).

*Comparison of protein levels of thrombomodulin (TM), PAI-1, TGF-β1, and Col I in the kidney tissues of mice among groups*

Western blot analysis showed that the relative expressions of PAI-1, TGF-β1, and Col I were increased, while thrombomodulin (TM) was decreased in the kidney tissues across all experimental groups compared to the sham operation group (P<0.05). Relative to the model group, the expressions of PAI-1, TGF-β1, and Col I were significantly reduced, and TM was increased in the high and low concentration

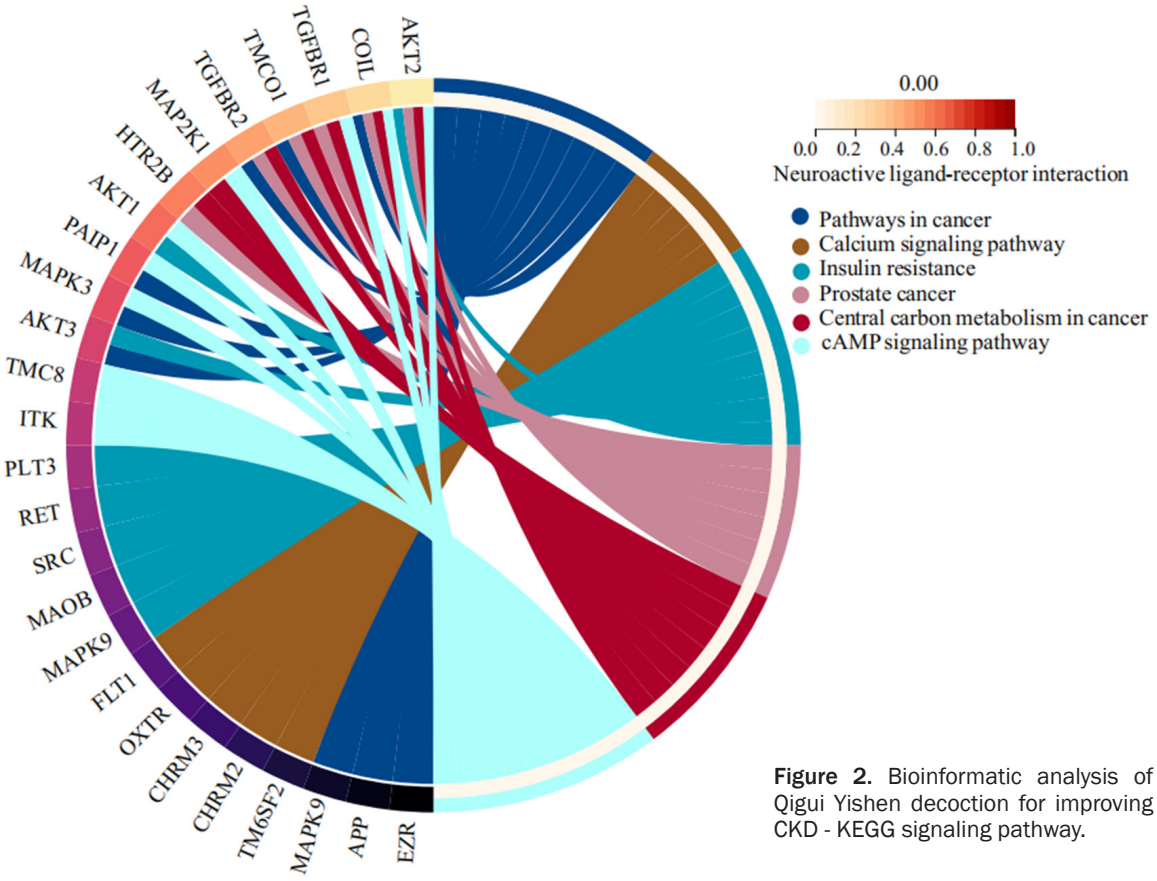


**Figure 2.** Bioinformatic analysis of Qigui Yishen decoction for improving CKD - KEGG signaling pathway.

**Table 1.** Comparison of Scr and BUN levels in mouse blood among groups

| Group | Number of animals (number) | Scr (umol/L) | BUN (mmol/L) |
|---|---|---|---|
| Sham operation group | 8 | 17.87±0.84 | 4.81±0.13 |
| Model group | 8 | 22.37±1.06# | 5.79±0.24# |
| High concentration group | 8 | 20.88±0.83# | 5.63±0.31# |
| Low concentration group | 8 | 22.20±0.71# | 5.64±0.17# |
| Benazepril Group | 8 | 21.38±0.91# | 5.60±0.29# |

Note: Compared with sham operation group, #$P$<0.05.

decoction and benazepril groups (P<0.05). Notably, the differences in expression levels between the low concentration and high concentration groups and the benazepril group were also significant (P<0.05), as shown in **Figure 5**.

## Discussion

Renal fibrosis is a fundamental concern of chronic kidney disease (CKD). Studies have shown that delaying renal fibrosis can effectively alleviate the progression of CKD [9]. The fibrotic process is complex, involving immune cells, pro-fibrotic mediators and parenchymal cells, and renal microvessels, many of which are related to inflammatory activation caused by kidney injury [10]. Understanding how to reduce the inflammatory state in kidney tissue and regulate fibrosis is of great significance to reduce CKD risk.

Western medical treatments for CKD typically involve pharmacotherapy with specific clinical indications. For example, myocardial and renal function impairments associated with CKD may be treated with immunosuppressants. Angiotensin Converting Enzyme Inhibitor (ACEI) can effectively reduce capillary pressure in glomeruli, prevent mesangial cell proliferation, and reduce ECM production [11]. ACEIs can weaken sympathetic nerve activity, improve vascular remodeling and insulin resistance function, and effectively protect the kidney. Benazepril hydrochloride, a common ACEI drug,

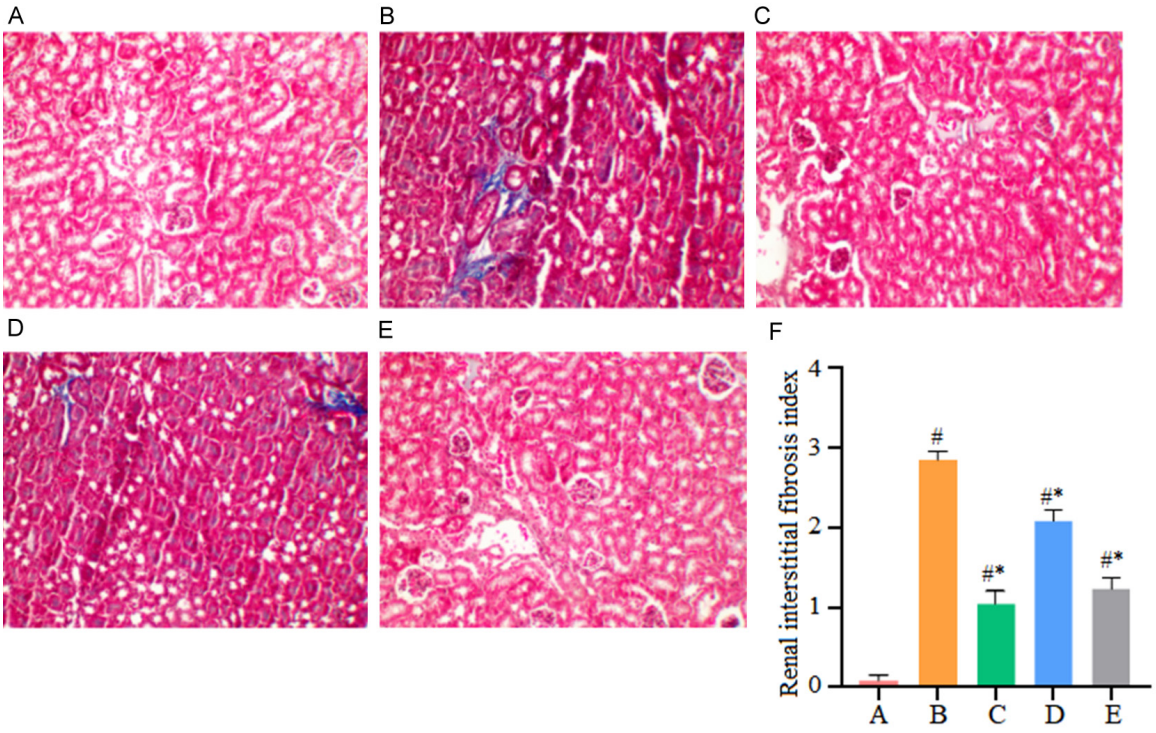


**Figure 3.** Comparison of Masson staining results (A-E) and renal interstitial fibrosis index of mice in each group (F). Note: Compared to the model group, $^{\#}P<0.05$; Compared to the model group, $^{*}P<0.05$. A: Sham operation group; B: Model group; C: High concentration group; D: Low concentration group; E: Benazepril group.

can effectively alleviate the course of renal fibrosis by inhibiting the effect of angiotensin II and reducing the production of TGF-β1 [12, 13]. However, these drugs can cause adverse reactions such as hyperkalemia and hypotension, which seriously affect the prognosis of patients and limit their clinical application.

From the TCM viewpoint, CKD-related kidney fibrosis, described as "kidney dysentery", stems from a deficiency in both the spleen and kidney and an excess in blood vessels [14]. The Qigui Yishen Decoction utilizes a carefully selected blend of herbs to treat conditions associated with renal and spleen deficiencies: Golden cherry seeds and euryale seeds serve as the primary herbs (Jun medicine), known for their ability to invigorate the spleen. Radix pseudostellariae, Astragalus membranaceus, and Atractylodes macrocephala are incorporated to nourish qi and strengthen the spleen [15]. Ligusticum, Angelica sinensis, and Achyranthes bidentata act as adjuvant herbs, enhancing blood circulation and clearing blockages in collaterals. Hedyotis diffusa is used to clear lower heat, while cicada slough is used to expel wind and further open collaterals. Achyranthes bidentata also has a role in tonifying the kidneys and dredging the collaterals, directing the effects of the herbs to the kidney meridian. Licorice harmonizes the various components, ensuring the formula promotes overall spleen and kidney health, enhances blood circulation, and clears collaterals [16]. However, the specific mechanism of action of the effective ingredients and anti-fibrotic or anti-inflammatory effects of Qi Gui Yi Shen Fang on CKD treatment remain unclear.

In this study, network pharmacology was used to explore the mechanism of Qigui Yishen Decoction in the treatment of CKD-related renal fibrosis. The results showed that Qigui Yishen decoction contained sitosterol, stigmasterol, mannitol, quercetin, and other active ingredients, which may function in the treatment of renal fibrosis. Sitosterol, with a similar structure to cholesterol, can reduce the production of pro-inflammatory factors, such as tumor necrosis factor (TNF) and interleukin (IL), and enhance the activity of anti-inflammatory factors, thereby reducing inflammation-induced

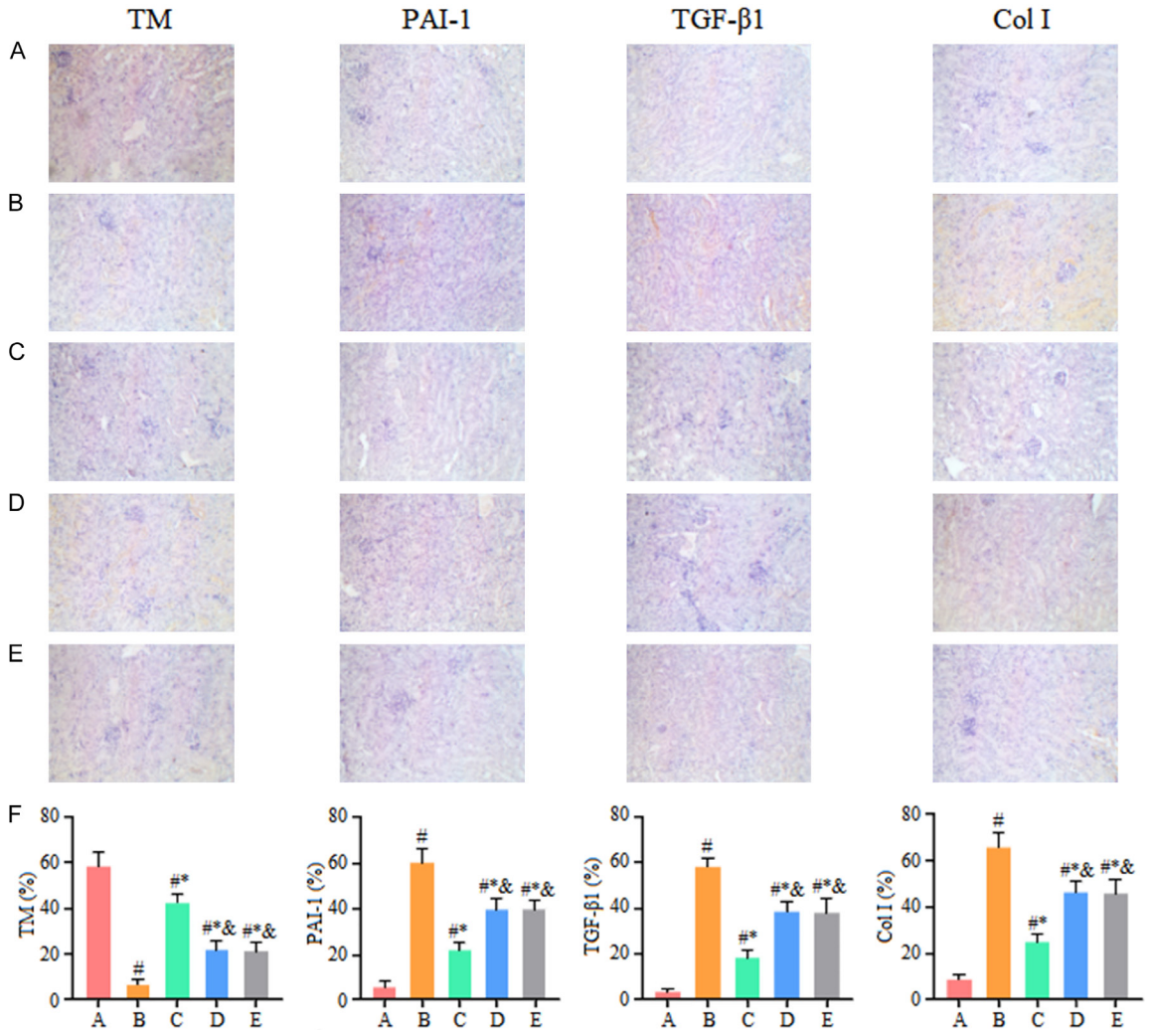


**Figure 4.** Immunohistochemical staining of mice kidney tissue (A-E, ×400) and expression levels of TM, PAI-1, TGF-β1, and Col I in mouse kidney tissue (F). Note: Compared to the sham operation group, $^{\#}P<0.05$; Compared to the model group, $^{*}P<0.05$; Compared to the Qigui Yishen high concentration group, $^{\&}P<0.05$. TM: thrombin regulatory protein; PAI-1: plasminogen activator inhibitor-1; TGF-β1: transforming growth factor-β1; Col I: collagen I. A: Sham operation group; B: Model group; C: High concentration group; D: Low concentration group; E: Benazepril group.

kidney damage. In addition, sitosterol is involved in regulating the activity of matrix metalloproteinases (MMPs) and their inhibitors (TIMPs), crucial in the synthesis and degradation of extracellular matrix [17]. Stigosterol may influence cell signaling pathways related to renal fibrosis, such as TGF-β signaling pathway and mitogen-activated protein kinase (MAPK) signaling pathway, thus regulating the proliferation and migration of renal cells [18]. Mannitol has an osmotic diuretic effect, which can promote the discharge of excess water from the body, reduce renal load, and potentially prevent the exacerbation of fibrosis due to prolonged renal strain [19]. Quercetin is one of the active extracted flavonoids with anti-inflammatory, anti-oxidative and anti-fibrotic activities [20]. The network pharmacology results revealed a multidirectional intersection between the targets of Qigui Yishen Decoction and the pathologic targets associated with CKD-induced renal fibrosis. This suggests that the decoction can exert a multifaceted therapeutic impact on CKD-related renal fibrosis through its diverse active ingredients and their multiple targets.

In this study, four specific targets, TM4SF19, PAIP1, TGF-β1, and Col I-AI, were identified as frequently involved in the pathway affecting the progression of renal fibrosis in CKD.

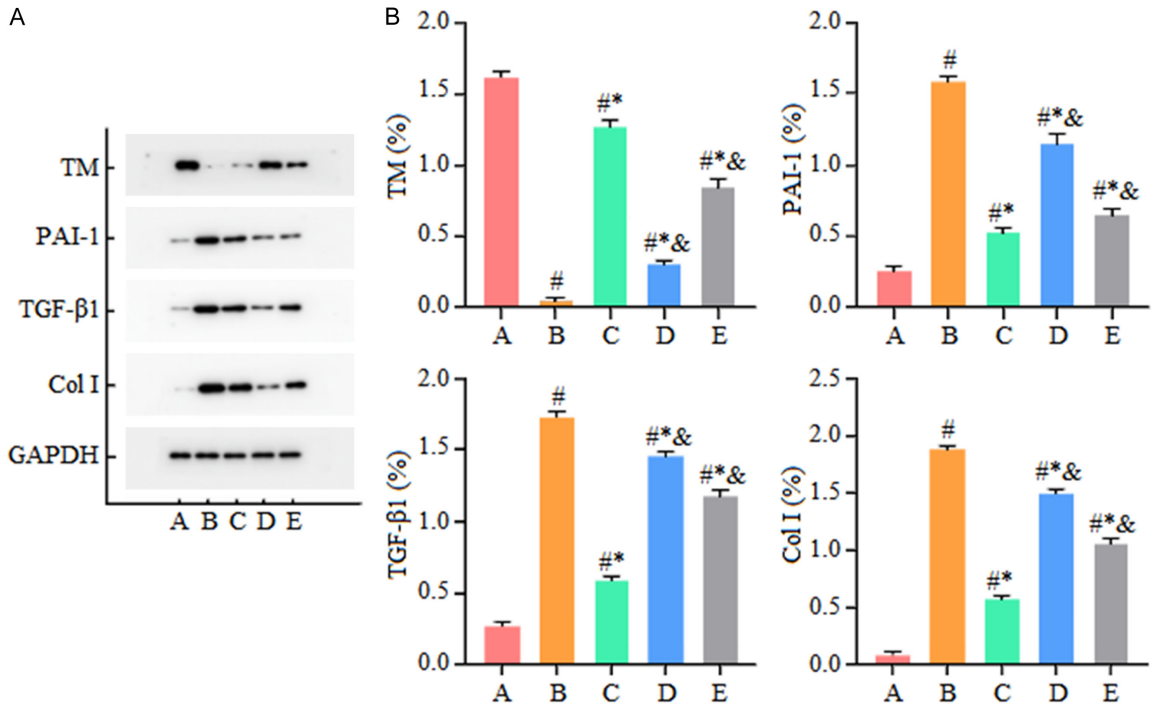


**Figure 5.** Western blot analysis (A) and the relative expression of TM, PAI-1, TGF-β1, and Col I in the kidney tissues of mice (B). Note: Compared to the sham operation group, $^{\#}P<0.05$; Compared to the model group, $^{*}P<0.05$; Compared to high concentration decoction group, $^{\&}P<0.05$. TM: thrombin regulatory protein; PAI-1: plasminogen activator inhibitor-1; TGF-β1: transforming growth factor-β1; Col I: collagen I; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase. A: Sham operation group; B: Model group; C: High concentration group; D: Low concentration group; E: Benazepril group.

Thrombomodulin (TM) is mainly distributed on the surface of vascular endothelial cells, with a lesser amount on the surface of mesothelial cells in other tissues such as pleura, pericardium, peritoneum, and arachnoid membrane. TM has a complex structure and participates in many physiologic processes. The mechanism of TM functions mainly involves the following two aspects. 1. Thrombin Binding and Protein C Activation: TM significantly enhances the activation rate of protein C (PC) when bound to thrombin, increasing it by up to 1000 times. Activated protein C (aPC), in turn, plays a crucial role in coagulation control by inactivating factors V and VIII, which helps reduce the risk of chronic diseases [21].

2. Interaction with EPCR and Downstream Effects: aPC can also bind to endothelial cell protein C receptor (EPCR) and affect the EPCR/APC-PAR-1 signaling pathway. This interaction activates sphingosine 1 phosphate receptor1 (S1P1) and triggers the downstream signaling process, thereby reducing the inflammatory response and vascular transparency [22]. Animal studies have also shown that damage to endothelial cells leads to an increase of TM cleavage products, which can affect the PC activation. Supplementing activated protein C (aPC) has been shown to reduce the inflammatory response and vascular permeability, reducing the mortality of endotoxemic mice [23].

PAI-1 is a major inhibitor of fibrinolytic activity in the blood circulation. Normally, PAI-1 expression is minimal in healthy kidney tissue across various species, including mice, rats, and humans. However, during an inflammatory response, PAI-1 expression rises notably in endothelial cells, mesangial cells, and tubular epithelial cells. PAI-1 is also a significant inhibitor of tissue plasminogen activator (tPA) and urokinase plasminogen activator (u-PA). It plays an important regulatory role in physiological processes such as thrombosis, ECM accumulation, and fibrinolysis [24, 25]. PAI-1 contributes to the progression of renal fibrosis primarily by inhibiting tPA and u-PA. This inhibition downregulates the plasminogen activation pathway, which suppresses the fibrinolytic process and

diminishes ECM breakdown. Consequently, this leads to ECM accumulation within the kidneys, exacerbating fibrotic conditions [26]. Extensive basic experiments have shown that TGF-β1 is a key driver mediating the proliferation, activation, and ECM deposition of myofibroblasts [27]. At the same time, TGF-β1 can also induce the transformation of renal tubular epithelial cells into mesenchymal cells, and the interstitial matrix produced by myofibroblasts. In addition, excessive and abnormal deposition of Col I is a hallmark of renal fibrosis. TGF-β1 can promote the synthesis of collagen (such as Col I) in cells, increase cell adhesion and chemotaxis [28], and then promote tissue fibrosis.

This study established an animal model of CKD renal fibrosis to validate the effects of Qigui Yishen Decoction on renal fibrosis and found that, after modeling, the Scr, BUN, and renal interstitial fibrosis index of each group of mice were significantly higher than those of the sham group. The results of immunohistochemistry showed that the relative expression of PAI-1, TGF-β1, and Col I were significantly increased, and the relative expression of TM was significantly decreased. After treatment, the relative expression of PAI-1, TGF-β1, and Col I in kidney tissue of mice in the high concentration decoction group, the low concentration decoction group and the benazepril group were decreased to varying degrees, and the relative expression of TM was increased. Scr and BUN are clinical indicators to reflect renal function. When renal function is damaged, both of them increase. Renal interstitial fibrosis index serves as a measure of the extent of renal fibrosis. In cases of CKD, severe kidney damage leads to significant renal fibrosis, resulting in elevated values of the renal interstitial fibrosis index [29, 30]. In this study, unilateral ureteral occlusion (UUO) was used to create a model of renal obstruction, which caused significant tissue edema and vascular compression, leading to ischemic injury and abnormal increases in clinical indicators of renal damage. At the same time, the enhanced inflammatory response in the kidney tissue exacerbated vascular intima damage and led to continuous TM lysis [31]. Therefore, the TM content in the kidney tissue of each group was decreased to varying degrees compared to the sham group. At the same time, with the aggravation of inflammation in renal tissue, TGF-β1 also promoted the accumulation of Col I by up-regulating the expression of PAI-1.

The results of this study indicate that Qigui Yishen decoction can regulate TGF-β1, hinder the synthesis of Col I, and thus delay the process of renal fibrosis, which is consistent with previous studies [32]. Also, the decoction can reduce the cleavage of TM in renal tissue, increase the expression of TM, reduce the expression of PAI-1, and play a role in reducing inflammation and fibrosis in renal tissues. These results reinforce the role of Col I, TGF-β1, TM, and PAI-1 as critical targets for Qigi-Yishen decoction in managing renal fibrosis. The study also revealed that a high concentration of Qigui-Yishen decoction outperforms benazepril, while the efficacy of its low concentration is comparable to that of the benazepril group. This suggests that Qigui-Yishen decoction is effective, highlighting the importance of determining the optimal therapeutic dosage.

Our study has limitations. First, TCM formulations usually contain a variety of chemical components, and the interactions and synergistic effects between these components may affect study outcomes and replicability. Second, the use of mice in this study poses a limitation due to possible variance in how different strains of mice respond to TCM. Individual differences in tolerance and metabolic processing of the compounds may affect the results. Therefore, future studies will further explore the specific mechanisms of different components of TCM on renal fibrosis and consider the interactions of other related signaling pathways. Animal models can be optimized, such as use of transgenic mice and disease model mice, or multi-omics technology can be considered to comprehensively analyze the effect of traditional Chinese medicine on renal fibrosis.

## Conclusion

Our study highlights the significant roles of thrombomodulin (TM) and plasminogen activator inhibitor-1 (PAI-1) in influencing the inflammatory state and fibrotic processes in renal tissue. Qigi-Yishen decoction can effectively reduce the TM cleavage in kidney tissues of CKD model mice, thereby increasing the expression of TM and reducing the expression of PAI-1, which collectively contribute to a delay in renal fibrosis progression.

In addition, by leveraging network pharmacology, we established a connection between the multiple components and targets of Qigui Yishen Decoction and the mechanisms of CKD-induced renal fibrosis. Our findings suggest that Qigui Yishen Decoction mitigates renal fibrosis through a multifaceted approach involving various components, targets, and pathways. This comprehensive action underscores the decoction's potential clinical importance in treating CKD, offering promising therapeutic avenues for managing this complex disease.

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## Disclosure of conflict of interest

None.

**Address correspondence to:** Xun Lu, Department of Traditional Chinese Medicine, The Affiliated Suzhou Hospital of Nanjing Medical University, No. 242 Guangji Road, Suzhou 215008, Jiangsu, China. Tel: +86-15862399704; E-mail: lx_305@163.com

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