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Research Article

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Jinkui Shenqi Pill and Its Active Component Diosgenin Mitigate UUO-Induced Renal Fibrosis Via Targeting the PI3K/AKT Axis

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Abstract

Background: *Chronic kidney disease (CKD) may result in renal fibrosis. It is a huge health crisis all over the world without any cure for it until now. JKSQ is a well-known TCM formula used clinically for CKD. JKSQ is used on the basis of warming the kidney and augmentation of deficiency, but the mechanisms of protection of renal fibrosis remain unknown. This study aims to completely understand the antifibrotic activity of JKSQ, as well as to seek out the active major parts and to learn about the parts and to see how their function plays in the PI3K/AKT signal pathway.*

Methods: *The chemical profile of JKSQ was characterized using UHPLC-Q-Exactive-Orbitrap-MS. Anti-fibrotic effects were assessed in a unilateral ureteral obstruction (UUO) rat model. Use network pharmacology and molecular docking to forecast active components, goals, and pathways. And the predicted one, the key component DIO, was proved both in vivo and in vitro. Fibrosis markers, inflammatory and oxidative stress indicators, and PI3K/AKT pathway activation were examined via histopathology, qRT-PCR, Western blotting, and ELISA. Pathway specificity was tested using the PI3K agonist 740Y-P.*

Results: *JKSQ administration significantly alleviated renal pathological damage, collagen deposition, and expression of fibrosis markers in UUO rats, while also reducing inflammation and oxidative stress. Network pharmacology and molecular docking results show that the PI3K/AKT pathway plays an important role, with AKT1, IL-6, TNF, and TP53 as core targets, and DIO exhibiting the strongest binding affinity. Both JKSQ and DIO blocked PI3K/AKT activation in vivo and in vitro, and this was reversed by 740Y-P, confirming pathway dependency.*

Conclusions: *JKSQ mostly stops kidney fibrosis from growing by messing with the PI3K/AKT signal pathway. The UHPLC-MS shows quite a lot of bioactivity segments were there in JKSQ; one majorly active element found was diosgenin when it was connected to this anti-fibrotic activity. These findings clarify the material basis and mechanism of JKSQ, providing a scientific rationale for its potential use in the clinical management of renal fibrosis.*

Keywords: Jinkui Shenqi Pill, Renal Fibrosis, Network Pharmacology, PI3K/AKT Signaling Pathway, Diosgenin

1. Background

Chronic kidney disease (CKD) has an increasing effect on worldwide population health and survival. CKD, according to the global incidence, is around 9.1%, and it is one of the leading causes of chronic noncommunicable disease deaths. [1]. Hemodialysis and kidney transplantation are the primary treatments for end-stage renal disease (ESRD) [2]. However, they incur a significant financial burden and negatively influence the patient's life.

When CKD gets progressively worse, it's usually linked to RF, where too much ECM material piles up first and then scars off [3]. Renal fibrosis mainly refers to the nephron loss process [4], inflammation [5], myofibroblast activation [6], and extracellular matrix deposition [7], ultimately leading to kidney tissue destruction and severe functional impairment. Currently, there is no anti-fibrotic therapy specifically targeting CKD; therefore, effective treatments to delay the progression of renal fibrosis are urgently needed.

Chinese herbal compound formulations are increasingly recognized for their therapeutic potential in chronic kidney disease and renal fibrosis, offering distinct advantages, including multiple components, multiple targets, and minimal toxic side effects [8], which has resulted in their increased usage. Jinkui Shenqi pill (JKSQ), a classic Chinese herbal formula from "Jin Kui Yao Lue" by Zhang Zhongjing, was developed in accordance with TCM principles and extensive clinical practice.

It consists of 8 herbs: Rehmannia glutinosa (Gaertn.) DC., Cornus officinalis Siebold & Zucc., Dioscorea polystachya Turcz., Alisma plantago-aquatica L., Wolfiporia cocos (F.A.Wolf), Paeonia × suffruticosa Andrews, Cinnamomum cassia (L.), and Aconitum carmichaelii Debx, making it a famous prescription recognized for improving blood circulation, dispersing blood stasis, promoting urination, activating blood and dredging meridians, invigorating yang, and warming kidneys [9]. Substantial evidence supports the high efficacy of JKSQ in treating kidney diseases.

Ma Chao et al. demonstrated that integrating JKSQ with conventional Western medicine was superior to conventional therapy alone in improving renal function and alleviating clinical symptoms in stage III-IV diabetic nephropathy patients [10]. Wang Shi-Min et al. found out that JKSQ exerted an inhibitory effect against renal injury in long-term renal failure rats by suppressing inflammatory processes and oxidative damage [11]. In addition, JKSQ has shown some improvement in diseases such as chronic glomerulonephritis [12] and lupus nephritis [13]. Therefore, we believe that JKSQ has the potential to improve renal fibrosis.

The concept of holism is central to the treatment of various diseases in TCM. Network pharmacology is a systems biology approach that analyzes the pharmacological effects of drugs on multiple targets and pathways. It can provide a bioinformatics network that demonstrates the interaction between disease genes and drug target genes, thereby elucidating the interactions between drugs and diseases [14], thereby offering a holistic view to elucidate drug-disease interactions, visualize mechanisms of action, and guide innovative drug development. To find out the mechanism of JKSQ against renal fibrosis, the current study used a network pharmacology approach, thereby offering novel scientific insights and perspectives for TCM-based chronic kidney disease therapy.

2. Methods

2.1. Materials

JKSQ was purchased from Beijing TRT Group, China. Diosgenin (Cat No. T2867, purity >99%) was purchased from TargetMol, Shanghai, China. TGF-β1 (Cat No. 100-21) was purchased from PeproTech, USA. 740Y-P (Cat No. HY-P0175) was purchased from MedChemexpress, USA.

2.2. The UHPLC-Q Exactive-Orbitrap-MS Preparation for Samples

The JKSQ sample was made by taking an exact amount of 100 mg of dried extract and extracting it with 15 ml of 70% aqueous methanol. The mixture was subjected to 30 minutes of ultrasonic time at 300 W and 40 kHz to dissolve, centrifuged at 12,000 rpm for 15 minutes, and the insoluble part was removed. The supernatant was filtered using a 0.22 μm filter, and the filtrate was analyzed via UHPLC-Orbitrap-MS/MS.

Chromatographic separation was performed using an ultimate HPLC system (Thermo Fisher Scientific, Waltham, MA, USA) equipped with an ACQUITY HSS T3 column (100 mm × 2.1 mm, 1.8 μm; Waters, Milford, MA, USA). Mobile phase: 0.1% formic acid aqueous solution (A), 0.1% formic acid in acetonitrile (B). Flow rate was 0.30 mL/min, gradient 0-5 min 97% A, 5-11 min 92-70% A, 11-20 min 70-20% A, 20-21 min 20-5% A, 21-27 min 5% A, and 28-34 min 97% A.

Mass spectrometric detection was done using the HESI source Q Exactive Focus Orbitrap MS (Thermo Fisher Scientific, USA). Positive as well as negative ion spectrums were taken at 100-1000 m/z with spray voltage 3.5 kV for positive and 3.0 kV for negative. The capillary and source were kept at 350°C and 360°C, while sheath and auxiliary gas (N_2) pressures were 50 psi and 10 psi, respectively. Sodium trifluoroacetate was employed for accurate mass calibration. Full-scan data-dependent MS/MS (Full MS-ddMS^2) acquisition was performed with a resolution of 35,000 for Full MS and 17,500 for ddMS^2, covering a scan range of m/z 100–1500. Normalized collision energies were stepped at 20, 40, and 60 eV. Compound identification was achieved by integrating second-order fragment ion data with MassFrontier software, the mzCloud online database, the mzVault local library of traditional Chinese medicine components, literature data, and authentic reference standards.

2.3. Animal Experiment

Male Sprague Dawley rats with a body weight of 200-220 g,

provided by Zhejiang Vital River Lab Animal Technology Co., were subjected to 1 week of acclimation and kept under SPF conditions (21 ± 1°C, 60% ± 5%) at a regular light-dark cycle (12:12). All the animal experiments were approved by the Zhejiang University IACUC-23-426. Rats were randomly assigned to 4 equal-sized groups (n=6): Sham group, UUO group, JKSQ-L group (1.8 g/kg), and JKSQ-H group (3.6 g/kg). The doses of JKSQ were determined based on the clinical human equivalent dose converted into the rat equivalent dose by the body surface area.

JKSQ was administered orally by gavage daily for 5 consecutive days, once daily. On day 6, anesthesia was induced by an intraperitoneal injection of 30 mg/kg sodium pentobarbital. Subsequently, a midline abdominal incision was performed to expose and separate the left ureter. Then the ureter was ligated near the renal end using 3-0 silk sutures and transected between the two ligation sites. In sham group rats only anesthesia was done and ureter separation was done but no ligation and transection of ureter was done. JKSQ was administered to all treatment groups beginning on day 1; the sham and UUO groups were similarly gavaged with 0.5% CMC-Na,, daily. Terminal collection of blood and renal tissues was performed 14 days after model establishment.

Another cohort of twenty rats (n=5) was randomly assigned to the following four groups: Sham, UUO, DIO-L (30 mg/kg), and DIO-H (60 mg/kg). The renal fibrosis model was constructed using the same method as described above. DIO giving to the treatment groups began on the first postoperative day, while the sham and UUO groups were gavaged with 0.5% CMC-Na once a day. Terminal collection of blood and renal tissues was performed 14 days after model establishment.

2.4. Renal Histopathology

After the kidney tissue was treated by 4% paraformaldehyde, it was dehydrated by a graded ethanol series, paraffin-embedded, and cut into 4 μm thickness, the remainder of the sections were stained with Hematoxylin and Eosin (H&E) and Masson's Trichrome stain for histology of renal injury and fibrosis. Renal tubular injury was graded as per tubular dilatation, necrosis, and cast deposition: 0 points, no injury; 1 point, <25% injury; 2 points, 25-50% injury; 3 points, 50-75% injury; 4 points, greater than 75% injury. The renal fibrosis area was examined by ImageJ 1.8.0 after Masson staining.

2.5. RNA Extraction and QRT-PCR

The total RNA of renal tissues and NRK-49F cells was extracted using Trizol reagent (Invitrogen, USA). RNA concentration and purity were assessed spectrophotometrically, with A260/A280 ratios between 1.8 and 2.0 indicating acceptable quality. Reverse transcribe one microgram of total RNA into the cDNA with TransScript First-Strand cDNA Synthesis SuperMix (TransGen Biotech Co., Ltd., China). Quantitative RT-PCR was performed at a final reaction volume of 20 μL using the QuantStudio 6 Flex Real-Time PCR System (Applied Biosystems, USA) with SYBR Green Supermix (Bio-rad, USA). The thermal cycles were as follows: 95°C for 30 s initial denaturation, followed by 40 cycles of 95°C for 5 s and 60°C for 30 s.. A melting curve analysis was performed to verify the specificity of the amplification. The amplification rates of each primer pair were verified from 95% to 105%. Gene expression was determined via the 2−ΔΔCt method and normalized to the endogenous reference gene Gapdh. Primer sequences used are in Table 1.

Gene	Primer Sequence (5'–3')
GAPHD	F: AGACAGCCGCATCTTCTTGT R: TCCCGTTGATGACCAGCTTC
α-SMA	F: CATCCGACCTTGCTAACGGA R: AGTCCAGCACAATACCAGTTGT
Col1a1	F: GGAGAGAGCATGACCGATGG R: AAGTTCCGGTGTGACTCGTG
FN1	F: AACGAGGAGGATGTGGCAGAG R: TACACGCTGGAGACACTGACTAAG
IL-6	F: AGAGACTTCCAGCCAGTTGC R: TGCCATTGCACAACTCTTTTC
TNF-α	GATCGGTCCCAACAAGGAGG R: CTTGGTGGTTTGCTACGACG
AKT1	F: ATCCCTTCCTTACAGCCCTCA R: AATCTCCGCACCGTAGAAGC
TP53	F: CCCAGGGAGTGCAAAGAGAG R: GTCTTCGGGTAGCTGGAGTG

Table 1: Primer Sequences

2.6. Western Blotting

The proteins from kidney tissue and cells were extracted with RIPA buffer with protease inhibitors and PMSF. Protein extract was performed by SDS-PAGE gel 7.5% running and then transferred to a PVDF membrane. The membranes were blocked with 5% non-fat milk at room temperature for 1.5 hours and incubated overnight

with the primary antibodies at 4°C. The primary antibodies included GAPDH (Diag Biotechnology, db11729, 1:2000), α-SMA (CST, 19245, 1:1000), FN1 (ProteinTech, 15613-1-AP, 1:2000), p-AKT (CST, 4060, 1:1000), t-AKT (CST, 4691, 1:1000), and PI3K (CST, 4257, 1:1000). After incubation with suitable secondary antibodies, the immunoreactive bands were visualized, and the imaging was detected with ECL for semi-quantitative detection. The semi-quantitative detection was carried out with ImageJ 1.8.0.

2.7. Network Pharmacological Analysis

We used the TCMSP database and analysis platform (https://old.tcmsp-e.com/tcmsp.php) to get all the chemical constituents of each TCM in JKSQ. We chose and defined the potential active ingredients on the basis of the standard values of OB≥30% and DL≥0.18. Search "renal fibrosis" in GeneCards and OMIM databases for genes related to renal fibrosis. A Venn diagram was used to identify the intersection between the active ingredients of JKSQ and genes related to renal fibrosis. The active ingredients were compared with the UniProt database. Interaction proteins were retrieved from the STRING database to construct a protein-protein interaction (PPI) network, and Cytoscape software was used for visualization. Key targets were selected based on their degree value. Biological information enrichment analysis was performed using the Metascape database, including GO analysis and KEGG pathway biological processes (BP), molecular functions (MF), and cellular components (CC), with a P-value < 0.05 as the screening criterion.

2.8. Molecular Docking

The most active ingredients' 3D structures were obtained from the PubChem database, and the targeted protein 3D structures were obtained from the RCSB database. Through the use of AutoDock Vina software for molecular docking, a binding affinity less than -5 kcal/mol was considered good binding activity between the protein and the compound. The docking results of the compound and the protein were visualized using PyMol software.

2.9. Preparation of JKSQ-Containing Serum

Male SD rats were randomly assigned to two groups (n=6) for the blank serum group and the JKSQ-containing serum group, respectively. The JKSQ-containing serum group received JKSQ (3.6 g/kg) by oral gavage once a day for 7 days, while the blank serum group received an equal volume of 0.5% CMC-Na solution following the same regimen. After the last administration, we collected the blood after an hour from the abdominal aorta. Then, the blood was centrifuged to obtain serum. The serum was heat-inactivated at 56°C for 30 minutes, followed by filtration through a 0.22 μm membrane. The filtered serum was aliquoted into cryotubes and stored at -80°C.

2.10. Cell Culture and Treatment

The NRK-49F cell line was sourced from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). The NRK-49F cells were cultured in DMEM/F12 medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin, and the cells were maintained in a 37°C, 5% CO_2 humidified cell incubator. Cells from passages 5 to 15 were used for all in vitro experiments. The cells were randomly divided into four groups: the control group, the TGF-β1 group, the TGF-β1+JKSQ group, and the TGF-β1+DIO group. The control group, TGF-β1 group, and TGF-β1+DIO group were cultured in medium supplemented with 10% blank serum, while the TGF-β1+JKSQ group was cultured in medium supplemented with 10% JKSQ-containing serum. The concentrations of DIO and TGF-β1 were 10 μM and 10 ng/ml, respectively. Finally, we used the PI3K agonist 740 Y-P (20 μM) in TGF-β1-treated NRK-49F cells. The cells were randomly divided into six groups: the control group, the TGF-β1 group, the TGF-β1+JKSQ group, the TGF-β1+JKSQ+740 Y-P group, the TGF-β1+DIO group, and the TGF-β1+DIO+740 Y-P group.

2.11. Statistical Analysis

Statistical analyses for all data were performed by GraphPad Prism 8.0. All numbers represent mean±SEM. We used t-tests for comparing two groups, and we used a two-way analysis of variance (ANOVA) for comparisons among three or more groups. Statistical significance was set at $p < 0.05$ (*/$P < 0.05$, **/$P < 0.01$, ***/$P < 0.001$). Differences with $p > 0.05$ were considered not significant (ns).

3. Results

3.1. Identification of the Chemical Composition in JKSQ

fragmentation behavior of diosgenin (compound 76) was analyzed to illustrate the identification process. In the positive ionization mode, a protonated molecular ion was detected at m/z 415.32025 $[M+H]^+$ (corresponding to $C_{27}H_{42}O_3$). The MS/MS spectrum showed a predominant fragment at m/z 271.0 $[M-C_8H_{16}O_2-H]+$ resulting from the neutral loss of $C_8H_{16}O_2$ from the precursor ion, followed by a further loss of H_2O to generate a fragment at m/z 253.0 $[M-C_8H_{16}O_2-H_2O-H]+$. Compound 76 was unambiguously identified as diosgenin when compared to an authentic reference standards. The TICs of JKSQW in positive and negative ion mode are shown in Fig. 1, and the information for each compound, along with its name, formula, m/z, and source, is shown in Table 2.

NO	Identification	Formula	RT (min)	Adduct	Detected mass (m/z)	Mass Error (ppm)	MS/MS fragments (m/z)	Compound Class
1	Adenine	$C_5H_5N_5$	1.06	M+H	136.06189	0.88	109.05074, 109.05074	Alkaloid
2	Adenosine	$C_{10}H_{13}N_5O_4$	1.63	M+H	268.10413	0.36	136.06189	Nucleoside
3	Guanine	$C_5H_5N_5O$	1.87	M+H	152.05673	0.28	110.03535,135.03032, 135.03032	Alkaloid
4	Guanosine	$C_{10}H_{13}N_5O_5$	1.87	M+H	284.09897	0.07	110.03503, 152.05676	Nucleoside
5	L-Phenylalanine *	$C_9H_{11}NO_2$	3.48	M+H	166.08636	0.63	120.08102, 149.05989	Amino Acid
6	Rehmannioside D	$C_{27}H_{42}O_{20}$	4.02	M-H	685.21674	0.21	685.21851, 341.10788, 179.05484	Iridoid Glycoside
7	Melittoside	$C_{21}H_{32}O_{15}$	4.20	M+NH_4	542.20758	-0.68	165.05469, 119.04916	Iridoid Glycoside
8	7-Methoxycoumarin	$C_{10}H_8O_3$	5.45	M+H	177.05469	0.38	121.06502, 149.05983	Coumarin
9	Ajugol	$C_{15}H_{24}O_9$	5.55	M+HCO_2	393.13889	-3.42	347.13309	Iridoid Glycoside
10	L-Abrine	$C_{12}H_{14}N_2O_2$	6.75	M+H	219.11284	0.18	188.07063, 132.08092	Amino Acid
11	8-O-Acetylharpagide	$C_{17}H_{26}O_{11}$	8.46	M+NH4	424.18118	-0.37	167.07056, 149.05983	Iridoid Glycoside
12	Morroniside	$C_{17}H_{26}O_{11}$	8.46	M+HCO^2	451.14429	-3.15	243.08621	Iridoid Glycoside
13	Bullatine G	$C_{22}H_{31}NO_3$	8.63	M+H	358.23770	0.09	340.22702	Diterpenoid Alkaloid
14	Oxypaeoniflorin	$C_{23}H_{28}O_{12}$	8.74	M+NH4	514.19202	0.23	179.07028, 317.10162	Monoterpene Glycoside
15	Cianidanol	$C_{15}H_{14}O_6$	8.76	M+H	291.08630	-0.02	139.039, 123.04425	Flavonoid
				M-H	289.07083	-3.21	245.0808	
16	Brevifolincarboxylic Acid	$C_{13}H_8O_8$	9.11	M+H	293.02924	0.16	219.02885, 191.03406	Phenolic Acid
17	Procyanidin B1	C30H26O12	9.36	M-H	577.13428	-1.51	407.07559, 289.07095	Flavonoid
				M+H	579.14972	0.03	301.07068, 409.09195	
18	Loganin	$C_{17}H_{26}O_{10}$	9.74	M+NH_4	408.18628	-0.34	179.07033, 109.06501	Iridoid Glycoside
				M+COOH	435.14923	-3.61	227.09138	
19	Verbenalin	$C_{17}H_{24}O_{10}$	9.75	M+HCO_2	433.13422	-2.16	225.07593	Iridoid Glycoside
20	Ferulaldehyde	$C_{10}H_{10}O_3$	9.76	M+H	179.07024	-0.19	161.05957, 147.04437	Phenylpropanoid
21	Asarylaldehyde	$C_{10}H_{12}O_4$	9.78	M+H	197.08087	0.16	151.03923	Phenylpropanoid
22	Sweroside	$C_{16}H_{22}O_9$	9.80	M+H	359.13364	-0.07	197.08095, 127.03915	Iridoid Glycoside
23	Epicatechin *	$C_{15}H_{14}O_6$	9.83	M+H	291.08630	-0.02	139.039, 123.04425	Flavonoid
				M-H	289.07083	-3.21	245.0808	
24	(+)-Magnoflorine	$C_{20}H_{24}NO_4$	9.94	M+	342.16995	-0.08	237.09041, 297.11206	Alkaloid
25	Purpureaside C	$C_{35}H_{46}O_{20}$	10.04	M-H	785.24872	-2.87	623.21826	Iridoid Glycoside
				M+NH_4	804.29083	-1.54	325.09161, 163.03894	
26	Talatisamine	$C_{24}H_{39}NO_5$	10.31	M+H	422.28992	-0.43	390.26343, 358.23715	Diterpenoid Alkaloid
27	Paeoniflorin	$C_{23}H_{28}O_{11}$	10.44	M+NH_4	498.19687	-0.24	179.07033, 151.07544	Monoterpene Glycoside
28	Ellagic Acid	$C_{14}H_6O_8$	11.30	M+H	303.01352	-0.07	201.01805, 275.01855	Tannin
				M-H	300.99808	-3.03	245.00816, 257.00839	
29	(-)-Catechin Gallate	$C_{22}H_{18}O_{10}$	11.34	M-H	441.08170	-2.32	289.07117	Flavonoid
30	Rosarin	$C_{20}H_{28}O_{10}$	11.34	M+NH_4	446.20193	-0.30	117.07018	Phenylpropanoid
31	Caffeic Acid	$C_9H_8O_4$	11.41	M+H	181.04945	-0.48	117.03371, 135.04414	Phenolic Acid
32	Verbascoside	$C_{29}H_{36}O_{15}$	11.40	M-H	623.19611	-3.26	461.16513	Phenylpropanoid Glycoside
				M+NH_4	642.23834	-1.41	163.03894, 325.09164	
33	Isoquercitrin	$C_{21}H_{20}O_{12}$	11.42	M-H	463.08664	-3.37	300.02667, 271.02393	Flavonoid Glycoside
34	Cynaroside	$C_{21}H_{20}O_{11}$	11.45	M-H	447.09219	-2.43	285.03943	Flavonoid Glycoside
35	1,2,3,4,6-Pentagalloylglucose	$C_{41}H_{32}O_{26}$	11.49	M-H	939.10773	-3.38	313.05624, 617.0777	Tannin
36	Plantamajoside	$C_{29}H_{36}O_{16}$	11.53	M-H	639.19104	-3.16	477.16052	Phenylpropanoid Glycoside

				$M+NH_4$	658.23303	-1.71	163.039, 325.09198	
37	Aurantio-Obtusin Beta-D-Glucoside	$C_{23}H_{24}O_{12}$	11.56	M+H	493.13391	-0.28	331.08115	Anthraquinone Glycoside
38	Taxifolin	$C_{15}H_{12}O7$	11.62	M-H	303.05029	-2.43	275.05527, 241.04971	Flavonoid
39	Pinoresinol 4-O-Glucoside	$C_{26}H_{32}O_{11}$	11.90	M-H	519.18597	-2.32	357.13348	Lignan Glycoside
40	Apigenin	$C_{15}H_{10}O_5$	12.07	M+H	271.06006	-0.15	153.01854	Flavonoid
41	Rhoifolin	$C_{27}H_{30}O_{14}$	12.07	M-H	577.15503	-2.17	413.08801	Flavonoid Glycoside
				M+H	579.17072	-0.20	271.06003, 153.01849	
42	Cornuside	$C_{24}H_{30}O_{14}$	12.16	M-H	541.15515	-2.09	309.06104	Iridoid Glycoside
43	Vanillin	$C_8H_8O_3$	12.24	M+H	153.05467	0.34	125.06016	Phenylpropanoid
44	Apigenin-7-O-Beta-D-Glucoside	$C_{21}H_{20}O_{10}$	12.24	M+H	433.11282	-0.22	153.01797, 271.05997	Flavonoid Glycoside
				M-H	431.09732	-2.43	268.03693	
45	Eriodictyol	$C_{15}H_{12}O_6$	12.48	M+H	289.07068	0.06	163.039, 153.01831	Flavonoid
46	Coumarin	$C_9H_6O_2$	13.11	M+H	147.04401	-0.36	103.05457	Coumarin
47	Apigenin 7-Glucuronide	$C_{21}H_{18}O_{11}$	13.11	M+H	447.09192	-0.61	271.06	Flavonoid Glycoside
				M-H	445.07690	-1.63	269.04489	
48	Berberine	$C_{20}H_{18}NO_4$	13.68	M+	336.12292	-0.31	278.08173, 292.09671	Alkaloid
49	Arglabin	$C^{15}H_{18}O_3$	13.83	M+H	247.13280	-0.29	187.11188, 131.08557	Sesquiterpene
50	Quercetin *	$C_{15}H_{10}O_7$	14.10	M+H	303.04987	-0.21	153.01836, 165.01808	Flavonoid
51	Wogonoside	$C_{22}H_{20}O_{11}$	14.29	M+H	461.10767	-0.38	285.07565	Flavonoid Glycoside
52	Mesaconitine	$C_{33}H_{45}NO_{11}$	14.46	M+H	632.30585	-1.10	572.28607, 105.03381	Diterpenoid Alkaloid
53	Benzoylpaeoniflorin	$C_{30}H_{32}O_{12}$	14.68	M+NH4	602.22247	-1.21	179.07021, 249.07558	Monoterpene Glycoside
54	Pedunculoside	$C_{36}H_{58}O_{10}$	14.94	M+COOH	695.39972	-2.13	487.34183	Triterpenoid Saponin
55	Naringenin *	$C_{15}H_{12}O_5$	14.98	M+H	273.07581	0.20	153.01828	Flavonoid
56	Hypaconitine	$C_{33}H_{45}NO_{10}$	15.03	M+H	616.31116	-0.75	338.17493, 524.26453	Diterpenoid Alkaloid
57	Cinnamaldehyde	C_9H_8O	15.15	M+H	133.06477	-0.12	95.04956, 105.0702	Phenylpropanoid
58	Kaempferol	$C_{15}H_{10}O_6$	15.19	M+H	287.05484	-0.59	153.0184, 258.05203	Flavonoid
59	Hispidulin	$C_{16}H_{12}O_6$	15.24	M+H	301.07059	-0.24	286.04703	Flavonoid
60	Ginsenosidero	$C_{48}H_{76}O_{19}$	15.24	M-H	955.48840	-2.51	569.38361, 731.43433	Triterpenoid Saponin
61	Isorhamnetin	$C_{16}H_{12}O_7$	15.38	M-H	315.05017	-2.73	300.02652	Flavonoid
				M+H	317.06543	-0.47	302.04175, 153.0184	
62	Araloside A	$C_{47}H_{74}O_{18}$	15.75	M-H	925.47803	-2.39	569.38354	Triterpenoid Saponin
63	Paeonol	$C_9H_{10}O_3$	15.84	M+H	167.07018	-0.57	125.05988, 121.065	Monoterpene
64	Chikusetsu Saponin 4A	$C_{42}H_{66}O_{14}$	15.90	M-H	793.43597	-2.53	793.43573, 455.35324	Triterpenoid Saponin
65	Tectochrysin	$C_{16}H_{12}O_4$	16.56	M+H	269.08075	-0.34	226.06252	Flavonoid
66	Rheic Acid	$C_{15}H_8O_6$	16.83	M-H	283.02417	-2.26	239.03406	Anthraquinone
67	6-Demethoxytangeretin	$C_{19}H_{18}O_6$	17.27	M+H	343.11752	-0.25	313.07047, 285.0759	Flavonoid
68	Neobavaisoflavone	$C_{20}H_{18}O_4$	17.41	M+H	323.12762	-0.51	267.0654	Flavonoid
69	Quillaic Acid *	$C_{30}H_{46}O_5$	17.87	M+H	487.34137	-0.88	451.3204, 187.14809	Triterpene
70	Dihydroartemisinic Acid	$C^{15}H^{24}O_2$	18.21	M+H	237.18481	-0.40	107.08587	Sesquiterpene
71	Momordin Ic	$C_{41}H_{64}O_{13}$	18.39	M-H	763.42505	-3.11	483.34595	Triterpenoid Saponin
72	Genistein	$C_{15}H_{10}O_5$	18.70	M-H	269.04477	-2.90	241.05017	Flavonoid
73	Alisol C 23-Acetate	$C_{32}H_{48}O_6$	19.10	M+H	529.35205	-0.60	451.32043, 227.14253	Triterpene
74	18 Beta-Glycyrrhetintic Acid	$C_{30}H_{46}O_4$	19.79	M-H	469.33115	-2.52	409.30994	Triterpene
75	Abietic Acid	$C_{20}H_{30}O_2$	19.93	M+H	303.23172	-0.46	123.11691	Diterpene
76	Diosgenin *	$C_{27}H_{42}O_3$	19.99	M+H	415.32025	0.21	415.3202, 271.20527, 253.19466	Triterpene

77	Alisol A	$C_{30}H_{50}O_5$	19.99	M+HCO_2	535.36267	-2.54	471.34698	Triterpene
78	Corosolic Acid	$C_{30}H_{48}O_4$	20.00	M+H	473.36240	-0.30	205.15874	Triterpene
79	Alisol A 24-Acetate	$C_{32}H_{52}O_6$	20.23	M+HCO_2	577.37323	-2.36	531.36786	Triterpene
80	Dehydrotumulosic Acid	$C_{31}H_{48}O_4$	20.47	M-H	483.34644	-3.20	273.18503, 421.30939	Triterpene
				M+H	485.36224	-0.61	311.23669	
81	Poricoic Acid A	$C_{31}H_{46}O_5$	20.61	M+H	499.34158	-0.43	325.21609, 223.14806	Triterpene
				M-H	497.32617	-2.17	423.28943, 211.14832	
82	Glabrolide	$C_{30}H_{44}O_4$	20.72	M-H	467.31543	-2.68	423.3262	Triterpene
				M+H	469.33118	-0.13	95.08591	
83	Alisol B 23-Acetate	$C_{32}H_{50}O_5$	21.00	M+H	515.37311	0.02	339.26779, 97.06527	Triterpene
84	Acetyl-11-Keto-Beta-Boswellic Acid	$C_{32}H_{48}O_5$	21.50	M+H	513.35742	-0.05	173.13219	Triterpene
85	Beta-Elemonic Acid	$C_{30}H_{46}O_3$	21.84	M+H	455.35181	-0.36	313.25278	Triterpene
86	Ursolic Acid	$C_{30}H_{48}O_3$	22.21	M+H	457.36774	0.26	95.08591	Triterpene
87	Alisol B	$C_{30}H_{48}O_4$	22.40	M+Na	495.34680	4.69	353.24652, 293.22641	Triterpene
88	Bryodulcosigenin	$C_{30}H_{50}O_4$	22.62	M+Na	497.36224	4.25	437.34128, 295.24203	Triterpene
89	3-O-Acetyl-16Alpha-Hydroxytrametenolic Acid	$C_{32}H_{50}O_5$	22.63	M-H	513.35706	-2.91	451.32135,	Triterpene
90	Pachymic Acid	$C_{33}H_{52}O_5$	23.02	M+H	529.38849	-0.49	451.35693, 295.24216	Triterpene
				M-H	527.37256	-3.11	465.33594,	
*Indicates that the compound matches the reference standard								

Table 2: Identification of the Chemical Constituents in the JKSQ using UHPLC-Q Exactive-Orbitrap-MS.

3.2. JKSQ Relives Renal Fibrosis in UUO Rats

To evaluate JKSQ's treatment potential on renal fibrosis, we built a UUO rat model. After H&E staining showed that the renal tubular atrophy, the tubular deposition and the inflammatory cell infiltration are more serious than the Sham group.

After JKSQ treatment, the severity of renal lesions in the rats was significantly reduced. Masson staining was used to assess the degree of renal fibrosis, and the results showed that the Sham group exhibited minimal collagen deposition in the renal interstitium, while the UUO group displayed marked fibrous collagen deposition. JKSQ treatment significantly reduced collagen fiber accumulation (Fig. 2 A-C). JKSQ treatment significantly mitigated renal fibrosis by reducing the expression of core fibrosis markers, including α-SMA, Col1a1, and FN1. JKSQ treatment significantly reduced mRNA levels of α-SMA, Col1a1, and FN1. (Fig. 2 D-F). Western blotting analysis indicated that the expression of α-SMA and FN1 was significantly upregulated in the UUO group compared with the Sham group, and JKSQ evidently decreased the protein expression of α-SMA, FN1 in UUO group (Fig. 2 G-I). These findings imply that JKSQ effectively alleviates renal fibrosis following UUO surgery.

3.3. JKSQ Attenuates the Inflammatory Response and Oxidative Stress in UUO Rats.

Inflammatory responses play a critical role in the initiation and progression of renal fibrosis. We measured levels of IL-6 and TNF-α, which are potent inflammatory agents. JKSQ treatment significantly reduced IL-6 and TNF-α mRNA levels, as shown by qRT-PCR results (Fig. 3 A-B). We measured serum IL-6 and TNF-α concentrations using ELISA.

The study showed that rats treated with JKSQ had significantly lower serum levels of IL-6 and TNF-α compared to the UUO group (Fig. 3 C-D), suggesting that JKSQ can effectively improve the inflammatory response. To assess oxidative stress, a hallmark of UUO-induced renal fibrosis, we evaluated the amounts of MDA and SOD in the rats' kidneys. As shown in Fig. 3E and F, JKSQ treatment attenuated the UUO-induced increase in renal MDA levels and elevated the renal SOD content. (Fig. 3 E-F). The above results suggest that JKSQ can considerably reduce inflammation and oxidative stress throughout the renal fibrosis process.

3.4. Network Pharmacology-Based Investigation of JKSQ in the Treatment of Renal Fibrosis

A network pharmacology strategy was implemented to elucidate JKSQ's anti-fibrotic mechanisms, commencing with the retrieval of candidate compounds from the TCMSP database. This process identified 207 potential component targets of JKSQ. A total of 1990 unique targets associated with renal fibrosis were retrieved from the GeneCards and OMIM databases using the keyword “renal fibrosis.” We applied a Venn diagram to discover 123 overlapping targets among the components and diseases (Fig. 4A).

We constructed a protein-protein interaction (PPI) network using interaction data obtained from the STRING database to visualize

the complex interactions among the targets. The top four key targets, AKT1, IL-6, TNF, and TP53, were selected based on degree values (Fig. 4B). A "drug component-disease-target" network was drawn using various active components in JKSQ and disease-related targets, revealing that quercetin, kaempferol, diosgenin, beta-sitosterol, and stigmasterol may be the core active components of JKSQ in treating renal fibrosis (Fig. 4C).

3.5. GO and KEGG Enrichment Analysis of JKSQ in the Treatment of Renal Fibrosis

Bioinformatics enrichment analysis was performed using the Metascape database. Gene Ontology (GO) analysis revealed that the biological processes (BP) significantly enriched for the renal fibrosis targets of JKSQ included response to inorganic substances, response to reactive oxygen species, and response to oxidative stress. The main molecular functions (MF) were transcription factor binding, DNA-binding transcription factor binding, and kinase binding (Fig. 5A). Furthermore, the KEGG pathway analysis results indicated that the PI3K-Akt pathway, MAPK pathway, and TNF pathway were enriched in JKSQ-treated renal fibrosis. (Fig. 5B).

3.6. Molecular Docking of The Key Active Components and Key Targets of JKSQ

The above results indicate that the identified active components (quercetin, kaempferol, diosgenin, beta-sitosterol, and stigmasterol) and key targets (AKT1, IL-6, TNF, and TP53) play essential roles in the JKSQ-mediated treatment of renal fibrosis. Therefore, the binding ability of the key active components and targets was tested via molecular docking. It can be seen from the docking results that the active component and key targets exhibit good binding activity, with a binding energy lower than -5.00 kcal/mol, and diosgenin demonstrated the best binding activity with the key targets (Fig. 6 A). The binding schematic diagram in Fig. 6B indicates that diosgenin can form stable complexes with these four key targets (Fig. 6B). These results suggest that JKSQ and diosgenin can influence renal fibrosis-related pathways through these targets to improve renal fibrosis.

3.7. The Effect of JKSQ on Key Targets and the PI3K/AKT Signaling Pathway

According to the results of PPI analysis in Network drug, we measured the mRNA level changes of the top 4 key targets (TP53, IL-6, TNF-α, and AKT1). The results showed that the mRNA expression levels of TP53, IL-6, TNF-α, and AKT1 in the UUO group were significantly higher than those in the Sham group.

After treatment with JKSQ, the expression of mRNA of TP53, IL-6, TNF-α, and AKT1 was down-regulated in a dose-dependent way (Fig. 7 A-D). According to the prediction results of KEGG, the PI3K/AKT signaling pathway seems to be the main pathway that JKSQ affects renal fibrosis. So we used Western blotting to examine the p-AKT/AKT expression change and PI3K pathway protein in the rat kidney from each group. JKSQ treatment reversed the elevated expression of p-AKT and PI3K in the kidneys induced by UUO (Fig. 7 E-G). In conclusion, JKSQ may improve renal fibrosis by the PI3K/AKT pathway.

3.8. JKSQ and DIO Protect NRK-49F from TGF-β1-Induced Fibrosis Through the PI3K/AKT Signaling Pathway

Next, we examined the in vitro antifibrotic effects of JKSQ and DIO and their regulation of the PI3K/AKT pathway. Fibrosis in NRK-49F cells was induced using TGF-β1. We quantified the mRNA expression of fibrosis markers in each cell group. Compared with the CON group, the mRNA levels of α-SMA, Col1a1, and FN1 were significantly upregulated in the TGF-β1 group. This upregulation was reversed after treatment with JKSQ and DIO (Fig. 8 A-C).

Western blot analysis showed an upregulation of p-AKT and PI3K in the TGF-β1 group, which was significantly suppressed following treatment with JKSQ and DIO. (Fig. 8 D-F). To confirm the role of the PI3K/AKT signaling pathway in the treatment of renal fibrosis, we used the PI3K agonist 740Y-P to activate the signaling pathway. 740Y-P treatment antagonized both the antifibrotic effects of JKSQ and DIO and their suppression of the PI3K/AKT pathway (Fig. 8 G-L). These findings indicate that JKSQ and DIO exert antifibrotic effects mainly by inhibiting PI3K/AKT signaling.

3.9. Diosgenin Improves Renal Fibrosis in UUO Rats

The above experiments demonstrated that DIO might be the key active component in the JKSQ formula used to treat renal fibrosis, so we used the UUO model of rats to test the DIO in the treatment of kidney fibrosis. H&E staining results revealed no renal damage in the Sham group, while tubular atrophy, tubular necrosis, tubular casts, inflammatory cell infiltration, etc. were observed in UUO groups. Treatment with DIO greatly decreased renal injury in rats (Fig. 9 A-B). Masson staining demonstrated evident collagen deposition in the kidneys of UUO rats, and DIO treatment markedly reduced this deposition (Fig. 9 A and C).

Histopathological analysis results showed that DIO greatly reduced the renal histopathology of UUO rats; qRT-PCR analysis showed that DIO treatment decreased the mRNA expression of α-SMA, Col1a1, and FN1 (Fig. 9 D-F). Furthermore, in the kidneys of UUO rats, the expression levels of α-SMA and FN1 were significantly increased. These increases were markedly attenuated by DIO treatment. (Fig. 9 G-I). Collectively, these data show that DIO ameliorates UUO-induced renal fibrosis.

4. Discussion

Based on the research findings, it can be known that JKSQ can alleviate the renal fibrosis of UUO rats. Through the combination of network pharmacology and molecular docking, it is speculated that the mechanism by which JKSQ alleviates renal fibrosis is related to the activation of the PI3K/AKT pathway, and the main bioactive compound is DIO.

Subsequent validation experiments confirmed that JKSQ exerts its anti-fibrotic effects via the PI3K/AKT pathway. Furthermore, 740Y-P, a PI3K agonist, was employed to validate the mechanism. And at the same time, it is found that DIO itself has anti-fibrosis.

Put together, they show that JKSQ could be used to treat renal fibrosis by DIO being a big part of it. Even more noteworthy is the fact that DIO was identified as a significant component of JKSQ in the UHPLC-MS analysis, along with all the other known biologically active components of JKSQ (quercetin, kaempferol, and beta-sitosterol). This indicates that JKSQ is composed of multiple components.

A very common problem among all kinds of CKD renal fibrosis is the last phase of any ailment, no matter what has caused it. We used the UUO model for the kidney scarring model in this experiment. The UUO model, an important animal model in renal fibrosis research, is widely used in kidney disease experiments. In this model, kidney injury includes tubule destruction, inflammatory cells in the interstitium, and extracellular matrix [15]. When the ureter is blocked, the intratubular hydrostatic pressure rises, which causes tubular cell necrosis in the collecting duct and the distal and proximal tubules [16].

According to our research, tubular atrophy, necrosis, and a significant number of tubular casts are present in the kidneys of UUO rats. Tubular damages in the UUO rats were also observed to be less after JKSQ and DIO treatments. Inflammatory cell infiltration around tubules is considered an early stage of renal fibrosis [17]. Key pro-inflammation factors, mostly from monocytes, are main drivers in the subsequent fibrosis stage [18]. We used qRT-PCR and ELISA to detect the expression of main inflammatory-related factors. The JKSQ group's UUO rats showed significantly lower levels of IL-6 and TNF-α in their kidneys and serum after 28 days. It is about fibroblast stimulation and ECM overproduction; this leads to deep interstitial aggregation of tubule-capillary, which eventually deteriorates kidney function [19], interstitial fibrosis. ECM is full of collagen I and fibronectin. [20] identified alpha-smooth muscle actin-positive cells and measured α-SMA, Col1a1, and FN1 expression levels. JKSQ and DIO greatly reduce fibrosis.

According to the chemical characteristics of the JKSQ as analyzed using UHPLC-MS, 90 main components were discovered, and this serves as the foundation for the network pharmacology analysis that will follow. And thus a comprehensive profile can result in the current possibility of being discovered for the active ingredient of the active compound and the target of that compound, where network pharmacology is a newly emerged method that included and embodied that network between the organism, the drugs, and the disease. And it also shows the interaction and mechanism of action of the disease gene and drug target from the whole network perspective.

Thus, a complete approach like TCM is also of great significance in the research on drugs for the treatment of complex diseases like renal fibrosis. And following the network pharmacology analysis, we get four active ingredients, which are quercetin, kaempferol, diogenin, and beta-sitosterol. Through molecular docking, it is proved that the active components have good binding activities to the major target. The flavonoid quercetin can be found in many kinds of medicinal herbs; it can alleviate the fibrosis of kidneys in different ways, such as by inhibiting the TLR4/MyD88 pathway or promoting SIRT1/PINK1/Parkin-promoted mitophagy [21]. And as for the evidence, it's found that kaempferol alleviates renal fibrosis and epithelial-mesenchymal transition (EMT) in hypertensive CKD models via inhibition of the sonic hedgehog pathway [22]. Beta-sitosterol, a compound found in lipid-rich plants [23], has been proved to help prevent renal ischemia/reperfusion damage by alleviating inflammation and oxidative stress [24].

Diosgenin is a widely distributed sterolin of natural saponin, belonging to the Dioscorea and Leguminosae families, shown to exhibit different biological activities such as anticancer activity [25], antithrombotic effect [26], anti-inflammatory activity [27], and antioxidant capacity [28]. Diosgenin reduces diabetic nephropathy by elevating mitophagy and boosting mitochondrial dynamics through CaMKK2 [29]. A number of recent studies have found that diosgenin could treat I/R-related renal fibrosis via regulating the NOX4/p65 pathway [30]. And following experiments in vivo, it was found that diosgenin could reduce TGF-β1–induced fibrosis in NRK-49F cells and UUO surgery with improvement on diosgenin for renal fibrosis. It shows that diosgenin was an antifibrosis element.

From KEGG enrichment analysis, we discover that JKSQ can alleviate renal fibrosis mainly by inhibiting PI3K/AKT signal transduction. The PI3K/AKT signaling pathway plays a role in many different physiological functions like apoptosis [31], proliferation [32], and metabolism [33]. PI3K, as an intracellular phosphoinositide kinase, transduces signals that control cell growth, proliferation, metabolism, migration, and secretion [34]. When tyrosine kinase receptors activate PI3K, it catalyzes the conversion of phosphatidylinositol 4,5-bisphosphate (PIP2) to phosphatidylinositol 3,4,5-trisphosphate (PIP3), which recruits downstream effector proteins such as AKT to the membrane.

AKT is then phosphorylated and activated by PDK1, which regulates various functions like apoptosis, proliferation, and differentiation [35]. Many studies continuously pointed out the PI3K/AKT pathway is essential in promoting renal fibrosis [8; 36; 37]. Following that, we discovered that the PI3K/AKT pathway is activated in UUO rats, which is clearly inhibited by JKSQ. JKSQ and DIO reduce fibrotic marker expression, as seen in the TGF-β1-induced NRK-49F cell model. They downregulate the expression of p-AKT and p-PI3K. To be steadfast with this pathway, we will use 740Y-P, an activator of PI3K. And the result showed that the anti-fibrotic effect of JKSQ and DIO, along with the PI3K/AKT pathway being suppressed, had been reversed if 740Y-P was introduced together. And the results demonstrated that 740Y-P reversed the anti-fibrotic impact of JKSQ and DIO, along with the inhibition of the PI3K/AKT pathway. Summarizing, the benefits of JKSQ treatment on renal fibrosis mainly depend upon the pharmacological inhibition of the PI3K/AKT pathway.

5. Conclusion

The investigation with the chemical was carried out via UHPLC-MS; the multi-component profile of JKSQ is established, and

diosgenin as a prominent proportion among those bioactive compounds is recognized. According to this chemical foundation, we employed network pharmacology and experimental methodologies to find that JKSQ can retard the progression of renal fibrosis. From a molecular perspective, JKSQ might hinder renal fibrosis via regulating the PI3K/AKT pathway. DIO is one of its major activities, and providing a theoretical basis and scientific evidence for its clinical application.

Abbreviations

740Y-P, PI3K agonist; AKT, protein kinase B; α-SMA, alpha-smooth muscle actin; BP, biological process; CC, cellular component; CKD, chronic kidney disease; Col1a1, collagen type I alpha 1 chain; DIO, diosgenin; DL, drug-likeness; ECM, extracellular matrix; ECL, enhanced chemiluminescence; ELISA, enzyme-linked immunosorbent assay; ESRD, end-stage renal disease; FN1, fibronectin 1; GO, Gene Ontology; H&E, hematoxylin and eosin; IL-6, interleukin-6; JKSQ, Jinkui Shenqi pill; KEGG, Kyoto Encyclopedia of Genes and Genomes; MDA, malondialdehyde; MF, molecular function; OB, oral bioavailability; PDK1, phosphoinositide-dependent kinase 1; PI3K, phosphoinositide 3-kinase; PIP2, phosphatidylinositol 4,5-bisphosphate; PIP3, phosphatidylinositol 3,4,5-trisphosphate; PPI, protein-protein interaction; PMSF, phenylmethylsulfonyl fluoride; qRT-PCR, quantitative real-time polymerase chain reaction; RF, renal fibrosis; SEM, standard error of the mean; SOD, superoxide dismutase; SPF, Specific Pathogen Free; TCM, Traditional Chinese Medicine; TCMSP, Traditional Chinese Medicine Systems Pharmacology; TGF-β1, transforming growth factor-beta 1; TNF-α, tumor necrosis factor-alpha; TP53, tumor protein p53; UUO, unilateral ureteral obstruction.

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Authors' contributions

Luyao Wu: Conceptualization, Methodology, Resources, Visualization, Writing - original draft. Writing - review & editing, **Xiaoying Xu:** Conceptualization, Writing - original draft, Writing - review & editing, Investigation, Methodology, Formal Analysis. **Haoxiang Fang:** Conceptualization, Methodology, Resources, Validation. **Yingying Dong:** Conceptualization, Methodology, Resources, Writing - original draft. **Haowen Yang:** Methodology, Validation. **Siqi Shen:** Methodology, Validation. **Wenxiu Xu:** Methodology, Validation. **Yanfei Shao:** Funding acquisition, Writing - review & editing, Conceptualization, Supervision, Resources.

Ethics approval and consent to participate

The research plan and the reporting of animal studies adhered to the ARRIVE 2.0 guidelines. All animal research was authorized by the Institutional Animal Care and Use Committee at Zhejiang University.

Competing interest

All authors declare no conflicts of interest.

Reference

1. Pradhan, N., & Dobre, M. (2023). Emerging preventive strategies in chronic kidney disease: Recent evidence and gaps in knowledge. *Current Atherosclerosis Reports, 25*, 1047–1058.
2. Humphreys, B. D. (2018). Mechanisms of renal fibrosis. *Annual Review of Physiology, 80*, 309–326.
3. Liu, Y., Su, Y. Y., Yang, Q., & Zhou, T. (2021). Stem cells in the treatment of renal fibrosis: A review of preclinical and clinical studies of renal fibrosis pathogenesis. *Stem Cell Research & Therapy, 12*, 333.
4. Zhou, D., & Liu, Y. (2016). Renal fibrosis in 2015: Understanding the mechanisms of kidney fibrosis. *Nature Reviews Nephrology, 12*, 68–70.
5. Tang, P. M., Zhang, Y. Y., Mak, T. S., Tang, P. C., Huang, X. R., et al. (2018). Transforming growth factor-β signalling in renal fibrosis: From Smads to non-coding RNAs. *Journal of Physiology, 596*, 3493–3503.
6. Liu, M., Liu, L., Bai, M., Zhang, L., Ma, F., et al. (2018). Hypoxia-induced activation of Twist/miR-214/E-cadherin axis promotes renal tubular epithelial cell mesenchymal transition and renal fibrosis. *Biochemical and Biophysical Research Communications, 495*, 2324–2330.
7. Wei, X., Hou, Y., Long, M., Jiang, L., & Du, Y. (2023). Advances in energy metabolism in renal fibrosis. *Life Sciences, 312*, 121033.
8. Li, R., Shi, C., Wei, C., Wang, C., Du, H., et al. (2023). Fufang Shenhua tablet inhibits renal fibrosis by inhibiting PI3K/AKT. *Phytomedicine, 116*, 154873.
9. Chen, Z. P., Huang, Y. Q., Cui, Z. L., Miao, G. Z., & Cui, Y. Z. (2023). Treatment of diabetic nephropathy with Jingui Shenqi Pills based on the "state-target strategy." *World Chinese Medicine, 18*, 277–280.
10. Ma, C., Feng, X., & Xu, L. H. (2023). Clinical effect of Jinkui Shenqi Pill combined with western medicine in treatment of III–IV diabetic nephropathy. *World Journal of Complex Medicine, 9*, 149–152.
11. Wang, S. W., Ma, M. K., Han, J., & Geng, X. H. (2021). Effect of Jingui Shenqi Pill on Keap1/Nrf2/ARE pathway and renal function in rats with chronic renal failure. *Journal of Tropical Medicine, 21*, 1129–1133, 1237.
12. Wu, S., Li, J. X., Wang, W. S., Liu, Y. X., Xu, W. W., et al. (2023). Mechanism of Jingui Shenqi Pill and Guifu Dihuang Pill in treating chronic glomerulonephritis. *Clinical Journal of Traditional Chinese Medicine, 35*, 938–945.
13. Wu, G. W., & Xia, C. L. (2021). Clinical efficacy evaluation of Jin Kui Shen Qi Wan in patients with lupus nephritis. *Ningxia Medical Journal, 43*, 375–378.
14. Casas, A. I., Hassan, A. A., Larsen, S. J., Gomez-Rangel, V., Elbatreek, M., et al. (2019). From single drug targets to synergistic network pharmacology in ischemic stroke. *Proceedings of the National Academy of Sciences of the*

United States of America, 116, 7129–7136.
15. Chevalier, R. L., Forbes, M. S., & Thornhill, B. A. (2009). Ureteral obstruction as a model of renal interstitial fibrosis and obstructive nephropathy. *Kidney International, 75*, 1145–1152.
16. Martínez-Klimova, E., Aparicio-Trejo, O. E., Tapia, E., & Pedraza-Chaverri, J. (2019). Unilateral ureteral obstruction as a model to investigate fibrosis-attenuating treatments. *Biomolecules, 9*.
17. Liu, Y. (2011). Cellular and molecular mechanisms of renal fibrosis. *Nature Reviews Nephrology, 7,* 684–696.
18. Mack, M. (2018). Inflammation and fibrosis. *Matrix Biology*, 68–69, 106–121.
19. Djudjaj, S., & Boor, P. (2019). Cellular and molecular mechanisms of kidney fibrosis. *Molecular Aspects of Medicine, 65*, 16–36.
20. Asada, N., Takase, M., Nakamura, J., Oguchi, A., Asada, M., et al. (2011). Dysfunction of fibroblasts of extrarenal origin underlies renal fibrosis and renal anemia in mice. *Journal of Clinical Investigation, 121*, 3981–3990.
21. Song, L., Zhang, W., Tang, S. Y., Luo, S. M., Xiong, P. Y., et al. (2024). Natural products in traditional Chinese medicine: Molecular mechanisms and therapeutic targets of renal fibrosis and state-of-the-art drug delivery systems. *Biomedicine & Pharmacotherapy, 170*, 116039.
22. Guan, Y., Quan, D., Chen, K., Kang, L., Yang, D., et al. (2023). Kaempferol inhibits renal fibrosis by suppression of the sonic hedgehog signaling pathway. *Phytomedicine, 108*, 154246.
23. Wang, H., Wang, Z., Zhang, Z., Liu, J., & Hong, L. (2023). β-Sitosterol as a promising anticancer agent for chemoprevention and chemotherapy: Mechanisms of action and future prospects. *Advances in Nutrition, 14*, 1085–1110.
24. Koc, K., Geyikoglu, F., Cakmak, O., Koca, A., Kutlu, Z., et al. (2021). The targets of β-sitosterol as a novel therapeutic against cardio-renal complications in acute renal ischemia/reperfusion damage. *Naunyn-Schmiedeberg's Archives of Pharmacology, 394*, 469–479.
25. Sethi, G., Shanmugam, M. K., Warrier, S., Merarchi, M., Arfuso, F., et al. (2018). Pro-apoptotic and anti-cancer properties of diosgenin: A comprehensive and critical review. *Nutrients, 10*.
26. Wang, D., & Wang, X. (2022). Diosgenin and its analogs: Potential protective agents against atherosclerosis. *Drug Design, Development and Therapy, 16*, 2305–2323.
27. Prasad, M., Jayaraman, S., Rajagopal, P., Veeraraghavan, V. P., Kumar, P. K., et al. (2022). Diosgenin inhibits ER stress-induced inflammation in aorta via iRhom2/TACE mediated signaling in experimental diabetic rats: An in vivo and in silico approach. *Chemical Biology Interactions, 358*, 109885.
28. Nie, K., Gao, Y., Chen, S., Wang, Z., Wang, H., et al. (2023). Diosgenin attenuates non-alcoholic fatty liver disease in type 2 diabetes through regulating SIRT6-related fatty acid uptake. *Phytomedicine, 111*, 154661.
29. Zhong, Y., Jin, R., Luo, R., Liu, J., Ren, L., et al. (2023). Diosgenin targets CaMKK2 to alleviate type II diabetic nephropathy through improving autophagy, mitophagy and mitochondrial dynamics. *Nutrients, 15*.
30. Chiang, C. H., Lan, T. Y., Hsieh, J. H., Lin, S. C., Chen, J. W., et al. (2024). Diosgenin reduces acute kidney injury and ameliorates the progression to chronic kidney disease by modifying the NOX4/p65 signaling pathways. *Journal of Agricultural and Food Chemistry, 72*, 17444–17454.
31. Li, Y., Xia, J., Jiang, N., Xian, Y., Ju, H., et al. (2018). Corin protects H_2O_2-induced apoptosis through PI3K/AKT and NF-κB pathway in cardiomyocytes. *Biomedicine & Pharmacotherapy, 97*, 594–599.
32. Xin, Y., Min, P., Xu, H., Zhang, Z., Zhang, Y., et al. (2020). CD26 upregulates proliferation and invasion in keloid fibroblasts through an IGF-1-induced PI3K/AKT/mTOR pathway. *Burns & Trauma*, 8, tkaa025.
33. Revathidevi, S., & Munirajan, A. K. (2019). Akt in cancer: Mediator and more. *Seminars in Cancer Biology*, 59, 80–91.
34. Fruman, D. A., Chiu, H., Hopkins, B. D., Bagrodia, S., Cantley, L. C., et al. (2017). The PI3K pathway in human disease. *Cell*, 170, 605–635.
35. Huang, X., Liu, G., Guo, J., & Su, Z. (2018). The PI3K/AKT pathway in obesity and type 2 diabetes. *International Journal of Biological Sciences, 14*, 1483–1496.
36. Wang, Y., Ping, Z., Gao, H., Liu, Z., Xv, Q., et al. (2024). LYC inhibits the AKT signaling pathway to activate autophagy and ameliorate TGFβ-induced renal fibrosis. *Autophagy, 20*, 1114–1133.
37. Tuoheti, K., Bai, X., Yang, L., Wang, X., Cao, Y., et al. (2024). Forsythiaside A suppresses renal fibrosis and partial epithelial-mesenchymal transition by targeting THBS1 through the PI3K/AKT signaling pathway. *International Immunopharmacology, 129*, 111650.

Figure legends

Figure 1: ***Total ion chromatogram (TIC) of UHPLC-Q Exactive-Orbitrap-MS in the aqueous extract of JKSQ***. Total ion chromatogram in positive (A) and negative (B) ion mode.

Figure 2: ***JKSQ improves renal fibrosis in UUO rats***. (A) H&E and Masson staining of rat kidneys from each group. Scale bar = 50μm. (B) Tubular injury score of renal tubules assessed by HE staining. (C) Relative positive area of kidneys assessed by Masson staining. (D-F) Relative mRNA expression levels of fibrosis markers α-SMA, Col1a1 and FN1 by qRT-PCR. (G) Western Blotting analysis of α-SMA and FN1 expression levels in kidnyes tissues. (H-I) Relative expression levels of α-SMA and FN1. N=6 rats per group, *P<0.05, **P<0.01, *P<0.001.

Figure 3: ***JKSQ improves inflammation response and oxidative stress in UUO rats***. (A-B) Relative mRNA expression levels of IL-6 and TNF-α by qRT-PCR. (C-D) ELISA was used to measure the levels of IL-6 and TNF-α in the serum of rats in each group. (E) MDA expression levels in the kidneys. (F) SOD expression levels in the kidneys. N=6, *P<0.05, **P<0.01, *P<0.001.

Figure 4: ***Network pharmacology prediction of JKSQ treatment for renal fibrosis***. (A) The intersecting targets between JKSQ and renal fibrosis. (B) Visualization analysis of key targets using Cytoscape. (C) "Drug component-disease-target" network diagram.

Figure 5: ***GO and KEGG enrichment analysis of JKSQ in the treatment of renal fibrosis***. A. GO enrichment analysis of BP, CC and MF. B. The top 20 KEGG pathways. The size of the bubbles represents the gene count in the pathway, and the color represents the P value.

Figure 6: ***Molecular docking of the key active components and key targets of JKSQ***. A. Heatmap of the binding energy results of the molecular docking between the key active components and key targets of JKSQ. B. Visualization of the docking results of diosgenin with AKT1, TNF, TP53, and IL6.

Figure 7: ***Effects of JKSQ on key targets and the PI3K/AKT signaling pathway***. (A-D) The mRNA expression levels of AKT1, IL-6, TNF-α, and TP53 in the kidneys of rats from each group were detected by qRT-PCR. (E) The expression levels of p-AKT, t-AKT, and PI3K in kidney tissues from each group were detected by Western Blotting. (F-G) The relative quantitative expression levels of p-AKT/t-AKT and PI3K. N=6, *P<0.05, **P<0.01, *P<0.001.

Figure 8: ***JKSQ and DIO protect NRK-49F from TGF-β1-induced fibrosis through the PI3K/AKT signaling pathway***. (A-C) qRT-PCR was used to measure the mRNA expression levels of α-SMA, Col1a1, and FN1 in cells treated with JKSQ or DIO. (D) Western blotting was used to detect the expression levels of p-AKT, t-AKT, and PI3K in cells of each group. (E-F) Relative quantitative expression levels of p-AKT/t-AKT and PI3K. (G-I) qRT-PCR was used to measure the mRNA expression levels of α-SMA, Col1a1, and FN1 in cells treated with 740Y-P. (J) Western blotting was used to detect the expression levels of p-AKT, t-AKT, and PI3K in cells of each group. (K-L) Relative quantitative expression levels of p-AKT/t-AKT and PI3K. N=3, *P<0.05, **P<0.01, ***P<0.001.

Figure 9: DIO improves renal fibrosis in UUO rats. (A) H&E staining and Masson staining of kidneys from each group of rats. Scale bar = 50μm. (B) Pathological damage score of renal tubules from H&E staining. (C) Scoring of renal fibrosis area from Masson staining. (D-F) Relative mRNA expression of fibrosis markers α-SMA, Col1a1, and FN1. (G) Detection of α-SMA and FN1 expression levels in kidney tissues of each group by Western Blotting. (H-I) Relative quantitative expression levels of α-SMA and FN1. N=5, *P<0.05, **P<0.01, ***P<0.001.