

Original Article

Wenpi decoction attenuates cisplatin-induced chronic kidney disease via inhibition of the NLRP3/caspase1 signaling pathway: an experimental study

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Abstract: Objective: This study aimed to elucidate the mechanisms underlying the protective effects of Wenpi Decoction (WPD) against chronic kidney disease (CKD) progression using animal experimental approaches. Methods: The active components of WPD and their relevant therapeutic targets for CKD were identified based on the Traditional Chinese Medicine Systems Pharmacology (TCMSP) platform. A protein-protein interaction (PPI) network was constructed using the STRING database. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were performed in R to identify key biological processes and signaling pathways. The associations among NLRP3 (NOD-like receptor protein 3), CKD, inflammatory pathways, and immune cell infiltration were further evaluated using network-based analyses, and the causal relationship between NLRP3 and CKD was investigated through Mendelian randomization analysis. The effects of WPD were then validated in a cisplatin-induced CKD mouse model. Results: Network pharmacology analysis identified 751 potential target proteins and indicated that inflammation-related signaling pathways may represent major mechanisms underlying the effects of WPD. NLRP3 was highly expressed in CKD patients and showed potential diagnostic value. Further analyses suggested that NLRP3 was involved in inflammatory pathways and immune cell infiltration. Mendelian randomization confirmed a causal relationship between NLRP3 and CKD. Animal experiments demonstrated that WPD improved renal function, attenuated renal fibrosis, and downregulated the renal expression levels of NLRP3, IL-6, TNF- α , and caspase-1. Conclusion: Inflammation-related pathways, particularly the NLRP3/caspase-1 signaling axis, may play a critical role in the therapeutic effects of WPD in the cisplatin-induced CKD model. WPD effectively alleviated renal dysfunction and fibrosis, potentially via suppressing activation of the NLRP3/caspase-1 signaling pathway.

Keywords: Wenpi Decoction, network pharmacology, CKD, chronic kidney disease model

Introduction

Chronic kidney disease (CKD) has emerged as a major global public health concern, with its prevalence continuously increasing alongside socioeconomic development [1]. A comprehensive epidemiological study (2020) analyzing CKD data from 1990 to 2017 estimated that approximately 697.5 million individuals worldwide are affected by CKD, corresponding to a global prevalence of approximately 9.1%. Notably, China alone accounted for approximately 132 million of these cases [2]. According to clinical projections, by 2040, CKD is expected to rise from the 16th to the 5th leading cause of premature death globally [3].

Chronic glomerulonephritis, acute kidney injury, diabetes, and hypertension are major contributing factors in the pathogenesis of CKD [4]. These conditions can cause severe renal injury, often accompanied by impaired repair capacity, leading to microcirculatory dysfunction, chronic inflammatory cell infiltration, nephron loss, and renal fibrosis. Thereby, they promote CKD progression and may ultimately cause renal failure [5].

Traditional Chinese medicine (TCM) plays an important role in the clinical treatment of CKD, demonstrating established efficacy and a favorable safety profile [6-9]. According to TCM theory, CKD is primarily attributed to the pathogen-

esis of “deficiency of vital qi and excess of pathogenic factors”. Therefore, therapeutic strategies emphasize “supporting the healthy qi and eliminating pathogenic factors”, with commonly employed strategies including spleen and kidney tonification, promotion of blood circulation and diuresis, and detoxification and resolution of blood stasis [10].

Wenpi Decoction (WPD) is a classical TCM formula characterized by the functions of warming and tonifying spleen yang and eliminating cold accumulation. It has shown remarkable clinical efficacy in the treatment of both acute and chronic kidney diseases [11]. However, the precise molecular mechanisms underlying the renoprotective effects of WPD remain unclear. As WPD consists of seven herbal components, the synergistic interactions among these ingredients and their collective contribution to its therapeutic effects warrant further investigation.

Inflammation plays a critical role in the onset and progression of CKD. Inflammatory responses promote aberrant fibroblast accumulation and extracellular matrix deposition, thereby exacerbating renal tissue injury and accelerating renal fibrosis. As an immune-mediated inflammatory process, renal inflammation contributes to fibrotic progression through multiple mechanisms, including complement activation, cytokine release, and regulation of inflammation-related pathways [12].

The NLRP3 inflammasome has been implicated in the regulation of CKD progression. In patients with CKD, increased NLRP3 inflammasome activation has been observed in various renal cell types, including tubular epithelial cells, macrophages, monocytes, and podocytes. It primarily accelerates CKD progression and related complications through the NLRP3/IL-1 β (Interleukin-1 beta)/IL-18 signaling axis [13]. This concept is supported by multiple experimental studies. For instance, compared with control mice, NLRP3 knockout mice exhibited markedly attenuated renal tissue injury and fibrosis 14 days after unilateral ureteral obstruction (UUO), accompanied by decreased renal expression levels of caspase-1 and IL-18 [14]. Moreover, the NLRP3 inflammasome contributes to CKD progression through other mechanisms, including induction of mitochondrial dysfunction and pyroptosis [15, 16]. Recently,

several studies have demonstrated that both herbal monomers and compound TCM formulations may exert renoprotective effects by suppressing NLRP3 inflammasome activation [10, 17, 18].

Network pharmacology, an emerging discipline based on disease-gene-drug target interaction networks [19], provides a powerful framework for exploring the biological mechanisms underlying TCM formulas. By analyzing compound-target-disease relationships, this approach facilitates mechanistic interpretation and scientific validation for TCM theories, enabling the identification of bioactive constituents and related signaling pathways at the molecular level [20]. It has become an increasingly comprehensive and efficient strategy for characterizing complex interactions among drugs, biological system, organs, diseases, and target proteins [21].

Cisplatin-induced animal models, typically established through repeated intraperitoneal administration, are widely used and considered reliable models for mimicking CKD progression [22, 23]. These models provide a valuable experimental platform for evaluating potential therapies for renal injury and disease progression.

Based on this background, this study employed network pharmacology to identify potential therapeutic targets and signaling pathways associated with WPD. These predicted mechanisms were then validated through experimental studies using a cisplatin-caused CKD mice model.

Materials and methods

Identification of active components and targets in WPD

The herbal components of WPD, including *Angelicae Sinensis Radix* (Danggui), *Zingiberis Rhizoma* (Ganjiang), *Rhei Radix et Rhizoma* (Dahuang), *Ginseng Radix et Rhizoma* (Ren shen), *Glycyrrhizae Radix et Rhizoma* (Gan Cao), *Radix Aconiti Lateralis Preparata* (Fuzi), and *Natrii Sulfas* (Mangxiao), were identified based on the HERB database. Additional molecular information, including SMILES structures and molecular formulas, was retrieved from and cross-validated using the PubChem database.

Candidate compounds were initially screened according to the criteria of oral bioavailability

(OB) $\geq 30\%$ and were then subjected to further evaluation using SwissADME (<http://www.swissadme.ch/>). Only compounds that simultaneously fulfilled the following two criteria were retained as active ingredients: (1) High predicted gastrointestinal absorption; and (2) Compliance with at least three of the five drug-likeness filters (Veber, Egan, Muegge, Lipinski, Ghose), with each criterion marked as “Yes”.

The potential protein targets of the retained active components were predicted using SwissTargetPrediction. Only targets showing a non-zero probability (probability > 0) were retained as putative therapeutic targets.

Identification of CKD-related targets

CKD-related disease targets were collected from three major databases: GeneCards, Therapeutic Target Database (TTD), and Drugbank database. The keyword “Chronic kidney disease” was used for database retrieval. For GeneCards, targets with a relevance score ≥ 10 were included. Duplicate targets were removed after data integration.

Identification of overlapping targets

The intersection between WPD-related predicted targets and CKD-associated disease targets was identified using the Venny 2.1 tool. These overlapping targets were considered potential key targets responsible for the therapeutic effects of WPD against CKD.

Construction of the PPI network

The overlapping targets were imported into STRING database to generate protein-protein interaction (PPI) network. The interaction score was set to the highest confidence level (score ≥ 0.900). Considering the large number of overlapping targets, the top 50 targets ranked by degree centrality (defined based on the median degree value) were selected for PPI network visualization.

GO and KEGG enrichment analysis

The overlapping protein targets were standardized into gene symbols using the UniProt database. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were performed on the DAVID database. The top 20 significantly enriched

KEGG pathways and the top 10 significantly enriched terms from the GO category were selected for visualization.

Finally, a “Herb-Ingredient-Target” network incorporating the top 50 targets was constructed using Cytoscape software to visualize the multi-level interactions among WPD components, active ingredients, and molecular targets.

Validation of the association between NLRP3 and CKD using public databases

Raw gene expression microarray data from the dataset GSE30122 were retrieved from the Gene Expression Omnibus (GEO) database. Differential expression analysis was performed to compare NLRP3 expression levels between CKD patients and healthy controls. The diagnostic potential of NLRP3 for CKD was further evaluated using receiver operating characteristic (ROC) curve analysis, with the area under the curve (AUC) calculated.

Identification of NLRP3-associated biological pathways using gene set enrichment analysis (GSEA) and gene set variation analysis (GSVA)

Data Processing: Raw microarray expression data were normalized using the limma package, and genes were subsequently ranked according to their differential expression levels (log2 fold change) between the CKD and control groups.

GSEA: Gene set enrichment analysis was performed using the GSEA software (version 4.3.2; Broad Institute) based on the “Hallmark” and “KEGG pathway” gene sets obtained from the Molecular Signatures Database (MSigDB).

GSVA: The normalized expression matrix was converted into a pathway activity score matrix using the GSVA package (version 2.6.1) in R. These pathway activity scores were then used to evaluate differences in pathway activation among samples.

Protein interaction network analysis of NLRP3 and correlation analysis with immune cell infiltration

The GeneMANIA online platform was employed to construct a protein interaction network for NLRP3. This analysis aimed to identify genes associated with NLRP3 through co-expression,

physical interactions, and other functional relationships, thereby predicting potential regulatory networks and biological functions.

To investigate the association between NLRP3 expression and immune cell infiltration, the following analyses were performed:

Quantification of immune cell infiltration: The CIBERSORT algorithm (implemented in R package) was used to estimate the relative proportions of 22 immune cell subtypes in each sample.

Correlation analysis: Spearman correlation coefficients were calculated to evaluate the relationship between NLRP3 expression levels and the infiltration abundance of individual immune cell populations.

Visualization of results: The linkET R package was used to graphically display the correlation results.

Mendelian randomization (MR) analysis

Data for the NLRP3 gene (ID: eqtl - a - ENSG00000162711) were retrieved from the IEU OpenGWAS database (<https://gwas.mrcieu.ac.uk/>). Subsequently, the exposure dataset were screened to identify suitable instrumental variables. First, single-nucleotide polymorphisms (SNPs) strongly associated with the exposure factor were selected using a genome-wide significance threshold of $P < 5 \times 10^{-8}$. SNPs exhibiting linkage disequilibrium (LD) were removed to ensure the independence of instrumental variables. When two SNPs located within the same region and showed strong correlation, only the SNP with the highest statistical significance was retained. Linkage disequilibrium SNPs were removed according to $r^2 = 0.001$.

Weak instrumental variables, which exhibit insufficient correlation with the exposure factor or explain only a limited proportion of phenotypic variation, may introduce bias into MR analyses. Therefore, the F-statistic was calculated for each instrumental variable, and SNPs with an F-statistic > 10 were retained to minimize the influence of weak instruments.

Outcome data for CKD were obtained from the IEU database (<https://gwas.mrcieu.ac.uk/>). Two-sample Mendelian randomization analysis

was performed to evaluate the causal relationship between NLRP3 and CKD using the Two-SampleMR R package. Multiple MR approaches were applied, including the inverse-variance-weighted (IVW) method, MR-Egger regression, weighted median method, simple mode, and weighted mode.

The MR results were interpreted as follows: ① A statistically significant P value (< 0.05) in the IVW analysis was considered evidence supporting a potential causal association between the exposure factor and disease outcome, whereas the results of other MR methods were primarily used for consistency evaluation; ② Odds ratios (ORs) were calculated to estimate the effect size, with $OR > 1$ indicating an increased risk and $OR < 1$ indicating a protective effect. Similarly, positive β coefficients indicated risk-increasing effects, whereas negative β coefficients suggested protective effects (**Table 1**).

Animal experiment validation

Wenpi Decoction formula consists of seven herbal components: Rhei Radix et Rhizoma (rhubarb) 12 g; Angelicae Sinensis Radix (Chinese angelica) and Zingiberis Rhizoma (dried ginger) 9 g each; and Ginseng Radix et Rhizoma (ginseng), Natrii Sulfas (mirabilite), Aconiti Lateralis Radix Praeparata (prepared aconite root), and Glycyrrhizae Radix et Rhizoma (licorice) 6 g each.

For clinical administration as a decoction, the total herbal concentration was 54 g per 300 mL, corresponding to 0.18 g/mL. The clinical human dose was calculated as 54 g per 70 kg body weight, i.e., 0.771 g/kg. The corresponding mouse dose was converted using the standard body surface area-based interspecies dose conversion method, resulting in an equivalent dose of 7.02 g/kg.

For oral gavage in mice, the gavage volume was set at 1 mL per 100 g body weight. Therefore, the final concentration of the prepared solution for gavage was 0.702 g/mL. Based on the dose per unit body weight, the high-dose WPD concentration was prepared at 1.404 g/mL, whereas the low-dose WPD concentration was prepared by diluting the high-dose solution 4-fold, resulting in a final concentration of 0.351 g/mL.

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Table 1. Results of mendelian randomization analysis (MRresult.csv)

Exposure	Outcome	Method	SNP (n)	b	S.E	P	OR
NLRP3	Chronic kidney disease	MR Egger	20	0.0448011813165742	0.0215487688146645	0.0521839662580351	1.04581991069956
NLRP3	Chronic kidney disease	Weighted median	20	0.0273916794830155	0.0109884928314051	0.0126754358002769	1.02777028046933
NLRP3	Chronic kidney disease	Inverse variance-weighted	20	0.0216535891163648	0.00935552318337479	0.0206389601616561	1.02188972942557
NLRP3	Chronic kidney disease	Simple mode	20	0.011955536330925	0.0192779210100437	0.542515050392366	1.01202728941917
NLRP3	Chronic kidney disease	Weighted mode	20	0.0271821593729847	0.00960538029649721	0.0107026294173511	1.02755496448438

Table 2. Primer sequences

Gene	Primer F 5'-3'	Primer R 5'-3'
36b4	GAAACTGCTGCCTCACATCCGG	GCTGGCACAGTGACCTCACACG
Nlrp3	ATCAACAGGCGAGACCTCTG	GTCCTCCTGGCATACCATAGA
Klotho	GGGACACTTTCACCCATCACT	ACGTTGTTGTAACATATCGCTGG
Collagen-I	TAAGGGTCCCAATGGTGAGA	GGGTCCCTCGACTCCTACAT
Fibronectin	ATGTGGACCCCTCCTGATAGT	GCCCAGTGATTTCAGCAAAGG
Ngal	TGGCCCTGAGTGTATGTG	CTCTTGTAGCTCATAGATGGTGC
Kim-1	ACATATCGTGGAATCACACGAC	ACTGCTCTTCTGATAGGTGACA
Il-1 β	GAAATGCCACCTTTTGACAGTG	TGGATGCTCTCATCAGGACAG
Tnf- α	CAGGCGGTGCCTATGTCTC	CGATACCCCGAAGTTCAGTAG
Il-6	CTGCAAGAGACTTCCATCCAG	AGTGGTATAGACAGGTCTGTTGG

and stored for subsequent experiments.

Both kidneys were excised and weighed. One kidney was fixed in 10% formalin, embedded, and subsequently sectioned for histopathological examination and immunohistochemical (IHC) analysis. The remaining kidney tissue was immediately snap-frozen and stored in EP tubes for molecular analyses.

Construction of a cisplatin-caused CKD mouse model

All animal experiments were approved by the Ethics Committee of Nanjing Integrated Traditional Chinese and Western Medicine Hospital (Ethics No.23276). A total of 24 mice were randomly divided into four groups (n = 6 per group): the control group (CON), cisplatin-induced CKD model group (CDDP), cisplatin + low-dose WPD group (WPD-L), and cisplatin + high-dose WPD group (WPD-H).

Mice in the CDDP, WPD-L, and WPD-H groups received intraperitoneal (i.p.) injections of cisplatin at a dose of 8 mg/kg once per week for four consecutive weeks. Mice in the CON group received equivalent volumes of normal saline via i.p. Starting from the day after the first cisplatin injection, mice in the WPD-L and WPD-H groups received daily oral gavage administration of WPD for eight weeks. The CON and CDDP groups received equivalent volumes of saline by oral gavage. Mice were monitored for general health, and body weight was recorded every week until study completion.

Sample collection

Mice were euthanized with pentobarbital sodium (150 mg/kg, intraperitoneal injection) prior to sample collection. After euthanasia, blood samples were obtained, and serum was separated and stored for subsequent biochemical analyses.

Twenty-four-hour urine samples were collected and processed for further analysis. After centrifugation at low speed and low temperature to remove debris, supernatants were collected

Biochemical index assays

Blood urea nitrogen (BUN) serum creatinine (SCr) Kim-1 and NGAL levels were measured to evaluate renal function. Serum BUN and SCr levels were detected using assay kits (Nanjing Jiancheng Bioengineering Institute) according to the manufacturer's instructions. The concentrations of BUN and SCr were determined based on the corresponding optical density (OD) value.

Histological analysis

Fresh kidney tissues were collected and fixed in paraformaldehyde. After fixation, the samples were embedded, sectioned, and stained with hematoxylin and eosin (H&E). The pathological changes were subsequently observed under a microscope. Images were captured using a Nikon DS-U3 imaging system at 20 $\times$ magnification.

RT-qPCR

Total RNA was extracted using TRIzol reagent. Next, cDNA was synthesized using PrimeScript reverse transcriptase (TaKaRa) according to the manufacturer's instructions. Quantitative PCR was performed on an ABI Sequence Detection system (Applied Biosystems, CA). Gene expression levels were normalized to 36B4 RNA expression and calculated using the $2^{-\Delta\Delta CT}$ algorithms. The primers are listed in **Table 2**.

Western blot analysis

Kidney tissues were lysed in RIPA buffer, and protein concentrations were quantified using a commercial protein quantification kit. Equal

amounts of protein samples were denatured by boiling for 5 min, separated by SDS-PAGE, then transferred onto PVDF membranes. Subsequently, the membranes were incubated with corresponding primary antibodies at 4°C overnight for 12 h, followed by incubation with HRP-conjugated secondary antibodies. Protein signals were quantified using AlphaEaseFC software (version 3.1.2, Alpha Innotech Corporation, CA, USA). Primary antibodies against β -actin (Cat. No. 66009-1-Ig, 1:5,000 dilution), IL-1 β (Cat. No. 16806-1-AP, 1:1000 dilution), NLRP3 (Cat. No. 19771-1-AP, 1:1,000), and pro-caspase-1 (Cat. No. 81482-1-RR, 1:2,000 dilution) were purchased from Proteintech Group. Antibodies against cle-Caspase-1 (Cat. No. A0964, 1:2000) were purchased from Santa Cruz Biotechnology.

Statistical analysis

Statistical analyses were performed using SPSS 22.0. Continuous data were presented as mean $\pm$ standard deviation (SD). Comparisons between the two groups were performed using independent-samples t-tests. Categorical data were presented as frequencies and percentages and analyzed using Pearson's chi-square test. Differences among multiple groups were assessed using ANOVA followed by post hoc LSD test. A two-sided P value < 0.05 was considered statistically significant.

Results

Network pharmacology analysis of WPD and CKD-related targets

The active components and potential target proteins of WPD were obtained from the HERB Database (<http://herb.ac.cn/>). Based on the Genecards, TTD, and Drugbank databases, 4,960 predicted CKD-related targets were identified. According to the screening criteria of $DL \geq 0.18$ and $OB \geq 30\%$, 248 active ingredients were selected, corresponding on 1,157 potential targets (Tables S1, S2). After integration of component-target relationships, 751 overlapping targets were identified as candidate targets associated with the therapeutic effects of WPD on CKD (Figure 1A).

A "Herb-Active Ingredient-Target" interaction network was constructed using Cytoscape 3.9.1 to visualize the interactions between

WPD components and the top 50 candidate targets (Figure 1B). The network demonstrated that multiple components interact with multiple targets, and vice versa. Moreover, a PPI based on the top 50 candidate targets was constructed using the STRING database (Figure 1C). The results showed extensive interactions among these targets. The top 10 core targets included AKT, TNF, IL-6, ALB, SRC, IL-1 β , EGFR, CTNNB1, STAT3, and HIF-1 α (Figure 1D).

Functional enrichment analysis of CKD-related targets

To further investigate the potential mechanisms underlying the effects of WPD in CKD, the 751 overlapping targets were subjected to functional enrichment analysis. The top 30 significantly enriched GO terms are presented in Figure 2A. The enriched molecular functions included identical protein binding, protein tyrosine kinase activity, and ATP binding. The enriched cellular components include the plasma membrane, cell surface, and cytosol. The enriched biological processes were primarily associated with protein phosphorylation, inflammatory response, among other biological activities (Figure 2A). Among the enriched pathways, the PI3K-Akt, MAPK, cAMP, Ras, Rap1 signaling pathways were closely associated with CKD progression (Figure 2B).

Validation of the association between NLRP3 and CKD using public databases

The expression of NLRP3 was significantly higher in CKD patients than in healthy controls ($P < 0.05$). Moreover, ROC curve analysis demonstrated that NLRP3 exhibited potential diagnostic value for CKD, with an AUC of 0.665 (Figure 3).

Gene set enrichment analysis of NLRP3

Gene set enrichment analysis indicated that NLRP3 was primarily associated with inflammation-related signaling pathways (Figure 4).

Interaction network analysis of NLRP3 and its association with immune cell infiltration

As indicated by NLRP3-related interaction network, NLRP3 was mainly involved in inflammatory regulation and was closely related to immune cell populations, particularly macrophages and lymphocytes (Figure 5).

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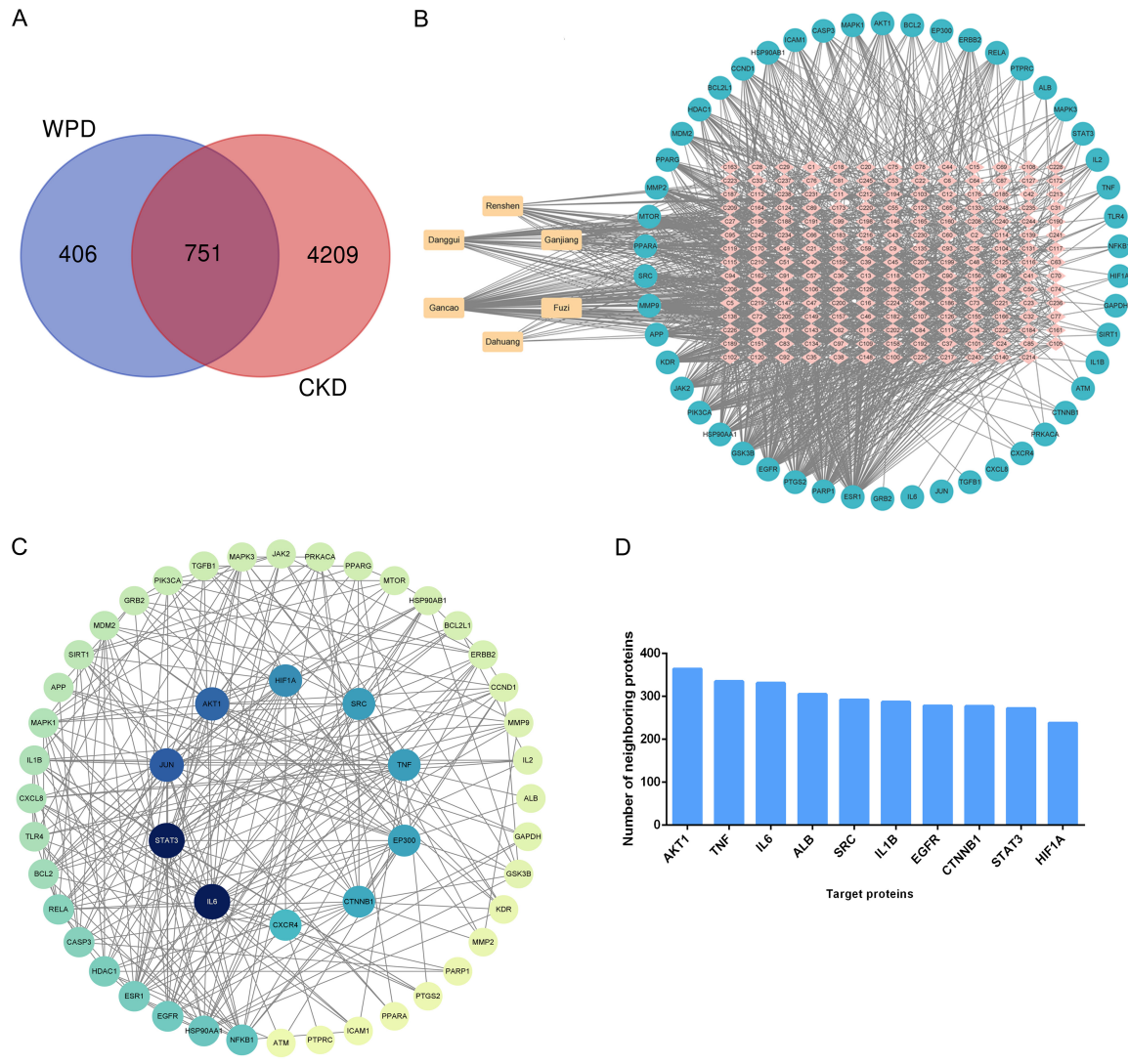


Figure 1. Identification of common targets and network analysis. A. Venn diagram showing the overlapping target genes between WPD and CKD; B. Herb-Active Ingredient-Target interaction network based on the top 50 candidate targets; C. Protein-protein interaction (PPI) network of the top 50 overlapping targets constructed using the STRING database; D. Bar chart showing the top 10 potential therapeutic targets identified from the PPI network.

Mendelian randomization analysis of the relationship between NLRP3 and CKD

To evaluate the potential correlation between NLRP3 and CKD, two-sample MR analysis was performed. A scatter plot was generated by integrating the effects of SNPs on NLRP3 expression and CKD risk. Using the IVW method, the overall causal effect of genetically predicted NLRP3 expression on CKD was assessed. The MR analysis suggested that NLRP3 may represent a risk factor for CKD (Figure 6).

Effect estimation of instrumental variables on CKD risk: To evaluate the contribution of indi-

vidual SNPs to CKD risk, a forest plot was generated (Figure 7). The IVW method was used to estimate the overall effect of NLRP3 expression on CKD risk. SNPs located on the left side of the null line represented protective effects, whereas those on the right side indicated increased risk.

MR Randomness Assessment: To assess potential bias and the distributional symmetry of SNP effects, a funnel plot was generated based on β coefficients and standard errors (SEs) of individual instrumental variables (Figure 8). The SNPs were roughly symmetrically distributed around the IVW estimate, indicating no apparent directional bias.

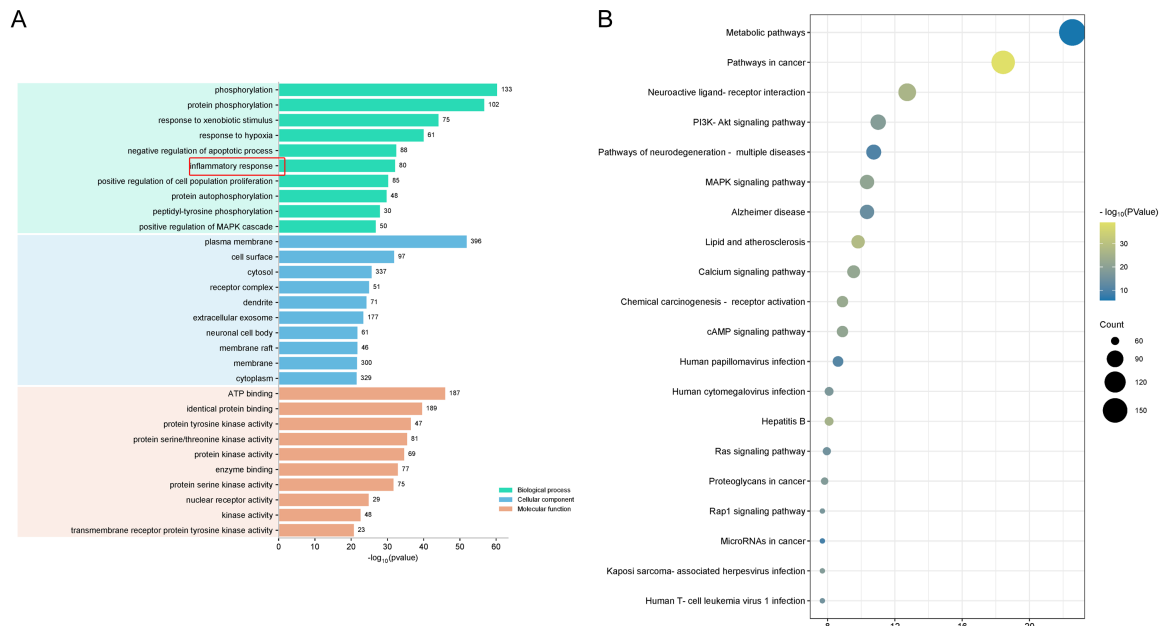


Figure 2. Functional enrichment analysis of overlapping targets. A. Gene Ontology (GO) enrichment analysis showing the significantly enriched terms in molecular function (MF), cellular component (CC), and biological process (BP) categories; B. Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis showing the significantly enriched signaling pathways.

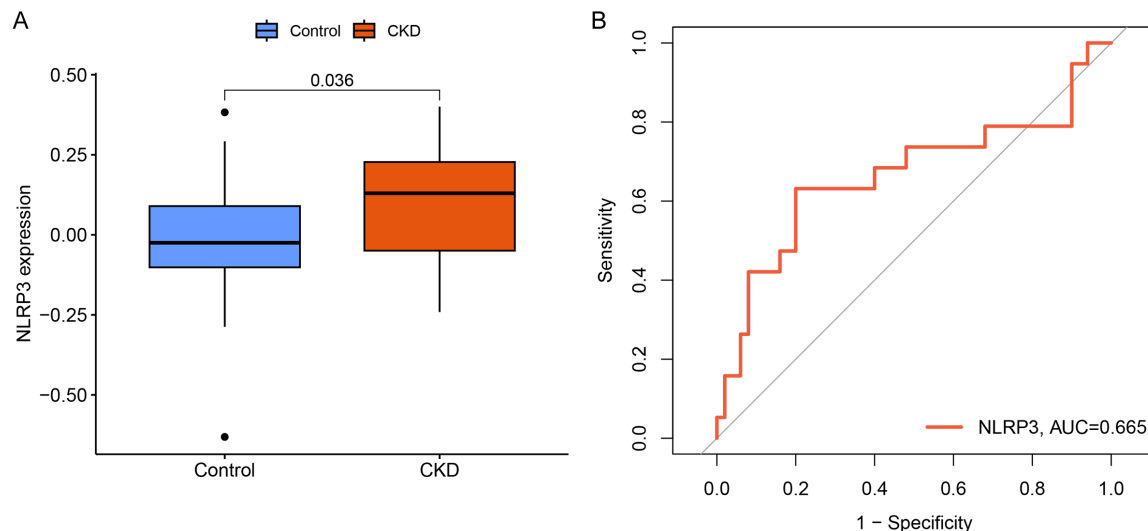


Figure 3. Association Between NLRP3 and CKD. A. Comparison of NLRP3 expression levels between CKD patients and healthy controls; B. Receiver operating characteristic (ROC) curve analysis evaluating the diagnostic performance of NLRP3 for CKD.

Sensitivity analysis

To evaluate the robustness of the MR findings, sensitivity analyses were performed, mainly including heterogeneity assessment, horizontal pleiotropy testing, and leave-one-out analysis.

Heterogeneity test: In this MR analysis, two-sample MR method integrates SNP-exposure and SNP-outcome datasets from different sources, leading to potential heterogeneity among samples. Therefore, Cochran's Q test was performed using the *mr_heterogeneity*

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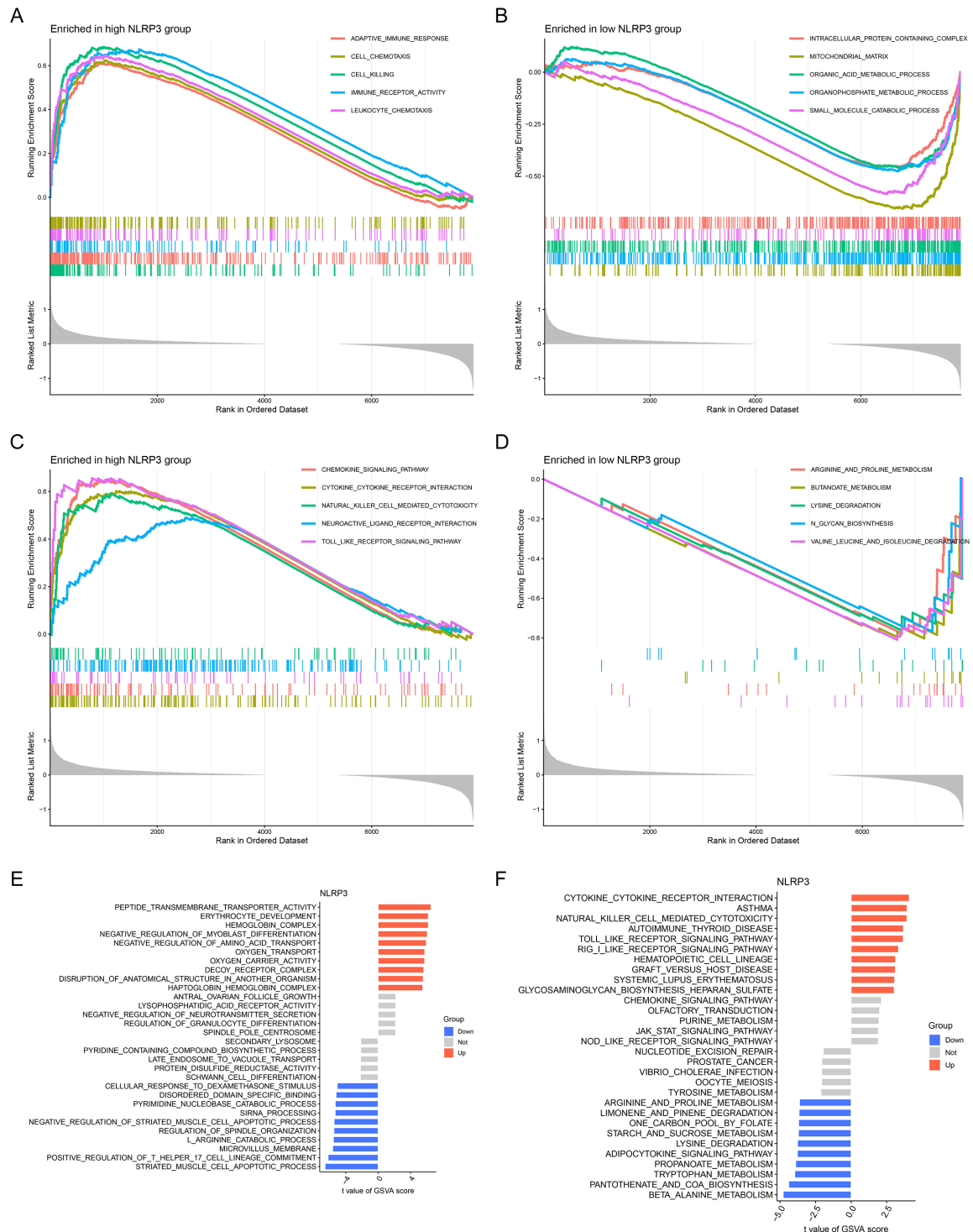


Figure 4. Gene set enrichment analysis of NLRP3-related pathways. A-D. Gene Set Enrichment Analysis (GSEA) showing the pathways associated with NLRP3 expression; E, F. Gene Set Variation Analysis (GSVA) illustrating the differences in pathway activity associated with NLRP3.

function in the *TwoSampleMR* R package. The results are presented in **Table 2**. For NLRP3, all Q_{test} P values were > 0.05 , indicating no significant heterogeneity among the included instrumental variables (**Table 3**).

Horizontal pleiotropy test: To evaluate the presence of confounding factors, horizontal pleiotropy was assessed using the *mr_pleiotropy_test* function in the *TwoSampleMR* R package. The intercept test showed P values > 0.05 , indi-

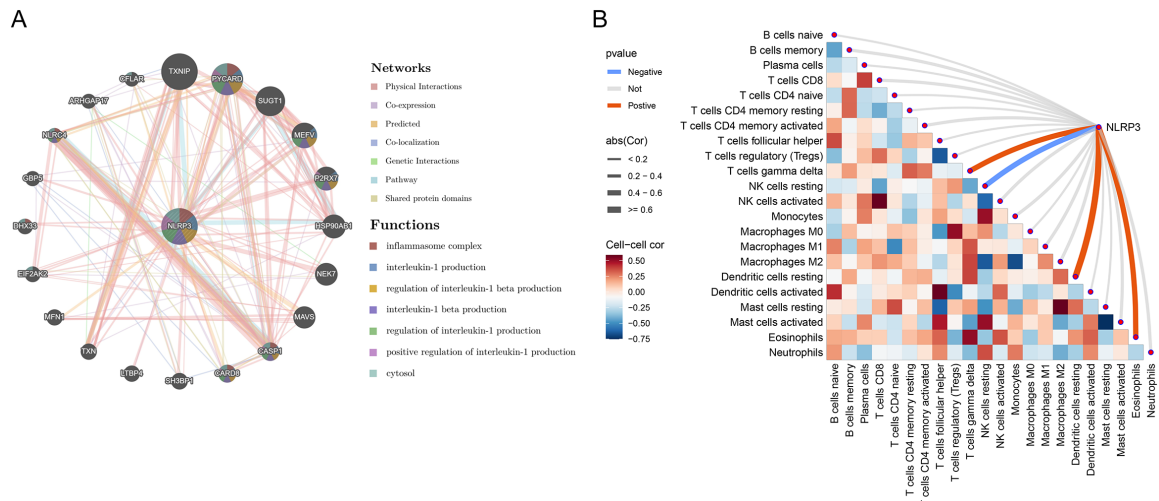


Figure 5. Interaction network of NLRP3 and its correlation with immune cell infiltration. A. Protein-protein interaction network of NLRP3 protein and its associated genes; B. Correlation between NLRP3 and immune cell infiltration.

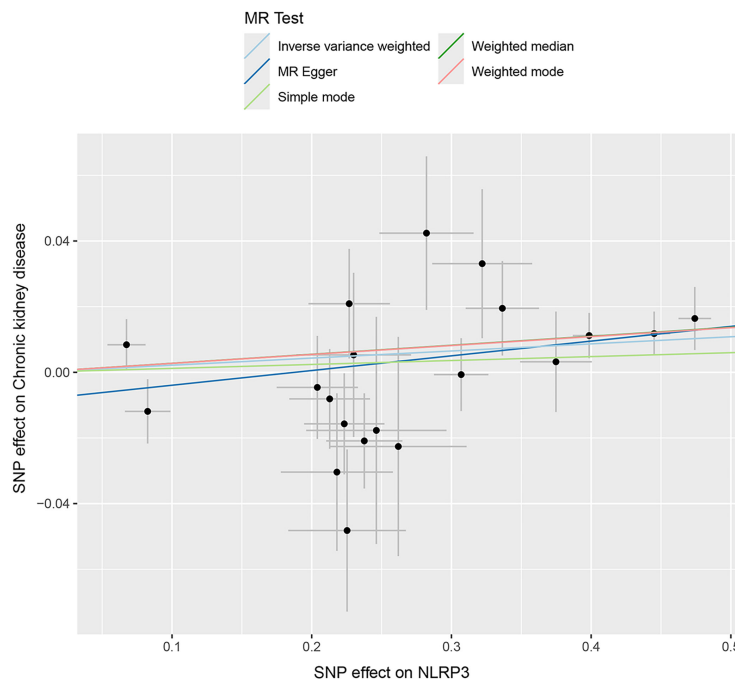


Figure 6. Scatter plot showing the causal association between NLRP3 expression and CKD risk based on mendelian randomization analysis.

cating no evidence of horizontal pleiotropy. In addition, MR-PRESSO analysis was performed using the *mr_presso* function. The MR-PRESSO global test also showed no significant pleiotropic effects ($P > 0.05$), further supporting the robustness of the MR results (Table 4).

Leave-one-out analysis: To determine whether any single SNP exerted a substantial influence

on the overall causal estimate, a leave-one-out analysis was performed using the *mr_leaveoneout* function in the *TwoSampleMR* R package. Each SNP was sequentially removed, and the pooled effect estimate was recalculated using the IVW method. The results remained stable after removing individual SNPs, with no substantial deviations from the overall estimate (Figure 9).

Effects of WPD on cisplatin-induced CKD

After 8 weeks of intervention, mice in the CDDP group exhibited a significant reduction in body weight compared with the CON group. WPD-H treatment significantly alleviated body weight loss induced by cisplatin ($P < 0.001$) (Figure 10A).

Compared with the CON group, kidney weight in the CDDP group was significantly reduced, indicating kidney atrophy. Both the WPD-L and WPD-H groups exhibited higher kidney weights than the CDDP group (Figure 10B).

Serum BUN and Scr levels were significantly elevated in the CDDP group compared with the control group ($P < 0.001$). Both WPD-L and

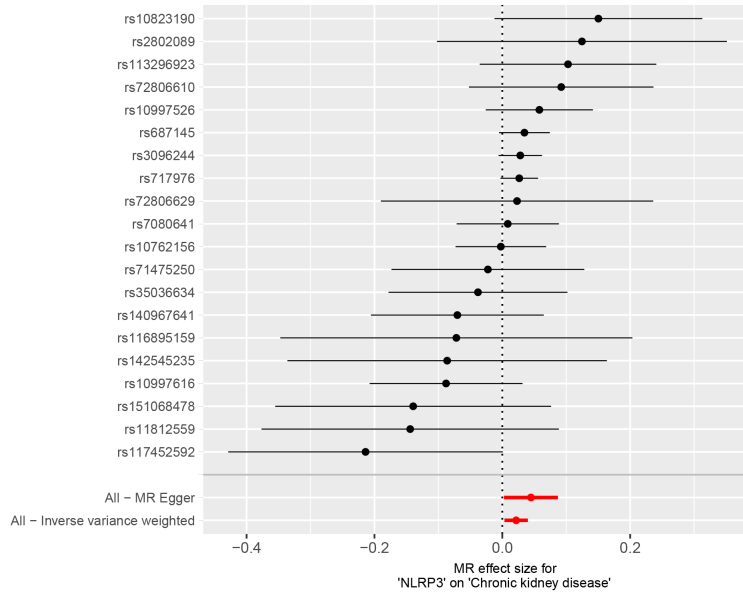


Figure 7. Forest plot.

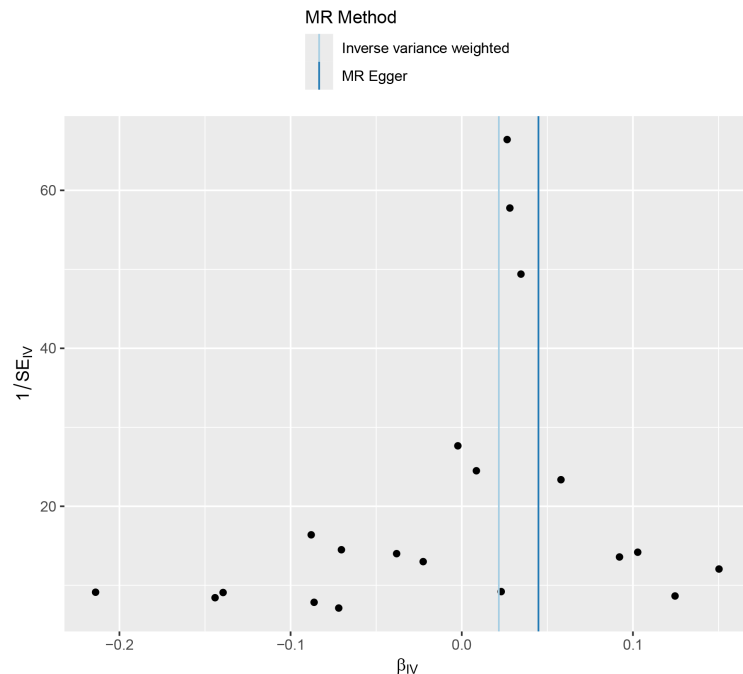


Figure 8. Funnel plot assessing potential bias in mendelian randomization analysis.

WPD-H treatments significantly reduced BUN levels compared with the CDDP group ($P < 0.001$), while WPD-H treatment also significantly decreased SCr levels ($P < 0.01$) (Figure 10C, 10D).

Histological analysis showed that the CDDP group showed severe tubular injury and fibro-

sis, whereas both WPD-L and WPD-H groups showed varying degrees of improvement in renal pathological changes ($P < 0.05$) (Figure 10E).

Levels of Kim-1 and NGAL were significantly increased in the CDDP group compared with the CON group ($P < 0.001$). Both the WPD-L and WPD-H groups exhibited significantly reduced Kim-1 and NGAL levels compared with the CDDP group ($P < 0.01$) (Figure 10F, 10G).

WPD inhibited cisplatin-induced kidney fibrosis in CKD mice

After 8 weeks of intervention, the CDDP group exhibited the most extensive renal fibrosis, showing a significantly increased fibrotic area compared with the CON group ($P < 0.001$). Both WPD-L and WPD-H treatments significantly reduced renal fibrosis compared with the CDDP group ($P < 0.001$) (Figure 11A). The expression levels of fibrosis-related markers, fibronectin and collagen-I, were significantly increased in the CDDP group compared with the CON group ($P < 0.001$). Both WPD-L and WPD-H treatments significantly decreased fibronectin and collagen-I levels compared with the CDDP group ($P < 0.05$) (Figure 11B, 11C).

Comparison of renal NLRP3 inflammasome and inflammatory mediators across intervention groups

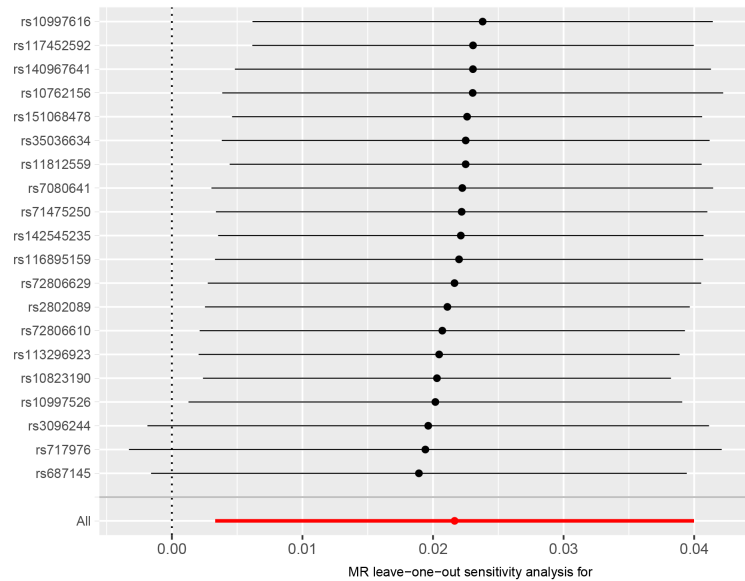
After 8 weeks of intervention, renal protein levels of NLRP3, caspase-1, and cleaved caspase-1 were significantly increased in the CDDP group compared with the CON group ($P < 0.05$). The WPD-H group exhibited significantly lower expression levels of NLRP3, caspase-1, and cleaved caspase-1 (p17) than the CDDP group ($P < 0.05$) (Figure 12A, 12B). The mRNA levels

Table 3. Results of the forward MR heterogeneity test

Outcome	Exposure	Method	Q	Q_df	Q_pval
Chronic kidney disease	NLRP3	MR Egger	21.559051940265	18	0.252151281174507
Chronic kidney disease	NLRP3	Inverse variance-weighted	23.253703208605	19	0.226352039192401

Table 4. Horizontal pleiotropy

Outcome	Exposure	Egger_intercept	S.E	P value
Chronic kidney disease	NLRP3	-0.008419158	0.00707794322714591	0.249700497058389

**Figure 9.** Forest plot of the leave-one-out test.

of IL-1 β , IL-6, TNF- α , and NLRP3 were obviously elevated in renal tissue from the CDDP group compared with the CON group ($P < 0.05$). Both WPD-L and WPD-H treatments significantly reduced renal NLRP3 mRNA levels compared with the CDDP group ($P < 0.01$). Furthermore, WPD-H treatment significantly decreased renal mRNA levels of TNF- α and IL-6 compared with the CDDP group ($P < 0.05$) (**Figure 12C-F**).

Discussion

WPD, a classical TCM formula, has been widely applied in clinical practice and has demonstrated therapeutic efficacy in treating CKD and digestive system disorders [11]. However, the precise molecular mechanisms underlying the effects of WPD on CKD remain unclear. Network pharmacology offers an efficient and systematic approach to explore the potential therapeutic mechanisms of TCM formulas in treating

CKD, which is consistent with the holistic characteristics of TCM. To further validate the predicted mechanisms derived from network pharmacology analysis, we conducted experimental studies. In this study, a cisplatin-induced CKD mouse model was established to evaluate the therapeutic effects of WPD intervention.

Our findings demonstrated that WPD intervention effectively alleviated CKD progression in the cisplatin-induced CKD model. WPD-treated mice exhibited elevated body weight maintenance, improved kidney function, and reduced renal injury. Renal fibrosis is a common pathological feature of various

kidney diseases and is closely associated with progressive renal function decline. In this study, WPD significantly attenuated renal fibrosis in the CKD model mice, indicating its potential protective effects against CKD progression.

Network pharmacology analysis revealed that inflammation-related signaling pathways may play an important role in WPD's therapeutic effects on CKD. Previous studies have demonstrated the significant role of NLRP3 inflammasome in renal fibrosis, acute kidney injury (AKI), and CKD progression [24-27]. Increased NLRP3 expression has been reported in various kidney disease models, including 5/6 nephrectomy and cisplatin-induced renal injury [28, 29]. Moreover, elevated NLRP3 expression has been observed in renal biopsy specimens from patients with both acute and chronic kidney diseases [30]. These findings suggest that NLRP3 inflammasome activation contributes to

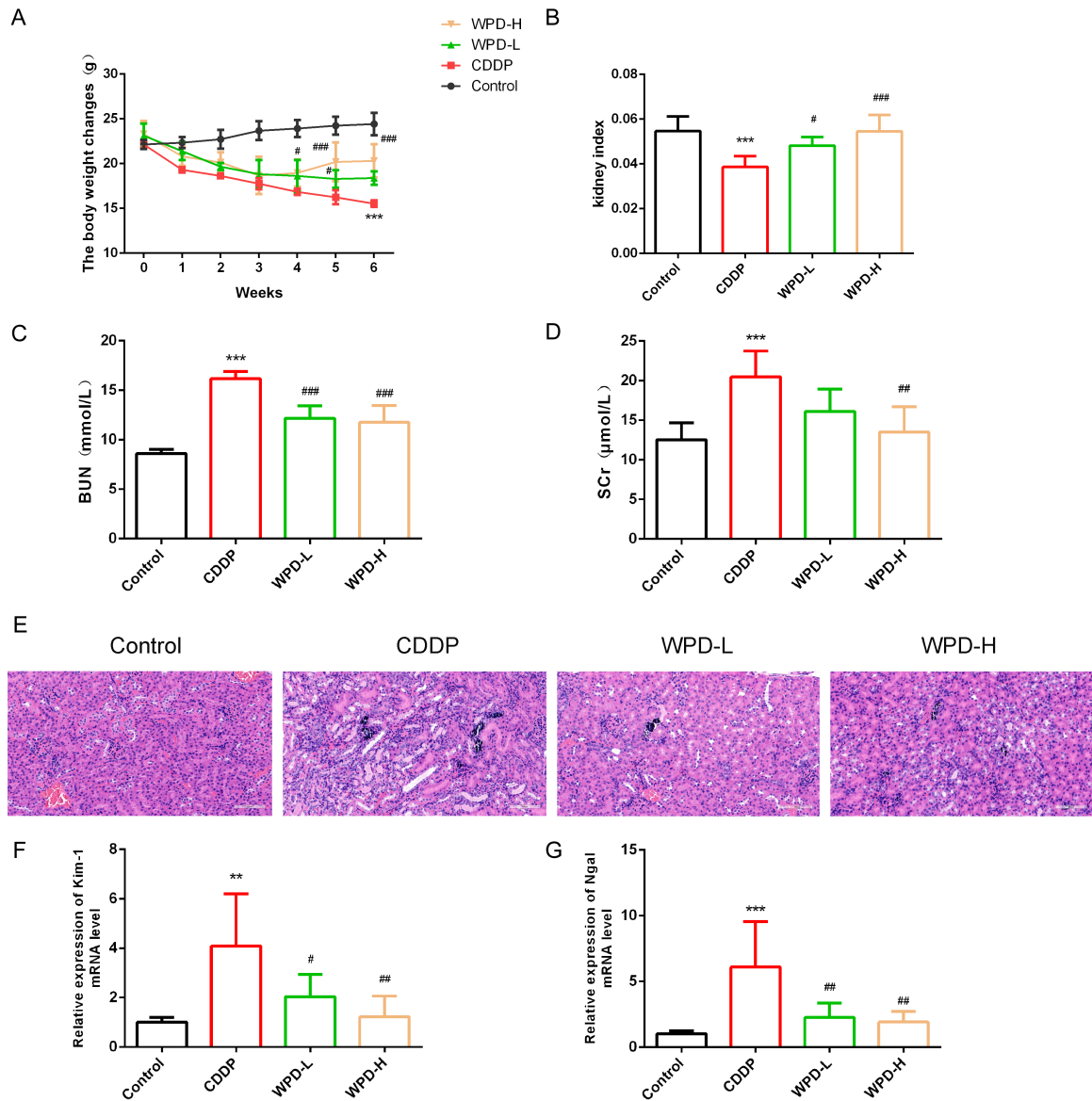


Figure 10. Effects of WPD on physiological parameters, renal function, renal injury, and histopathological changes after 8 weeks of intervention. A. Body weight; B. Kidney weight; C. Serum blood urea nitrogen (BUN) levels; D. Serum creatinine (Scr) levels; E. Renal histopathology (H&E); F. Renal Kim-1 expression levels; G. Renal NGAL expression levels. Notes: **P < 0.01, ***P < 0.001 vs. Control group; #P < 0.05, ##P < 0.01, ###P < 0.001 vs. CDDP group.

renal injury progression, whereas its inhibition may alleviate renal damage. Therefore, targeting the NLRP3 inflammasome represents a promising therapeutic strategy for CKD treatment.

Our study reveals that WPD intervention in the cisplatin-induced CKD model attenuated disease progression by suppressing the activation of the NLRP3/Caspase-1 pathway and reducing the production of inflammatory cytokines. These findings are consistent with previous studies demonstrating that UUO model increases the expression levels of caspase-1,

IL-1 β , and IL-18, promoting NLRP3 inflammasome activation. Conversely, NLRP3 knockout mice subjected to UUO exhibited reduced tubular injury and renal fibrosis [31]. Additionally, studies using caspase-1 or NLRP3 knockout mice have demonstrated alleviated kidney injury in ischemia-reperfusion-induced AKI models [31, 32]. Evidence further suggests that NLRP3 deficiency protects against AKI by inhibiting inflammation and ferroptosis [33].

WPD, a classical TCM formula composed of seven herbs in specific proportions, is clinically

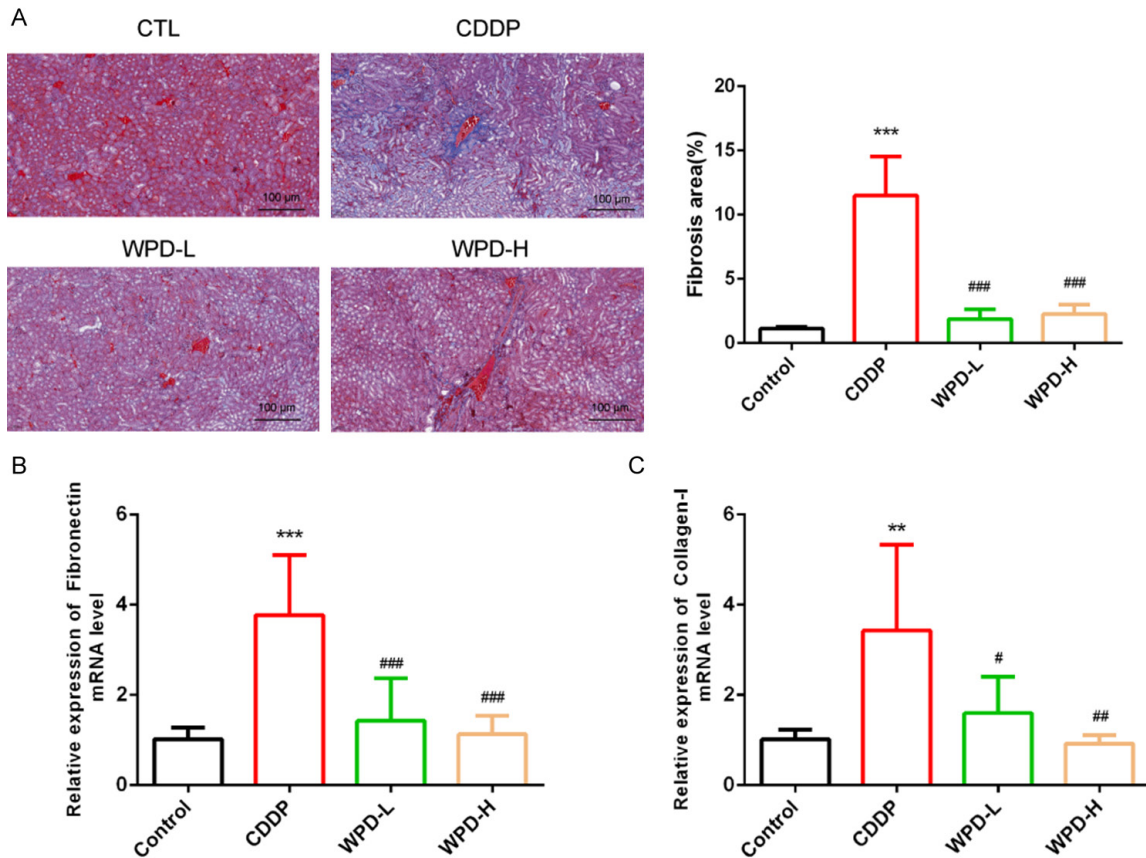


Figure 11. Effects of WPD on renal fibrosis after 8 weeks of intervention. A. Representative images and quantification of renal fibrosis area; B. Renal fibronectin expression levels; C. Renal Collagen-I expression levels. Notes: **P < 0.01, ***P < 0.001 vs. Control group; #P < 0.05, ###P < 0.01, ###P < 0.001 vs. CDDP group.

used for treating acute and chronic kidney diseases, as well as gastrointestinal disorders such as constipation, intestinal obstruction, and ulcerative colitis. Emerging evidence suggests that WPD may regulate intestinal function, and alterations in gut microbiota may suppress NLRP3 inflammasome activation, thereby improving renal fibrosis and renal function, as well as slowing CKD progression [34-36]. Our study demonstrated that WPD intervention significantly reduced the expressions levels of inflammatory mediators, including IL-1 β , IL-6, and TNF- α . Studies have also shown that inhibition of the NLRP3/IL-1 β signaling pathway may reduce CKD-related atrial fibrillation [37]. Furthermore, targeting IL-6 has been proposed as a protective strategy against post-AKI renal fibrosis by modulating the DNMT1/Wnt/ β -catenin axis, providing a potential therapeutic approach for preventing AKI-to-CKD transition [38]. Experimental evidence also demon-

strated that TNF- α inhibition improves proteinuria, reduces inflammatory responses, decreases tubular cell apoptosis, and attenuates renal fibrosis in glomerulonephritis mouse models [39].

Although this study provides valuable insights into the mechanisms underlying the therapeutic effects of WPD against CKD, several limitations should be acknowledged. First, CKD can result from diverse etiologies, including sepsis and autoimmune diseases. Therefore, further validation using additional CKD models is required to confirm the therapeutic targets and efficacy of WPD beyond cisplatin-induced renal injury. Second, current animal models cannot fully replicate the complex pathological characteristics of human CKD, and further validation through randomized controlled clinical trials are warranted. Finally, a comprehensive safety assessment, including liver function evaluation,

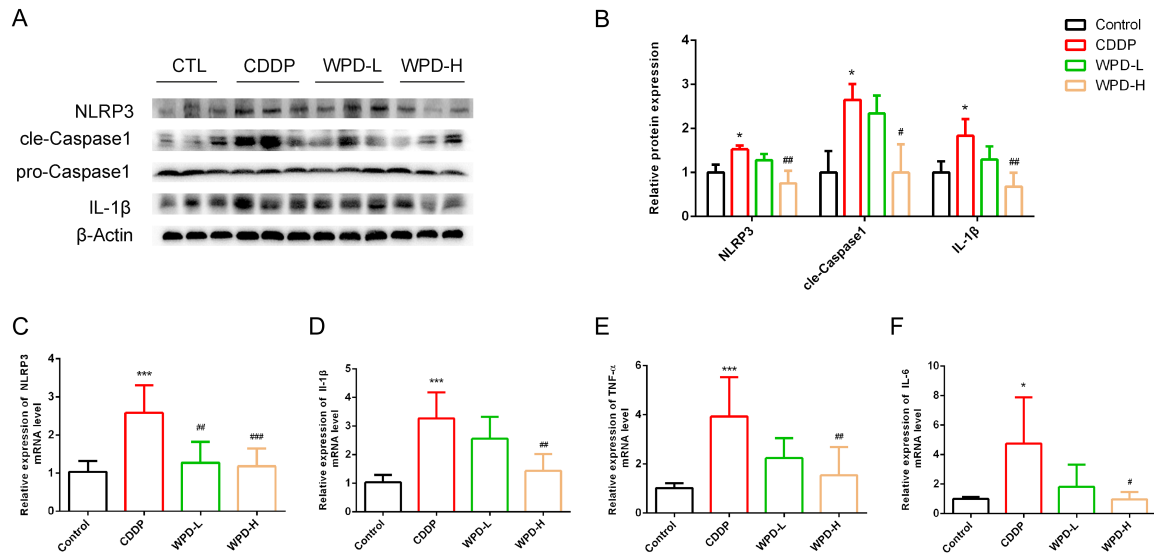


Figure 12. Effects of WPD on the renal NLRP3 inflammasome pathway and inflammatory cytokines after 8 weeks of intervention. A, B. Representative Western blot images and quantitative analysis of renal NLRP3, caspase-1, and cleaved caspase-1 (p17) protein levels; C. Renal NLRP3 mRNA levels; D. Renal IL-1β mRNA levels; E. Renal TNF-α mRNA levels; F. Renal IL-6 mRNA levels. Notes: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. CON group; while # vs. CDDP group.

hematological analysis, potential side effects, and long-term safety monitoring, should be conducted in future studies.

Conclusion

This study integrated network pharmacology analysis with experimental validation to investigate the potential mechanisms underlying the therapeutic effects of WPD against CKD. Our findings demonstrated that WPD improved renal function and alleviated renal fibrosis in a cisplatin-induced CKD mouse model. These renoprotective effects may be mediated, at least partially, through inhibition of NLRP3/Caspase-1 signaling pathway activation.

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Disclosure of conflict of interest

None.

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References

- [1] Romagnani P, Agarwal R, Chan JCN, Levin A, Kalyesubula R, Karam S, Nangaku M, Rodriguez-Iturbe B and Anders HJ. Chronic kidney disease. Nat Rev Dis Primers 2025; 11: 8.
- [2] GBD 2023 Chronic Kidney Disease Collaborators. Global, regional, and national burden of chronic kidney disease in adults, 1990-2023, and its attributable risk factors: a systematic analysis for the Global Burden of Disease Study 2023. Lancet 2025; 406: 2461-2482.
- [3] Foreman KJ, Marquez N, Dolgert A, Fukutaki K, Fullman N, McGaughey M, Pletcher MA, Smith AE, Tang K, Yuan CW, Brown JC, Friedman J, He J, Heuton KR, Holmberg M, Patel DJ, Reidy P, Carter A, Cercy K, Chapin A, Douwes-Schultz D, Frank T, Goettsch F, Liu PY, Nandakumar V, Reitsma MB, Reuter V, Sadat N, Sorensen RJD, Srinivasan V, Updike RL, York H, Lopez AD, Lozano R, Lim SS, Mokdad AH, Vollset SE and Murray CJL. Forecasting life expectancy, years

- of life lost, and all-cause and cause-specific mortality for 250 causes of death: reference and alternative scenarios for 2016-40 for 195 countries and territories. *Lancet* 2018; 392: 2052-2090.
- [4] Huang X, Wang M, Yu B, Ren P, Bian X, Wang P, Yao X and Lin W. Transition from acute kidney injury to chronic kidney disease: molecular mechanisms and therapeutic interventions. *Mol Biomed* 2026; 7: 64.
- [5] Zhang T, Widdop RE and Ricardo SD. Transition from acute kidney injury to chronic kidney disease: mechanisms, models, and biomarkers. *Am J Physiol Renal Physiol* 2024; 327: F788-F805.
- [6] Guo S, Zhou L, Liu X, Gao L, Li Y and Wu Y. Baicalin alleviates cisplatin-induced acute kidney injury by inhibiting ALOX12-dependent ferroptosis. *Phytomedicine* 2024; 130: 155757.
- [7] Bai Y, Chang Y, Zhang L, Zhou W, Su Y and Zhou J. Yiqi-Wenyang-Tiaoshen decoction reduces cisplatin-induced acute kidney injury in rats through autophagy and apoptosis signaling pathways based on network pharmacology and experimental validation. *Mediators Inflamm* 2026; 2026: e5435119.
- [8] Wang X, Guo S, Chen J, Cheng R, Zhou F, Huang L, Yang L, Lu Z, Li S, Li C, Mao W, Tian R and Xu P. Integrated network pharmacology and molecular docking combined with metabolomics to identify the pharmacological mechanisms of Jiedu Huoxue decoction against cisplatin-induced acute kidney injury. *J Ethnopharmacol* 2025; 351: 120088.
- [9] Zheng D, Ruan X, Wu Q, Qiu Y and Ruan S. Yishen Jiangzhuo decoction attenuates cisplatin-induced acute kidney injury by inhibiting inflammation, oxidative stress and apoptosis through the TNF signal pathway. *Exp Ther Med* 2024; 28: 331.
- [10] Zhu J, Yuan A, Le Y, Chen X, Guo J, Liu J, Chen H, Wang CY, Lu D and Lu K. Yi-Qi-Jian-Pi-Xiao-Yu formula inhibits cisplatin-induced acute kidney injury through suppressing ferroptosis via STING-NCOA4-mediated ferritinophagy. *Phyto-medicine* 2024; 135: 156189.
- [11] Wang R, Shi QQ and Zhu CF. Mechanism of action of Wenpi decoction in homotherapy for heteropathy in treatment of chronic kidney disease and ulcerative colitis: a study based on network pharmacology and molecular docking. *Hunan Journal of Traditional Chinese Medicine* 2025; 41: 151-157.
- [12] Ren D, Liu Q, Liao H, Wang M, Wang Y, Guo W, Duan C, Zhou Y and Luo Z. Inflammation, cell death, and lncRNAs: unraveling the mechanisms of sepsis-associated acute kidney injury. *Front Immunol* 2026; 17: 1710624.
- [13] Zhou Y, Zhou L, Wang M, Xu L, Li T and Wang C. Shen Shuai II Recipe alleviates renal fibrosis in chronic kidney disease by improving of hypoxia and inhibition of TLR4/MyD88/NF- κ B/NLRP3 pathway. *Ren Fail* 2025; 47: 2502875.
- [14] Tan H, Li F, Cui Y, Li Z, Yan S and Deng Q. ATP/P2X7 receptor/NLRP3 pathway facilitates renal tubular epithelial-myofibroblast transdifferentiation and interstitial fibrosis in rats with unilateral ureteral obstruction. *Front Pharmacol* 2025; 16: 1598151.
- [15] Yu F, Meng Y, Wang X, Chen J, He J, Yi X, He Y and Chen K. Protein lactylation of citrate synthase promotes the AKI-CKD transition by activating the NLRP3 inflammasome. *Cell Rep* 2025; 44: 116084.
- [16] Han X, Yu H, Gao Q, Wang X, Zhang L, Zhao Q, Yu L, Zhang Y, Sui M and Li Y. Gut microbiota dysbiosis promotes chronic kidney disease-associated atrial fibrillation through activation of the NLRP3 inflammasome. *Europace* 2026; 28: euag037.
- [17] Yao Y, Wan R, An Y, He B, Tan Q, Xu H, Pan X, Liu S, Chen X and Li J. Kaempferol ameliorates renal injury by inhibiting Piezo1/HIF-1 α /ROS/NLRP3 signaling in chronic kidney disease. *Biochem Pharmacol* 2026; 243: 117546.
- [18] Lu M, Yin J, Xu T, Dai X, Liu T, Zhang Y, Wang S, Liu Y, Shi H, Zhang Y, Mo F, Sukhorukov V, Orekhov AN, Gao S, Wang L and Zhang D. Fuling-Zexie formula attenuates hyperuricemia-induced nephropathy and inhibits JAK2/STAT3 signaling and NLRP3 inflammasome activation in mice. *J Ethnopharmacol* 2024; 319: 117262.
- [19] Liu Q, Li X, Li Y, Luo Q, Fan Q, Lu A, Guan D and Li J. A novel network pharmacology strategy to decode mechanism of Wu ling Powder in treating liver cirrhosis. *Chin Med* 2024; 19: 36.
- [20] Liu Q, Luo Q, Fan Q, Li Y, Lu A and Guan D. Screening of the key response component groups and mechanism verification of Huangqi-Guizhi-Wuwu-Decoction in treating rheumatoid arthritis based on a novel computational pharmacological model. *BMC Complement Med Ther* 2024; 24: 4.
- [21] Jiang Q, Pu D, Hu Y, Wu R, Jiang C, Yuan S, Zhu X and Xu L. Network pharmacology-based investigation of potential targets of mulberry twig acting on polycystic ovary syndrome. *Medicine (Baltimore)* 2025; 104: e46163.
- [22] Araoka T, Toyohara K, Ryosaka M, Inui C, Matsuura M, Ma C, Watahiki J, Li Z, Iwasaki M, Watanabe A, Yokokawa R, Tabata Y, Izpisua Belmonte JC and Osafune K. Human iPSC-derived nephron progenitor cells treat acute kidney injury and chronic kidney disease in mouse models. *Sci Transl Med* 2025; 17: eadt5553.

- [23] Yang J, Yuan L, Li L, Liu F, Liu J, Chen Y, Fu P, Lu Y and Yuan Y. Trehalose activates autophagy to alleviate cisplatin-induced chronic kidney injury by targeting the mTOR-dependent TFEB signaling pathway. *Theranostics* 2025; 15: 2544-2563.
- [24] Yan YL, Wu YJ, Liao TQ, Yue BJ, Li MY, Chen C, Du JR and Long FY. Senkyunolide I alleviated renal fibrosis in UUO mice by regulating the Nrf2/xCT/GPX4 and NLRP3/caspase-1/GSDMD pathways to inhibit ferroptosis and pyroptosis. *FASEB J* 2025; 39: e71193.
- [25] Zeng J, Zhang Y and Huang C. Macrophages polarization in renal inflammation and fibrosis animal models (Review). *Mol Med Rep* 2024; 29: 29.
- [26] Li Z, Wang X, Peng Y, Yin H, Yu S, Zhang W and Ni X. *Nlrp3* deficiency alleviates lipopolysaccharide-induced acute kidney injury via suppressing renal inflammation and ferroptosis in mice. *Biology (Basel)* 2023; 12: 1188.
- [27] Huang G, Zhang Y, Zhang Y and Ma Y. Chronic kidney disease and NLRP3 inflammasome: pathogenesis, development and targeted therapeutic strategies. *Biochem Biophys Rep* 2022; 33: 101417.
- [28] Krishnan SM, Ling YH, Huuskes BM, Ferens DM, Saini N, Chan CT, Diep H, Kett MM, Samuel CS, Kemp-Harper BK, Robertson AAB, Cooper MA, Peter K, Latz E, Mansell AS, Sobey CG, Drummond GR and Vinh A. Pharmacological inhibition of the NLRP3 inflammasome reduces blood pressure, renal damage, and dysfunction in salt-sensitive hypertension. *Cardiovasc Res* 2019; 115: 776-787.
- [29] Gong ZG, Zhao Y, Wang ZY, Fan RF, Liu ZP and Wang L. Epigenetic regulator BRD4 is involved in cadmium-induced acute kidney injury via contributing to lysosomal dysfunction, autophagy blockade and oxidative stress. *J Hazard Mater* 2022; 423: 127110.
- [30] Vilaysane A, Chun J, Seamone ME, Wang W, Chin R, Hirota S, Li Y, Clark SA, Tschopp J, Trpkov K, Hemmelgarn BR, Beck PL and Muruve DA. The NLRP3 inflammasome promotes renal inflammation and contributes to CKD. *J Am Soc Nephrol* 2010; 21: 1732-1744.
- [31] Shigeoka AA, Mueller JL, Kambo A, Mathison JC, King AJ, Hall WF, Correia Jda S, Ulevitch RJ, Hoffman HM and McKay DB. An inflammasome-independent role for epithelial-expressed Nlrp3 in renal ischemia-reperfusion injury. *J Immunol* 2010; 185: 6277-6285.
- [32] Ting JP, Willingham SB and Bergstralh DT. NLRs at the intersection of cell death and immunity. *Nat Rev Immunol* 2008; 8: 372-379.
- [33] Wang B, Wang Y, Zhang J, Hu C, Jiang J, Li Y and Peng Z. ROS-induced lipid peroxidation modulates cell death outcome: mechanisms behind apoptosis, autophagy, and ferroptosis. *Arch Toxicol* 2023; 97: 1439-1451.
- [34] Magliocca G, Mone P, Di Iorio BR, Heidland A and Marzocco S. Short-chain fatty acids in chronic kidney disease: focus on inflammation and oxidative stress regulation. *Int J Mol Sci* 2022; 23: 5354.
- [35] Yi C, Sun W, Ding L, Yan M, Sun C, Qiu C, Wang D and Wu L. Short-chain fatty acids weaken Ox-LDL-induced cell inflammatory injury by inhibiting the NLRP3/caspase-1 pathway and affecting cellular metabolism in THP-1 cells. *Molecules* 2022; 27: 8801.
- [36] Speer T, Dimmeler S, Schunk SJ, Fliser D and Ridker PM. Targeting innate immunity-driven inflammation in CKD and cardiovascular disease. *Nat Rev Nephrol* 2022; 18: 762-778.
- [37] Song J, Navarro-Garcia JA, Wu J, Saljic A, Abutaha I, Li L, Lahiri SK, Keefe JA, Aguilar-Sanchez Y, Moore OM, Yuan Y, Wang X, Kamler M, Mitch WE, Ruiz-Hurtado G, Hu Z, Thomas SS, Dobrev D, Wehrens XH and Li N. Chronic kidney disease promotes atrial fibrillation via inflammasome pathway activation. *J Clin Invest* 2023; 133: e167517.
- [38] Guo X, Zhu Y, Sun Y and Li X. IL-6 accelerates renal fibrosis after acute kidney injury via DNMT1-dependent FOXO3a methylation and activation of Wnt/ β -catenin pathway. *Int Immunopharmacol* 2022; 109: 108746.
- [39] Lousa I, Reis F, Santos-Silva A and Belo L. The signaling pathway of TNF receptors: linking animal models of renal disease to human CKD. *Int J Mol Sci* 2022; 23: 3284.