Introduction

Diabetic nephropathy (DN) stands out as a prominent microvascular complication of diabetes mellitus and represents the primary cause of end-stage renal disease. Recent years have witnessed a surge in the incidence of DN.\(^1\) Due to the limited regenerative capacity of podocytes, their injury and loss contribute to the destruction of the glomerular filtration membrane, exacerbating the primary cause of end-stage renal disease. Recent years have witnessed a surge in the incidence of DN.\(^1\) This disease manifests progressively, with initial clinical stages marked by proteinuria and glomerular hyperfiltration. As the condition progresses, there is a gradual decline in the glomerular filtration rate.\(^2\) Studies have shown that diabetes-induced proteinuria primarily exhibits disruption of the fissure diaphragm’s integrity, loss of podocytes, and thickening of the glomerular basement membrane.\(^3\) Podocytes, being highly differentiated cells forming the glomerular filtration barrier alongside vascular endothelial cells and the basement membrane,\(^4\) play a crucial role. Impairment of this barrier has been linked to proteinuria, glomerular lesions, and renal function abnormalities.\(^5,6\) Due to the limited regenerative capacity of podocytes, their injury and loss contribute to the destruction of the glomerular filtration membrane, exacerbating the primary cause of end-stage renal disease. Recent years have witnessed a surge in the incidence of DN.\(^1\) This disease manifests progressively, with initial clinical stages marked by proteinuria and glomerular hyperfiltration. As the condition progresses, there is a gradual decline in the glomerular filtration rate.\(^2\) Studies have shown that diabetes-induced proteinuria primarily exhibits disruption of the fissure diaphragm’s integrity, loss of podocytes, and thickening of the glomerular basement membrane.\(^3\) Podocytes, being highly differentiated cells forming the glomerular filtration barrier alongside vascular endothelial cells and the basement membrane,\(^4\) play a crucial role. Impairment of this barrier has been linked to proteinuria, glomerular lesions, and renal function abnormalities.\(^5,6\) Due to the limited regenerative capacity of podocytes, their injury and loss contribute to the destruction of the glomerular filtration membrane, exacerbating the
nephropathic process. Given the side effects associated with podocyte-protective drugs like angiotensin-converting enzyme inhibitors (ACEI) and endothelin receptor antagonists, such as hyperkalemia, exploring new and safe drugs for DN treatment is imperative. Therefore, naturally produced drugs like traditional Chinese medicine (TCM) have attracted significant interest in recent years. Jiawei Shengjiangsan, an empirical formula for DN treatment in TCM, has a historical track record, yet its impact on DN and the underlying mechanisms remain unclear, warranting further research.

The etiology of DN is complex and diverse, with inflammatory mechanisms being a major known pathogenic factor. It has been reported that the downstream signaling pathway of Phosphatidylinositol 3-kinase (PI3K) significantly influences cell growth, survival, and motility. The PI3K/Akt pathway activation under diabetic conditions affects renal tubular cell growth, epithelial-mesenchymal transition (EMT), and lipid metabolism. Hyperglycemia induces damage to glomerular and tubular cells, resulting in increased expression of inflammatory mediators that, in turn, induce renal injury through various mechanisms. Inflammatory mediators can contribute to extracellular matrix deposition and myofibroblast differentiation and proliferation through signaling pathways like NF-κB and JAK/STAT. Current evidence indicates that activation of the PI3K/Akt/NF-κB signaling pathway significantly increases downstream inflammatory cytokines like TNF-α, IL-1β, and IL-6, triggering renal inflammation and accelerating DN development. Therefore, intervening in the PI3K/Akt/NF-κB signaling pathway emerges as a promising strategy for DN prevention and treatment.

Jiawei Shengjiangsan (JWSJS), known for its efficacy of ascending lucidity and descending turbidity, opening sweat pores, and activating channels, has shown promise in reducing proteinuria and improving renal function. Additionally, Song et al demonstrated that JWSJS reduced podocyte pyroptosis and attenuated renal injury in diabetic kidney disease (DKD) rats by down-regulating the TXNIP/NLRP3 signaling pathway. Moreover, mounting evidence suggests the involvement of the PI3K/Akt/NF-κB signaling pathway in renal disease.

This study explored the intervention mechanism of JWSJS on renal injury in diabetic nephropathy, with emphasis on the PI3K/Akt/NF-κB signaling pathway and its associated inflammatory cytokines.

**Materials and Methods**

**Animals**

Thirty SPF-grade male db/db mice and six db/m mice (8 weeks of age) were procured from Hunan Skryginda Laboratory Co., Ltd. (Changsha, China), Animal License No. SCXK (Hunan) 2019-0004. These animals were maintained in accordance with the standards for experimental animals in the barrier environment of the Laboratory Animal Center of Henan University of Traditional Chinese Medicine, with Ethical Review Approval No.: DWLL202203012, and conducted according to the guidelines for the ethical review of laboratory animal welfare People’s Republic of China National Standard GB/T 35892–2018.

**Drugs, Reagents, and Instruments**

JWSJS, consisting of Jiang Huang (Curcuma longa L.), Da Huang (Rheum officinale Baill), Jiang Can (Bombyx Batryticatus), Chan Tui (Cicadae Periostracum), Huang Qi (Astragalus mongholicus Bunge), and Hong Hua (Carthamus tinctorius L.) were obtained from the First Affiliated Hospital of Henan University of Traditional Chinese Medicine (Manufacturer: Sichuan New Green Pharmaceutical Science and Technology Development Co., Ltd.). Perindopril tert-butylamine tablets (Yashida), 4mg×30s, were also obtained from the same hospital. ELISA kits for neutrophil gelatinase-associated lipocalin (NGAL), interleukin 1β (IL-1β), tumor necrosis factor-alpha (TNF-α), vascular endothelial cell adhesion molecule 1 (VCAM-1), and monocyte chemotactic protein 1 (MCP-1) were procured from Jiangsu Enzyme Immuno. Antibodies for p-PI3K, p-Akt, p-NF-κB p65, and GAPDH were sourced from Servicebio. Other equipment utilized included a fully automatic biochemical analyzer (Shenzhen Radu Life Science and Technology), a vertical electrophoresis instrument (Servicebio), a panoramic section scanner (3DHISTECH), and a transmission electron microscope (Hitachi). (see Table 1)

**Grouping, Modeling, and Drug Administration**

It is well-established that db/db mice, characterized by spontaneous gene mutations, exhibit elevated blood glucose levels at 4–8 weeks of age, develop urinary protein at 8 weeks, and display behavioral traits such as gluttony, daily thirst, and
polyuria. Consequently, they represent a robust animal model for type 2 diabetic nephropathy.23,24 The db/m mice, serving as a wild-type blank control group, provided a comparative baseline for the db/db mice model.

Grouping and administration: After one week of acclimatization, thirty db/db mice were randomly assigned to the following groups: model group, high-dose group (JWSJS-H group) (14 g/kg/d), middle-dose group (JWSJS-M group) (7 g/kg/d), low-dose group (JWSJS-L group) (3.5 g/kg/d), and perindopril positive control group (0.48 mg/kg/d). The grouping was based on the body weight stratification method, with six mice in the model group and six mice in the db/m group forming the blank control group. Both normal and model groups received an equal volume of pure water through gavage once daily for 12 weeks. The experiments spanned 12 weeks, during which urine was collected in metabolic cages at weeks 0, 4, 8, and 12 to record the 24-hour urine volume and measure 24-hour urine albumin content using ELISA.

Observational Indicators and Methods

Biochemical Indicators Assay

After serum thawing, the Myriad automatic biochemistry instrument was used to assess biochemical indexes (serum creatinine [SCr] and blood urea nitrogen [BUN]) and evaluate renal injury levels.

Enzyme-Linked Immunosorbent Assay (ELISA)

Quantification of mice urinary 24-hour urine albumin,25 serum HbA1c,26 NGAL,27 TNF-α,28 IL-1β,29 VCAM-1,30 and MCP-131 in mice was conducted using the corresponding kits, strictly adhering to the manufacturer’s instructions.

Kidney Pathology

The fixed renal tissues were embedded in paraffin blocks, sectioned on a microtome (thickness of 0.4 μm), and stained with hematoxylin and eosin (HE staining). The pathological changes in the mice kidneys were observed under an optical microscope. Electron microscope samples underwent double fixation with 2% glutaraldehyde starvation acid, followed by processing and photography under a 12,000× field of view by histopathologists. Glomerular basement membrane thickness was quantified using ImageJ software.

Western Blot

Kidney tissue was lysed using RIPA lysis buffer (containing phosphatase and protease inhibitors). The supernatant, obtained by centrifugation at 12,000 × g for 10 min at 4 °C, was subjected to Bicinchoninic Acid Assay (BCA) to determine protein concentration. Subsequently, polyacrylamide gel electrophoresis was performed, followed by membrane transfection and sealing with 5% skimmed milk. PI3K antibody, Akt antibody, NF-κB p65 antibody, p-PI3K antibody, p-Akt antibody, p-NF-κB p65 antibody, and the internal reference GAPDH were added separately and incubated overnight at 4°C. Akt antibody, p-NF-κB p65 antibody, and the internal reference GAPDH were also incubated at 4°C overnight. After TBST washing, horseradish peroxidase-labeled secondary antibody was added and incubated at room temperature for 2 hours, followed by ECL luminescence color development. The optical density of the target bands was analyzed using a gel image analyzer, and the results were expressed as p-PI3K/GAPDH, PI3K/GAPDH, p-Akt/GAPDH, Akt/GAPDH, p-NF-κB p65/GAPDH, NF-κB p65/GAPDH.

Table 1 Composition of JWSJS

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Relative Proportion</th>
<th>Chinese Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Curcuma longa L.</td>
<td>6</td>
<td>Jiang Huang</td>
</tr>
<tr>
<td>Rheum officinale Baill</td>
<td>3</td>
<td>Da Huang</td>
</tr>
<tr>
<td>Bombyx Batryticatus</td>
<td>9</td>
<td>Jiang Can</td>
</tr>
<tr>
<td>Cicadae Periostracum</td>
<td>6</td>
<td>Chan Tui</td>
</tr>
<tr>
<td>Astragalus mongholicus Bunge</td>
<td>15</td>
<td>Huang Qi</td>
</tr>
<tr>
<td>Carthamus tinctorius L.</td>
<td>6</td>
<td>Hong Hua</td>
</tr>
</tbody>
</table>

Note: Composition of JWSJS.
Statistical Analysis
Statistical analysis was conducted using SPSS 21.0 software, and data were presented as mean ± standard deviation (SD). Normality and variance chi-square tests were initially performed on all data. Between-group ANOVA was carried out using one-way ANOVA, and post hoc two-by-two comparisons were conducted using LSD two-by-two comparisons. Statistical significance was considered when P < 0.05.

Results
JWSJS Improves SCr and BUN in DN Mice
Compared to the normal group, the model group exhibited significantly elevated serum SCr and BUN levels in mice (P<0.05). Conversely, both the perindopril group and the JWSJS dosage group demonstrated a reduction in SCr and BUN levels compared to the model group (P<0.05). Notably, the JWSJS dosage group exhibited a significant decrease in SCr compared to the western medication group (P<0.05), with the medium-dose group showing a decrease in BUN levels (P<0.05). However, no significant difference was observed between the high and low-dose groups (P>0.05). These results demonstrate renal function injury in the model group, with JWSJS treatment showing the potential to mitigate renal injury (Figure 1).

JWSJS Reduced 24-Hour Urinary Protein Leakage in DN Mice
Compared to the normal group, the model group displayed a significant increase in 24-hour urine protein content in mice (P<0.05). The perindopril group and all dose groups of JWSJS exhibited a reduction in 24-hour urine protein content compared to the model group (P<0.05). Additionally, the JWSJS-M group showed a decrease in 24-hour urine protein content compared to the Western medicine group (P<0.05). However, there was no significant difference between the JWSJS-H and JWSJS-L groups (P>0.05). These findings indicate an elevation in urinary protein content in the model group, with JWSJS treatment reducing protein leakage (Figure 2).

JWSJS Decreased Blood Glucose and Levels of TNF-α, IL-1β, VCAM-1, MCP-1, and NGAL in DN Mice
Compared to the normal group, mice in the model group exhibited significantly elevated serum levels of TNF-α, IL-1β, VCAM-1, MCP-1, NGAL, and HbA1c (P<0.05). In contrast, the perindopril group and JWSJS dose groups demonstrated a decrease in these levels compared to the model group (P<0.05). In comparison to the Western medicine group, the medium-dose group of JWSJS exhibited a significant decrease in TNF-α, IL-1β, VCAM-1, MCP-1, NGAL, and HbA1c levels (P<0.05), with no significant difference between the high- and low-dose groups (P>0.05). These results indicate
elevated blood glucose levels, renal inflammatory reactions, and renal function damage in the model group, with JWSJS treatment reducing blood glucose levels, alleviating renal inflammatory reactions, and protecting the kidneys (Figure 3).

**JWSJS Alleviated Renal Pathological Injury in DN Mice**

Results from HE staining revealed that mice in the blank group exhibited a uniform glomerular distribution with consistent cell numbers in both glomeruli and stroma. Tubular epithelial cells appeared rounded and plump, and there were no evident inflammatory changes or notable hyperplasia in the kidney interstitium. In the model group, renal tubular epithelial cells displayed vacuolar degeneration with tiny round vacuoles in the cytoplasm (black arrows), and glomerular capillaries showed slight bruising and dilation (yellow arrows). In comparison to the model group, the JWSJS-L and perindopril groups exhibited improved glomerular capillary stasis and dilation, with a more significant improvement observed in the JWSJS-H and JWSJS-M groups (yellow arrows) (Figure 4).

Electron microscopy results demonstrated that the glomerular basement membrane of mice in the blank group was intact, continuous, and uniformly thick. Podocytes were not swollen, and foot processes (FP) were well-structured and neatly arranged without noticeable fusion or widening. Conversely, the model group exhibited uneven thickness of the glomerular basement membrane, blurred local structure, swollen podocytes, shallow and dissolved intracellular stroma, proliferated and edematous endothelial cells, reduced FP abundance, localized swelling and fusion, and aggregation of red blood cells (RBC) in the vascular lumen. Treatment with JWSJS and perindopril led to varying degrees of improvement. For instance, in the JWSJS-H and JWSJS-L groups, although the thickness of the glomerular basement membrane was slightly uneven, podocyte swelling was significantly reduced, and FP arrangement remained neat with only a few instances of fusion and shortening. The
JWSJS-M and perindopril groups exhibited more uniform glomerular basement membrane thickness, evenly distributed intracellular stroma in podocytes, and neatly arranged FP with uniform lengths, with only occasional fusion. The endothelial cells maintained a fair structure with homogeneous cytoplasm (Figure 5).

**The Effects of JWSJS on p-PI3K, p-Akt, and p-NF-κB p65 Proteins in Mice**

Compared to the normal group, mice in the model group displayed reduced protein expression content of p-PI3K and p-Akt, coupled with elevated protein expression content of p-NF-κB p65 (P<0.05). In contrast, the Chinese and Western medicine treatment groups exhibited increased protein expression content of p-PI3K and p-Akt, along with decreased...
protein expression content of p-NF-κB p65 in comparison to the model group (P<0.05). Compared to the Western medicine group, the JWSJS-L and JWSJS-M groups showed elevated p-PI3K and p-Akt protein expression content, while the low- and high-dose groups displayed decreased p-NF-κB p65 protein expression content (P<0.05). The differences between the remaining groups were not statistically significant (P>0.05) (Figures 6 and Figure 7).

Figure 4 HE staining results of kidney in each group (×200).

Notes: Black arrows indicate the cytoplasm contains rounded vacuoles of minute size. Yellow arrows indicate glomerular capillaries showed slight bruising and dilation. (A) normal group; (B) model group; (C) JWSJS-H group; (D) JWSJS-M group; (E) JWSJS-L group; (F) perindopril group.

Figure 5 Electron microscopic results of kidney in each group (×12000).

Notes: (A) normal group; (B) model group; (C) JWSJS-H group; (D) JWSJS-M group; (E) JWSJS-L group; (F) perindopril group.
**Figure 6** Comparison of protein expression of p-Akt, p-PI3K and p-NF-κB p65 in renal tissue of mice in each group.

**Abbreviations:** N: normal group; Mo: model group; W: Perindopril group; L: JWSJS-L group; Me: JWSJS-M group; H: JWSJS-H group.

**Figure 7** Comparison of protein expression of p-PI3K (A), p-Akt (B), and p-NF-κB p65 (C) in renal tissue of mice in each group.

**Notes:** *P<0.05, compared with normal group; #P<0.05, compared with model group; ▲P<0.05, compared with western medicine group; ★P<0.05, compared with JWSJS-M group; ☆P<0.05, compared with JWSJS-L group.
Discussion

Shengjiangsan made its historical debut in the Ming Dynasty, documented in Zhang Heteng’s “The Complete Book of Summer Wounds”. Comprising Cicadae Periostracum, Curcuma longa L., Rheum officinale Baill, and Bombyx Batryticatus, this formulation holds specific therapeutic roles. Bombyx Batryticatus thermoregulatory properties and potential antidepressant effects. Flavonoids in Bombyx Batryticatus mainly include quercetin and kaempferol, among which kaempferol ameliorates renal injury and fibrosis by increasing the release of GLP-1 and insulin as well as inhibiting RhoA/Rho kinase in the mouse model of DN. Cicadae Periostracum clearing heat to release the exterior, and has anti-inflammatory, sedative analgesic antispasmodic, anti-convulsive, and anticoagulant effects. Cicadae Periostracum reduces proteinuria by inhibiting the overexpression of TLR4 in the renal tissues, inhibiting glomerular Mesangial cell proliferation, and attenuating mesangial matrix accumulation. Curcuma longa L. has the capacity to regulating qi and resolving stasis, and may possess antidepressant properties. Siwei Jianghuang Decoction Powder ameliorates renal injury in DN mice by regulating HIF-1α, VEGF, and TGF-β1 overexpression. The addition of Astragalus mongholicus Bunge and Carthamus tinctorius L. to the formula targets the disease mechanism of qi deficiency and blood stasis. Astragalus mongholicus Bunge, being the most abundant component, replenishing qi to rise yang and abating fever. Carthamus tinctorius L., with its pungent and warm nature, activates blood circulation, removes blood stasis, and clears the channels. The overall composition, predominantly featuring pungent herbs, capitalizes on their dispersing, developing, running away, and propagating nature. This imparts the formula with the efficacy of ascending clarity, descending turbidity, dredging collaterals, and opening sweat pores. Research has identified the therapeutic potential of Astragalus mongholicus Bunge and Carthamus tinctorius L. to the formula targets the disease mechanism of qi deficiency and blood stasis. Astragalusmongholicus Bunge, being the most abundant component, replenishing qi to rise yang and abating fever. Carthamus tinctorius L., with its pungent and warm nature, activates blood circulation, removes blood stasis, and clears the channels. The overall composition, predominantly featuring pungent herbs, capitalizes on their dispersing, developing, running away, and propagating nature. This imparts the formula with the efficacy of ascending clarity, descending turbidity, dredging collaterals, and opening sweat pores. Research has identified the therapeutic potential of Astragalus mongholicus Bunge and Carthamus tinctorius L. to the formula targets the disease mechanism of qi deficiency and blood stasis. Astragalus mongholicus Bunge, being the most abundant component, replenishing qi to rise yang and abating fever. Carthamus tinctorius L., with its pungent and warm nature, activates blood circulation, removes blood stasis, and clears the channels.

The early clinical manifestations of DN often lack specific symptoms, necessitating the use of laboratory tests for early detection of renal lesions. Among the various indicators for assessing renal injury, serum creatinine, blood urea nitrogen, and urinary microalbumin exhibit high sensitivity, while neutrophil gelatinase-associated lipocalin (NGAL) serves as an early marker for chronic renal injury. Additionally, elevated levels of glycated hemoglobin (HbA1c) play an indispensable role in DN diagnosis. Results from the current experimental study indicate that JWSJS effectively reduces serum SCr, BUN, NGAL, and HbA1c levels, diminishes 24-hour urinary protein excretion, and alleviates kidney injury in mice.

The pathogenesis of DN is intricate, with chronic inflammatory responses emerging as a major, yet unavoidable, contributing mechanism. The PI3K/AKT/NF-κB signaling pathway is closely intertwined with the development of inflammatory diseases. Activation of the PI3K/Akt signaling pathway in DN has been reported to initiate a cascade of immune-inflammatory responses, leading to damage and degeneration of the glomerular basement membrane. This process involves the mediation of swelling, fusion, and loss of podocytes, ultimately impairing glomerular filtration and causing leakage of urinary proteins. NF-κB, a crucial inflammatory transcription factor downstream of the PI3K/Akt pathway, is normally bound to its inhibitory protein IκB in the cytoplasm during the resting state, keeping the PI3K/Akt/ NF-κB signaling pathway inactive. However, under the high glucose conditions of diabetes, this pathway is easily activated. Consequently, rapid activation of NF-κB proteins occurs, leading to the release of substantial inflammatory factors, including TNF-α, IL-1, and IL-6, initiating an inflammatory cascade known as an “inflammatory storm”. This storm can result in severe and rapid damage to the kidneys. The findings of our study demonstrate that JWSJS can increase the expression of p-PI3K and p-Akt proteins while downregulating the expression of p-NF-κB p65 protein. This dual action serves to inhibit the activation of the PI3K/Akt/NF-κB signaling pathway. Consequently, JWSJS effectively reduces the expression of inflammatory factors such as TNF-α, IL-1β, VCAM-1, and MCP-1. This reduction in inflammatory response contributes to the attenuation of renal injury, protection of the kidney, improvement in renal
tubular epithelial cell vacuolar degeneration, amelioration of glomerular capillary stasis and dilatation, repair of glomerular vascular endothelial cells, mitigation of glomerular basement membrane damage, repair of podocyte injuries, and enhancement of the fusion and degeneration of foot processes.

In summary, JWSJS emerges as a promising therapeutic agent capable of mitigating renal injury, reducing renal inflammatory responses, and ameliorating pathological injuries in mice with diabetic nephropathy. This therapeutic effect is likely achieved through the inhibition of the PI3K/Akt/NF-κB signaling pathway. Nonetheless, further research is imperative to unveil the comprehensive molecular mechanisms underlying JWSJS’s efficacy in diabetic nephropathy.

**Conclusion**

JWSJS demonstrated efficacy in reducing renal inflammation in diabetic nephropathy mice, with its mechanism likely associated with the inhibition of the PI3K/Akt/NF-κB signaling pathway.

**Abbreviations**

DN, Diabetic nephropathy; ACEI, Angiotensin-converting enzyme inhibitors; TCM, Traditional Chinese medicine; P13K, Phosphatidylinositol 3-kinase; TNF-α, Tumor necrosis factor-alpha; IL-1β, Interleukin-1β; IL-6, Interleukin-6; NGAL, neutrophil gelatinase-associated lipocalin; VCAM-1, vascular endothelial cell adhesion molecule-1; MCP-1, monocyte chemotactic protein 1; SCr, serum creatinine; BUN, blood urea nitrogen; ELISA, Enzyme-Linked Immunosorbent Assay; BCA, Bicinchoninic Acid Assay; FP, foot processes.

**Author Contributions**

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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**Disclosure**

The authors report no conflicts of interest in this work.

**References**


