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Phloretin alleviates renal injury in streptozotocin-induced diabetic mice through NF- κ B/MAPK signaling pathways

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Abstract. Diabetic nephropathy (DN) is a complication of diabetes characterized by renal dysfunction, fibrosis, and inflammation. This study aimed to investigate the nephroprotective effects of phloretin (PHL) in a streptozotocin (STZ)-induced diabetic mouse model. Mice were treated with PHL (50 mg/kg/day) for eight weeks, after which renal function parameters, oxidative stress markers, histopathological changes, fibrosis- and inflammation-related protein expression, and key signaling pathways were analyzed. The results demonstrated that PHL ameliorated hyperglycemia, renal dysfunction (as evidenced by reduced BUN, serum creatinine, and urinary albumin), and dyslipidemia (lowered serum TG and TC). PHL also attenuated renal oxidative stress by enhancing antioxidant enzyme activities (SOD, CAT, GSH-Px) and reducing lipid peroxidation (MDA). Histopathological evaluation revealed that PHL alleviated glomerular damage, tubular necrosis, and inflammatory cell infiltration, while Masson's staining showed inhibition of renal interstitial fibrosis. Mechanistically, PHL downregulated the expression of fibrosis markers (fibronectin, collagen I, α -SMA) and proinflammatory cytokines (IL-6, IL-1 β , TNF- α). Furthermore, PHL suppressed the activation of the NF- κ B and MAPK pathways, as indicated by increased I κ B α levels and reduced phosphorylation of NF- κ B p65, p38, JNK, and ERK1/2. In conclusion, PHL protects against DN by improving renal function, reducing oxidative stress, mitigating fibrosis and inflammation, and inactivating the NF- κ B/MAPK pathways.

Key words: Diabetic nephropathy — Phloretin — Fibrosis — Inflammation — Oxidative injury

Introduction

Diabetic nephropathy (DN) is a serious microvascular complication of diabetes mellitus and a leading cause of end-stage renal disease (ESRD) worldwide, contributing significantly to morbidity and mortality among diabetic patients (Dwivedi and Sikarwar 2025). Clinically, DN is characterized by persistent albuminuria, a progressive decline in the glomerular filtration rate, and elevated blood pressure, ultimately leading to irreversible renal failure (Oshima et al.

2021). The pathological features include renal hypertrophy, glomerular basement membrane thickening, mesangial expansion, tubulointerstitial fibrosis, and glomerulosclerosis (Jeddi et al. 2025). Current therapeutic strategies, primarily focusing on glycemic and blood pressure control, along with the use of renin-angiotensin-aldosterone system inhibitors, only slow but cannot halt disease progression (Neuen et al. 2025). Kidney replacement therapy, including dialysis and transplantation, remains the last resort for patients with ESRD, which is often limited by high costs and organ shortage (Bavanandan et al. 2025). Therefore, exploring novel and effective therapeutic agents to prevent or reverse the progression of DN is urgently needed.

Accumulating evidence indicates that renal fibrosis, inflammation, and oxidative stress are key contributors to

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the pathogenesis of DN (Ansari et al. 2025). Chronic hyperglycemia promotes the upregulation of fibrotic factors such as collagen I, fibronectin, and α -smooth muscle actin (α -SMA) in the kidney, accelerating extracellular matrix (ECM) accumulation and mesangial expansion, thereby driving tubulointerstitial fibrosis and glomerulosclerosis (Liu et al. 2025). Moreover, hyperglycemic conditions enhance the release of proinflammatory mediators, including tumor necrosis factor α (TNF- α), interleukin-1 β (IL-1 β), and IL-6, in the kidney, leading to renal inflammation and exacerbation of DN (Altamimi et al. 2023; Yan et al. 2023). An imbalance between reactive oxygen species (ROS) production and antioxidant enzyme activity further contributes to oxidative injury (Hernandez et al. 2022). Importantly, exacerbated oxidative stress can amplify the release of both proinflammatory and profibrotic factors by activating key signaling pathways such as nuclear factor-kappa B (NF- κ B) and mitogen-activated protein kinases (MAPKs), which regulate diverse biological processes, including apoptosis, cell survival, oxidative stress, and inflammation, and have been demonstrated to play pivotal roles in DN progression (Li et al. 2022; Han X et al. 2024). For instance, Kang et al. reported that MAPKs can trigger NF- κ B-mediated inflammation in DN (Kang et al. 2017), while Ravera et al. highlighted that feedback activation of the NF- κ B/MAPK axis upregulates proinflammatory cytokines, thereby promoting renal fibrosis and dysfunction (Ravera et al. 2020). Supporting this, Han et al. (2022) showed that sclareol alleviates hyperglycemia-induced renal inflammation and fibrosis by inhibiting MAPK/NF- κ B signaling. Therefore, targeting agents capable of inactivating MAPK/NF- κ B signaling represents a promising therapeutic strategy for DN treatment. Phloretin (PHL), a natural dihydrochalcone flavonoid found in *Prunus mandshurica* and apple trees (Wu et al. 2019), exhibits a wide range of pharmacological properties, including anti-inflammatory, immunomodulatory, antioxidant, antitumor, antidiabetic, and cardioprotective effects (Anunciato Casarini et al. 2020). The therapeutic potential of PHL has been demonstrated in various disease models. For instance, it has been shown to protect against lipopolysaccharide-induced acute lung injury by inhibiting pulmonary oxidative stress and inflammation through suppression of the NF- κ B/MAPK pathways (Huang et al. 2016). In addition, PHL alleviates diabetic cardiomyopathy in streptozotocin (STZ)-induced murine models and high glucose-stimulated *in vitro* settings via its antifibrotic and anti-inflammatory activities (Ying et al. 2019). Notably, PHL also ameliorates hyperuricemia-associated renal injury by attenuating mitochondrial dysfunction, fibrosis, and inflammation (Cui et al. 2020). Despite these promising findings, the role of PHL in DN remains largely unknown.

This study aimed to investigate the pharmacological effects of PHL and its underlying mechanisms in a STZ-

induced DN mouse model. We hypothesized that PHL ameliorates renal fibrosis, oxidative stress, and inflammation by modulating the MAPK/NF- κ B signaling pathways. Our findings provide novel insights into the therapeutic potential of PHL and elucidate its molecular mechanisms of action in DN.

Materials and Methods

Animal models

A total of 30 male C57BL/6 mice (8-weeks old) were purchased from Charles River Laboratories. All mice were housed under specific pathogen-free conditions with a 12-h light/dark cycle, ambient temperature of $22 \pm 1^\circ\text{C}$, and provided with free access to water and food. All animal procedures were performed in strict accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The experimental protocol was approved by the Institutional Animal Care and Use Committee of the Hubei Provincial Center for Disease Control and Prevention (Approval No. 202411256; Approval Date: April 23, 2024). The mice were randomly divided into three groups ($n = 10$ in each group): control (Con), STZ-induced diabetic (STZ), and STZ-induced diabetic treated with PHL (STZ+PHL). Diabetes was induced as previously described (Song et al. 2020). Briefly, after a 12 h fast, mice in the STZ and STZ+PHL groups received daily *intraperitoneal* injections of STZ (50 mg/kg/day; Sigma-Aldrich) dissolved in citrate buffer (pH 4.5) for five consecutive days. Mice with blood glucose levels exceeding 13.9 mmol/l (250 mg/dl) measured 72 h after the first STZ injection were considered diabetic and included in subsequent experiments (Song et al. 2020). Control mice received an equal volume of citrate buffer. After successful modelling, mice in the STZ+PHL group were administered PHL (50 mg/kg/day; HPLC purity $\geq 98\%$, Yuanye Co., Ltd., Shanghai, China) dissolved in 0.5% (w/v) CMC-Na by oral gavage daily for eight weeks (Shen et al. 2017; Un et al. 2021). Fasting blood glucose was monitored weekly throughout the study. At the endpoint, body weight was recorded, and blood and urine samples were collected for renal function assessment. All mice were euthanized by CO₂ inhalation, and kidneys were promptly excised. After weighing, renal tissues were either snap-frozen in liquid nitrogen for biochemical analyses or fixed in 4% paraformaldehyde for histopathological studies.

Measurement of biochemical parameters

After collection, blood samples were centrifuged at $2,700 \times g$ for 20 min at 4°C to obtain serum. The levels of blood urea nitrogen (BUN), creatinine, total cholesterol (TC), and

triglycerides (TG) in serum, as well as urinary albumin in urine, were measured using commercially available assay kits according to the manufacturer's instructions (Saipai Biotechnology Co., Ltd, Wuhan, China). The absorbance was measured with a Multiskan GO microplate reader (Thermo Fisher Scientific).

Detection of oxidative stress markers

The activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), and the content of malondialdehyde (MDA) in renal tissues were measured using commercial assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's instructions. Briefly, renal tissues were homogenized in ice-cold physiological saline (1:9, w/v). The homogenates were then centrifuged at 12,000×g for 10 min at 4°C. The resulting supernatants were collected, and the absorbance of each sample was measured with a microplate reader for subsequent biochemical analyses.

Histology

Renal tissues were fixed in 4% paraformaldehyde for 48 h, dehydrated through a graded ethanol series, and embedded in paraffin. Sections (4 µm thickness) were cut using a rotary microtome (Leica RM2235). For hematoxylin and eosin (H&E) staining, sections were deparaffinized, stained with hematoxylin (Sigma-Aldrich) for 5 min and eosin (Sigma-Aldrich) for 2 min, then dehydrated and mounted with neutral balsam (Beyotime, Shanghai). For Masson's trichrome staining, sections were processed using a commercial kit (Masson Trichrome Stain Kit; Solarbio Science & Technology Co., Ltd., Beijing, China) according to the manufacturer's protocol to assess collagen deposition. Stained sections were examined and imaged under a light microscope (BX53, Olympus, Japan) by two blinded pathologists. Renal injury scores were quantified based on H&E staining using a semiquantitative scale as previously described (Sun et al. 2011), while the fibrotic area (%) was calculated from Masson's trichrome-stained sections using ImageJ software (v1.8.0, National Institutes of Health).

Immunofluorescence staining

Renal tissues were fixed in 4% paraformaldehyde, embedded in paraffin, and sectioned at 4 µm thickness. After deparaffinization and rehydration, antigen retrieval was performed by heating sections in citrate buffer (pH 6.0) at 95°C for 20 min. Sections were blocked with 10% donkey serum (Beyotime) containing 0.3% Triton X-100 for 1 h at room temperature, followed by incubation overnight at 4°C with primary antibodies against α-SMA (1:200, 19245, Cell

Signaling Technology (CST)) or phospho-NF-κB p65 (1:800, 3033, CST). After washing with Tris-buffered saline containing 0.1% Tween-20 (TBST), sections were incubated with Alexa Fluor 488-conjugated secondary antibodies (1:500, Abcam) for 2 h at room temperature in the dark. Nuclei were counterstained with DAPI (Beyotime) for 10 min. Sections were mounted with anti-fade mounting medium (Beyotime) and visualized using a fluorescence microscope (BX53). Quantitative analysis of α-SMA-positive area or phospho-NF-κB p65-positive cells was performed using ImageJ software by evaluating fluorescence intensity in five random fields per section.

Western blot

Renal tissues were homogenized in ice-cold RIPA lysis buffer (Thermo Fisher Scientific) supplemented with protease and phosphatase inhibitors. The homogenates were centrifuged at 12,000×g for 15 min at 4°C, and the supernatants were collected. Protein concentrations were determined using a BCA protein assay kit (Beyotime). Equal amounts of protein (30 µg per lane) were separated by 10% SDS-PAGE and transferred onto PVDF membranes. After blocking with 5% non-fat milk for 2 h at room temperature, the membranes were incubated overnight at 4°C with primary antibodies against the following targets: p38 MAPK (8690), phospho-p38 MAPK (4511), JNK (9252), phospho-JNK (4668), ERK1/2 (4695), phospho-ERK1/2 (4377), IκBα (4812), NF-κB p65 (8242), phospho-NF-κB p65 (3033), α-SMA (19245), Fibronectin (62601), Collagen I (72026), IL-6 (12912), IL-1β (31202), TNF-α (11948), and GAPDH (2118). All primary antibodies were purchased from CST and used at dilutions ranging from 1:1000 to 1:2000. After washing with TBST, membranes were incubated with HRP-conjugated secondary antibodies (1:2000, Abcam) for 2 h at room temperature. Protein bands were visualized using an ECL detection system (GE Healthcare) and imaged with a chemiluminescence imaging system (ChemiDoc MP, Bio-Rad). Band densities were quantified using ImageJ software, with GAPDH serving as the loading control.

Statistical analysis

All data are presented as the mean ± standard deviation (SD). Statistical analyses were performed using SPSS 21.0 (IBM Corp.). The normality of data distribution was assessed using the Shapiro-Wilk test. For comparisons among the three groups, one-way analysis of variance (ANOVA) was applied for data conforming to a normal distribution, followed by Tukey's *post hoc* test. If the data violated the normality assumption, the non-parametric Kruskal-Wallis test was used, followed by Dunn's *post hoc* test for multiple

comparisons. A p -value of less than 0.05 was considered statistically significant.

Results

PHL ameliorates renal dysfunction and lipid metabolism in diabetic mice

Initial measurements of blood glucose confirmed the anti-diabetic property of PHL. As illustrated in Figure 1A, STZ administration led to significantly elevated blood glucose levels over the 8-week period, which were effectively restored by PHL treatment. Furthermore, the increased kidney weight-to-body weight ratio induced by STZ was markedly reduced by PHL (Fig. 1B). We next evaluated the influence of PHL on renal dysfunction by assessing BUN, serum creatinine, and urinary albumin levels. The results demonstrated that PHL significantly inhibited the STZ-induced elevations in BUN, serum creatinine, and urinary albumin

(Fig. 1C–E), indicating a substantial improvement in renal function. Additionally, analysis of lipid metabolism revealed that STZ notably elevated serum TG and TC levels, alterations that were reversed by PHL administration (Fig. 1F,G). Collectively, these findings demonstrate that PHL

effectively attenuates renal dysfunction and normalizes lipid metabolism in STZ-induced diabetic mice.

PHL attenuates renal oxidative stress in diabetic mice

To evaluate the antioxidative effect of PHL in the context of DN, we measured key oxidative stress parameters in renal tissues. As shown in Figure 2, STZ injection significantly decreased the activities of the antioxidant enzymes SOD, CAT, and GSH-Px, while increasing the content of MDA, a marker of lipid peroxidation. Treatment with PHL effectively reversed these alterations, indicating that PHL alleviates oxidative renal injury by enhancing antioxidant capacity and reducing lipid peroxidation, thereby contributing to its protective role against DN.

PHL ameliorates diabetes-induced renal histopathological damage and fibrosis

Histopathological evaluation of renal tissues by H&E staining revealed substantial renal injury in STZ-treated mice, characterized by glomerular structural damage, widened renal *interstitium*, thickened capillary basement membranes, vacuolar degeneration and necrosis of tubular epithelial cells, and inflammatory cell infiltration compared with the

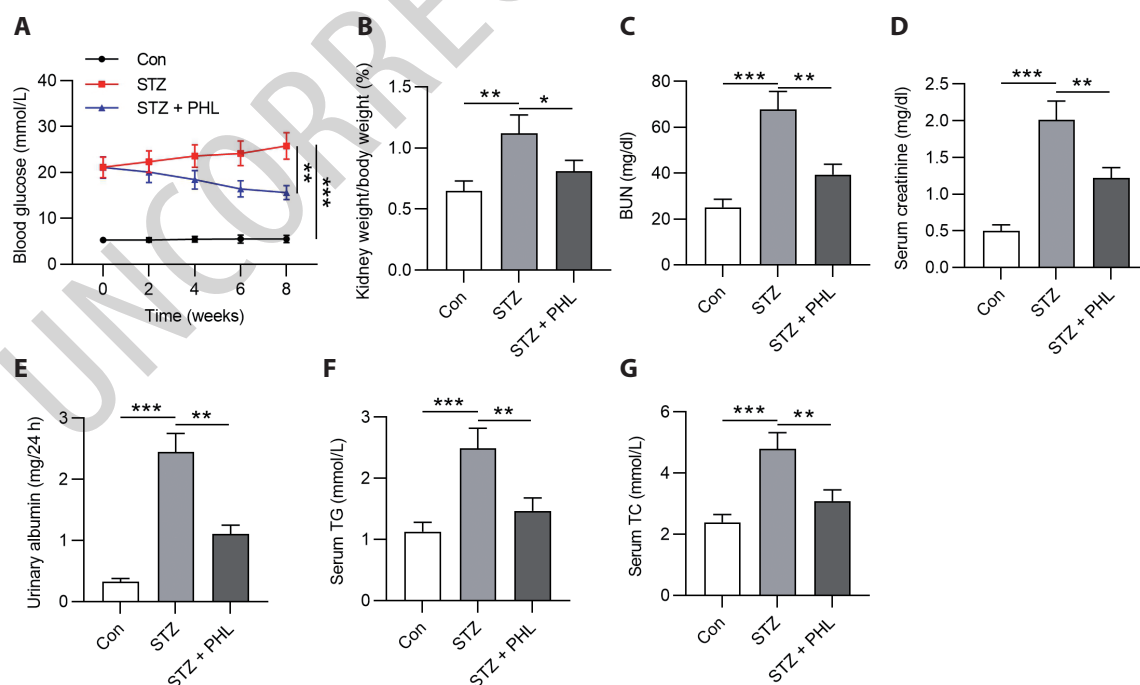


Figure 1. PHL ameliorates on renal dysfunction and lipid metabolism in diabetic mice. Data from all panels were obtained from mice in the following groups ($n = 10$ per group): control (Con), STZ-induced diabetic (STZ), and PHL-treated diabetic (STZ+PHL). **A.** Blood glucose levels. **B.** Kidney weight-to-body weight ratio. Serum levels of **(C)** BUN and **(D)** creatinine. **E.** Urinary albumin excretion. Serum levels of **(F)** triglycerides (TG) and **(G)** total cholesterol (TC). Data are presented as mean $\pm$ SD. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

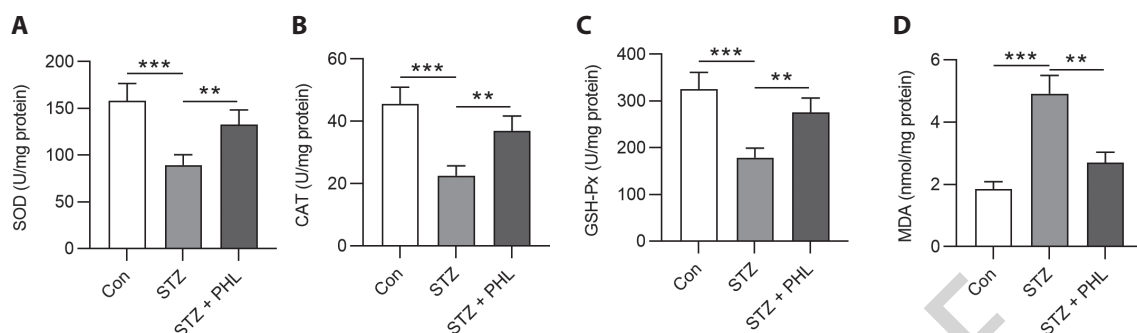


Figure 2. PHL attenuates renal oxidative stress in diabetic mice. Renal tissues were analyzed for (A) superoxide dismutase (SOD), (B) catalase (CAT), and (C) glutathione peroxidase (GSH-Px) activities, and (D) malondialdehyde (MDA) content using commercial kits. Data are presented as mean $\pm$ SD ($n = 10$ per group). ** $p < 0.01$, *** $p < 0.001$.

control group. These pathological alterations were markedly ameliorated by PHL treatment (Fig. 3A). Consistent with these observations, the increased kidney injury score in STZ-injected mice was significantly reduced by PHL (Fig. 3B). Masson's trichrome staining further demonstrated severe renal interstitial fibrosis with extensive collagen deposition in the STZ group, which was notably suppressed by PHL administration (Fig. 3C). Quantitative analysis confirmed a significant reduction in the fibrotic area following PHL treatment (Fig. 3D). Together, these results indicate that PHL effectively mitigates diabetes-induced renal histopathological injury and interstitial fibrosis.

PHL suppresses renal fibrosis and inflammation in diabetic mice

Immunofluorescence analysis revealed that STZ administration significantly increased the α -SMA-positive area in renal tissues, a marker of fibrosis, which was markedly attenuated by PHL treatment (Fig. 4A). Consistent with this finding, Western blot analysis demonstrated that the protein levels of fibrosis-related genes, fibronectin, collagen I, and α -SMA, were upregulated in the kidneys of STZ-induced diabetic mice, whereas PHL treatment significantly suppressed their expression (Fig. 4B). Furthermore, the effect of PHL on

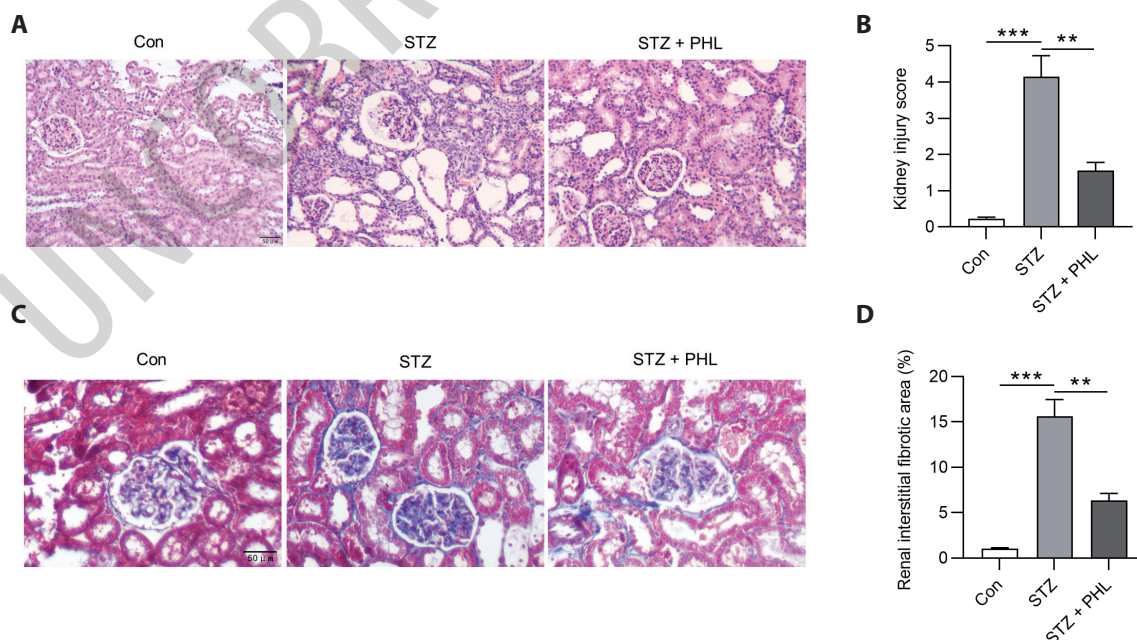


Figure 3. PHL ameliorates diabetes-induced renal histopathological damage and fibrosis. **A.** Representative H&E-stained renal sections. **B.** Quantitative kidney injury scores based on H&E staining. **C.** Representative images of Masson's trichrome-stained renal sections. **D.** Quantitative analysis of renal interstitial fibrotic area. Data are presented as mean $\pm$ SD ($n = 10$ per group). ** $p < 0.01$, *** $p < 0.001$.

inflammation was evaluated by measuring proinflammatory cytokines. As shown in Figure 4C, STZ injection led to elevated protein levels of IL-6, IL-1 β , and TNF- α in murine kidneys, an effect that was reversed by PHL. Collectively, these results indicate that PHL effectively inhibits renal fibrosis and inflammation in diabetic mice.

PHL attenuates DN by suppressing NF- κ B and MAPK signaling pathways

To elucidate the mechanism underlying the nephroprotective effect of PHL in DN, we examined key signaling pathways. Western blot analysis revealed that STZ injection downregulated I κ B α protein levels and upregulated phosphorylated NF- κ B p65 in murine kidneys, effects that were reversed by PHL treatment (Fig. 5A,B). Immunofluorescence staining further confirmed that STZ enhanced the fluorescence intensity of phosphorylated NF- κ B p65 in renal tissues, which was subsequently reduced by PHL (Fig. 5C), indicating inhibition of NF- κ B signaling. Additionally, PHL suppressed the STZ-induced phosphorylation of p38, JNK, and ERK1/2 (Fig. 6), demonstrating concurrent inactivation

of the MAPK pathway. These findings collectively indicate that PHL ameliorates DN by attenuating both NF- κ B and MAPK signaling pathways (Fig. 7).

Discussion

This study provides the first compelling evidence that PHL exerts significant nephroprotective effects in a STZ-induced DN mouse model. Our findings demonstrate that PHL treatment effectively ameliorated key pathological features of DN, including hyperglycemia, renal dysfunction, and dyslipidemia. Histopathological and biochemical analyses further revealed that PHL markedly attenuated renal injury, interstitial fibrosis, oxidative stress, and inflammation. Mechanistically, the therapeutic benefits of PHL were associated with the inhibition of both NF- κ B and MAPK signaling pathways. These results collectively indicate that PHL protects against DN through a multi-faceted mechanism involving anti-hyperglycemic, renoprotective, antifibrotic, antioxidant, and anti-inflammatory actions, primarily mediated *via* inactivation of the NF- κ B/MAPK axis.

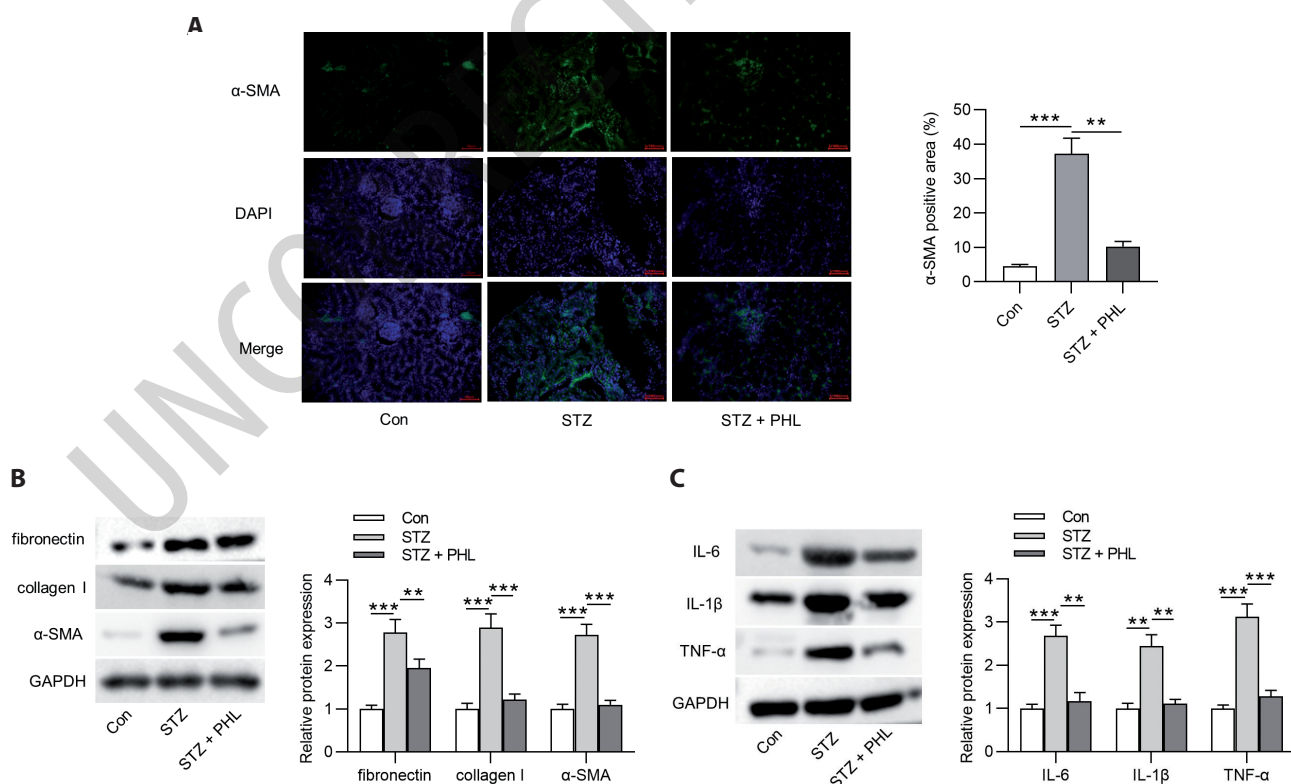


Figure 4. PHL suppresses renal fibrosis and inflammation in diabetic mice. **A.** Representative immunofluorescence images and quantitative analysis of α-SMA (a fibrosis marker) distribution in renal tissues. **B, C.** Western blot analysis of protein levels of fibrosis-related genes (fibronectin, collagen I, and α-SMA) and proinflammatory cytokines (IL-6, IL-1 β , and TNF- α) in renal tissues. Data are presented as mean $\pm$ SD ($n = 10$ per group). ** $p < 0.01$, *** $p < 0.001$.

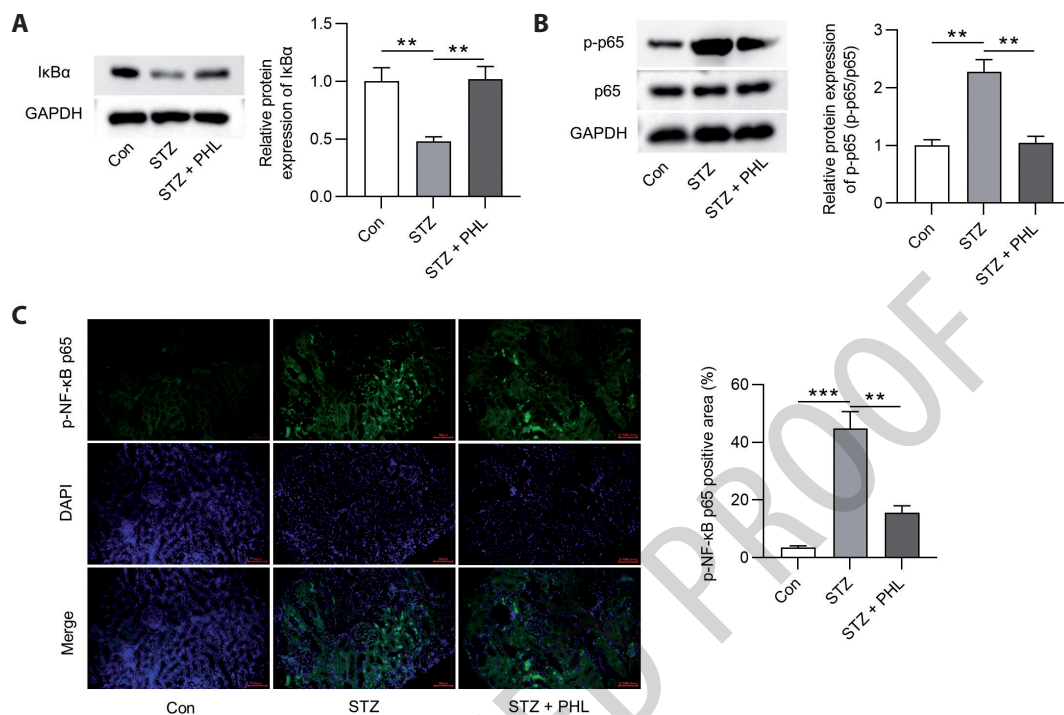


Figure 5. PHL suppresses NF-κB pathway activation in DN. **A, B.** Western blot analysis of IκBα, total p65, and phosphorylated p65 (p-p65) protein levels in renal tissues. **C.** Representative immunofluorescence images and quantitative analysis of p-NF-κB p65 expression in kidney sections. Data are presented as mean ± SD ($n = 10$ per group). ** $p < 0.01$, *** $p < 0.001$.

Our findings demonstrate that STZ induction led to a sustained increase in blood glucose levels, which was effectively counteracted by PHL treatment, indicating its anti-hyperglycemic property. This observation is consistent with previous reports confirming the glucose-regulatory role of PHL in diabetes and its complications (Shu et al. 2014; Xia et al. 2020; Mao et al. 2022). Histopathological assessment revealed that STZ provoked significant renal pathological injury, characterized by inflammatory cell infiltration, renal tubular epithelial cell necrosis, and glomerular structural damage, all of which were markedly alleviated by PHL. The kidney weight-to-body weight ratio, an indicator of renal edema (Murawski et al. 2010), was elevated in STZ-treated mice, and this increase was suppressed by PHL, suggesting its ability to ameliorate renal edema. Furthermore, STZ-induced elevations in urinary albumin, serum creatinine, and BUN, key biomarkers of renal dysfunction (Edelstein 2008), were significantly reversed by PHL, supporting its role in improving renal function. These results collectively indicate that PHL exerts nephroprotective effects against DN by mitigating renal dysfunction, attenuating histopathological damage, and regulating glycemia. In addition, abnormal lipid metabolism is a well-established pathological feature in DN (Han X et al. 2024).

Diabetic patients often exhibit dyslipidemia, characterized notably by elevated TC and TG levels (Li et al. 2023). In the present study, PHL suppressed STZ-induced increases in serum TG and TC, suggesting that its protective effect may also involve the correction of lipid metabolic abnormalities. It is worth noting that a recent study by Liu et al. (2022)

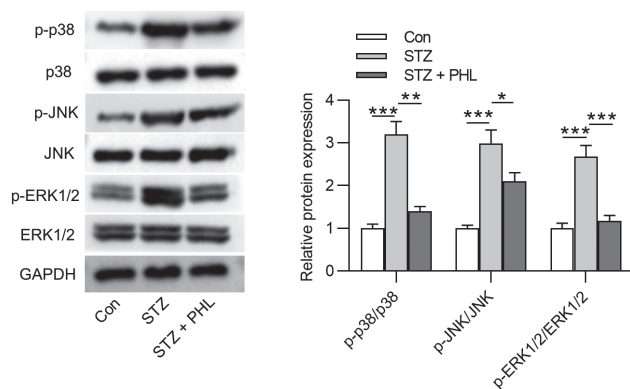


Figure 6. PHL suppresses MAPK pathway activation in DN. Western blot analysis of phosphorylated p38 (p-p38), JNK (p-JNK), and ERK1/2 (p-ERK1/2) protein levels in renal tissues. Data are presented as mean ± SD ($n = 10$ per group). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

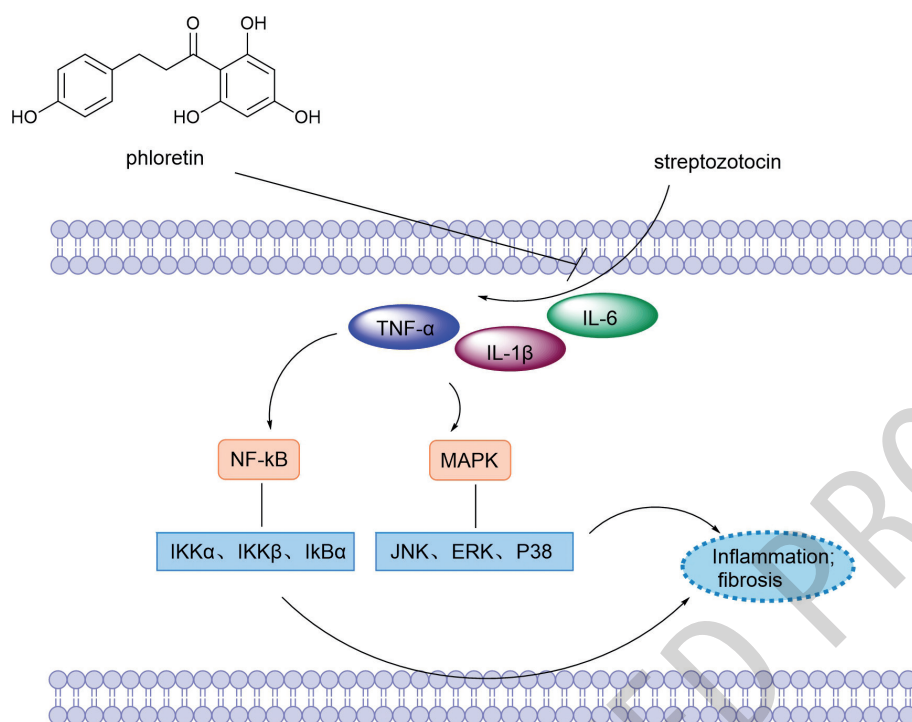


Figure 7. Proposed mechanism for the nephroprotective effect of PHL in DN. PHL alleviates renal injury in STZ-induced diabetic mice by suppressing renal fibrosis, oxidative stress, and inflammation through inactivation of the NF-κB and MAPK signaling pathways.

revealed that phloretin ameliorates diabetic nephropathy by preserving podocyte integrity through upregulation of nephrin and podocin, independent of hypoglycemic effects, further supporting the multifaceted renoprotective mechanisms of PHL beyond glycemic control.

In the present study, PHL was found to significantly alleviate STZ-induced renal interstitial fibrosis. This observation is consistent with the report by Cui et al. (2020), in which PHL suppressed renal interstitial fibrosis, ECM deposition, and tubular necrosis in a hyperuricemia-induced kidney injury model, thereby corroborating our findings. Fibronectin, a major non-collagen glycoprotein in the ECM and basement membrane, plays important roles in regulating cell growth, differentiation, polarity, and adhesion (Patten and Wang 2021). Diabetic kidneys often exhibit upregulation of collagen I/IV and α -SMA (Wang et al. 2018), and the elevation of these fibrotic genes promotes ECM accumulation, leading to tubulointerstitial fibrosis, mesangial expansion, glomerulosclerosis, and ultimately irreversible deterioration of renal function.

In this study, STZ injection increased the expression of fibrotic genes including fibronectin, collagen I, and α -SMA in mouse kidneys, which was effectively suppressed by PHL treatment. These results indicate that PHL alleviates renal fibrosis by downregulating the expression of key fibrotic genes in diabetic mice. Interestingly, the anti-fibrotic effect of PHL has also been observed in other pathological contexts, such as hyperuricemia-related kidney disease (Cui et al. 2020),

idiopathic pulmonary injury (Cho et al. 2017), and diabetic endothelial damage (Mao et al. 2022), supporting its broad therapeutic potential.

Oxidative injury and chronic inflammation are recognized as significant pathogenic factors in the pathophysiology of DN (Huang et al. 2020), and accumulating studies have validated the anti-inflammatory and antioxidative properties of PHL in various diabetic complications, including diabetes itself, diabetic neuropathy (Balaha et al. 2018), diabetic kidney disease (Liu et al. 2022), and diabetic cardiomyopathy (Ying et al. 2018). Under hyperglycemic conditions, the endogenous antioxidant system becomes disrupted, leading to excessive generation of ROS that contribute to the pathogenesis of DN (Dai et al. 2021). In the present study, STZ injection resulted in increased MDA content and decreased activities of the antioxidant enzymes CAT, GSH-Px, and SOD, alterations that were reversed by PHL treatment, indicating that PHL protects against renal oxidative injury in diabetic mice by enhancing antioxidant activity and inhibiting lipid peroxidation. Furthermore, hyperglycemia promotes the release of proinflammatory cytokines, thereby exacerbating renal inflammation in DN (Rayego-Mateos et al. 2020). Consistent with this, our results showed that STZ triggered renal inflammatory injury by elevating the levels of IL-6, IL-1 β , and TNF- α , an effect that was neutralized by PHL. These findings suggest that the renoprotective effects of PHL in DN are mediated, at least in part, through its anti-inflammatory and antioxidant activities.

The NF- κ B signaling pathway is widely recognized as a central mediator of inflammatory responses in various pathological conditions (Lawrence 2009). Upon activation of the NF- κ B pathway, I κ B α undergoes degradation, while p65 becomes phosphorylated, leading to the accelerated secretion of proinflammatory cytokines (Oeckinghaus et al. 2011). MAPKs, which include JNK, ERK, and p38 (Cargnello and Roux 2011), participates in a broad spectrum of biological processes such as cell survival, death, differentiation, proliferation, inflammation, apoptosis, and oxidative stress (Anerillas et al. 2020). A growing body of evidence supports the involvement of MAPK/NF- κ B pathways in regulating fibrosis, inflammation, and oxidative stress in DN (Furukawa et al. 2013; Das et al. 2020; Meng et al. 2020; Wang et al. 2021). Furthermore, previous studies have established the ability of PHL to modulate MAPK/NF- κ B signaling (Huang et al. 2015a, 2015b; Wu et al. 2020). In the present study, we observed decreased I κ B α levels and increased phosphorylation of p65 in the renal tissues of STZ-injected mice, indicating NF- κ B pathway activation in DN. Similarly, STZ promoted the phosphorylation of JNK, ERK, and p38, suggesting concomitant MAPK pathway activation. Importantly, PHL treatment reversed all these STZ-induced alterations, demonstrating that the nephroprotective effect of PHL in DN is mediated through the inactivation of both MAPK and NF- κ B signaling pathways.

While the STZ-induced diabetic model is a well-established and widely used method to study DN, it is important to consider its inherent limitations in interpreting our findings. A primary concern is the potential for STZ to cause direct, acute nephrotoxicity independent of hyperglycemia (Kintzel 2001). In this study, the sustained hyperglycemia over an 8-week period, coupled with the manifestation of chronic pathological features such as renal fibrosis, inflammation, and oxidative stress, which are hallmark characteristics of human DN (Lenzen 2008), strongly suggests that the observed renal injury was primarily driven by the diabetic state. Furthermore, the therapeutic effects of PHL, including the amelioration of hyperglycemia and the reversal of these chronic pathological processes, support its efficacy against DN. Nonetheless, we acknowledge that the initial renal insult might have been compounded by STZ-specific toxicity. Future studies employing genetic models of diabetes (e.g., db/db mice) would be valuable to further confirm the specific protective role of PHL in hyperglycemia-mediated nephropathy, independent of chemical inducer effects.

This study has several limitations that should be acknowledged. First, as noted in the preceding paragraph, the use of the STZ-induced diabetic model presents a potential confounder, as direct nephrotoxicity of STZ cannot be entirely ruled out. Second, this research was conducted exclusively in a male mouse model, which may limit the extrapolation of the findings to female subjects. Third, the investigation

focused on the NF- κ B/MAPK pathways; although they are central to the pathology, other signaling mechanisms might also be involved and warrant further exploration. Despite these limitations, the strengths of our work are notable. To our knowledge, this is the first study to comprehensively demonstrate the protective effects of PHL against diabetic renal injury, specifically by linking its efficacy to the amelioration of renal dysfunction, fibrosis, oxidative stress, and inflammation. Mechanistically, we provided clear evidence that the benefits of PHL are mediated through the inactivation of the NF- κ B and MAPK signaling pathways. These findings not only underscore the potential of PHL as a multifaceted therapeutic agent for DN but also establish a solid foundation for future translational research.

Ethics approval. All animal procedures were performed in strict accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The experimental protocol was approved by the Institutional Animal Care and Use Committee of the Hubei Provincial Center for Disease Control and Prevention (Approval No. 202411256; Approval date: April 23, 2024).

Authors contributions. XX conceived and designed the experiments; XX, ChG, XB carried out the experiments; XX, XB analyzed the data and drafted the manuscript. All authors agreed to be accountable for all aspects of the work. All authors have read and approved the final manuscript.

Conflicts of interests. The authors declare that they have no competing interests.

Availability of data and materials. All data generated or analyzed during the current study are available from the corresponding author on reasonable request.

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