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Original Research Article

Protective effect of syringaresinol on rats with diabetic nephropathy via regulation of Nrf2/HO-1 and TGF-β1/Smads pathways

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Abstract

Purpose: *To investigate the protective role of syringaresinol in a rat model of diabetic nephropathy (DN).*
Methods: *Streptozotocin was injected intraperitoneally into rats to establish the diabetic model. Streptozotocin-induced rats were orally administered syringaresinol, and pathological changes in kidneys were assessed using hematoxylin and eosin staining. Enzyme-linked immunosorbent assay (ELISA) was used to determine kidney injury indicators, 24-h urine proteins, blood urea nitrogen (BUN), and serum creatinine (SCR). Blood glucose was measured using a blood glucose meter, while levels of malonaldehyde (MDA), superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-PX) in kidney were also measured using ELISA.*
Results: *Pathological changes in the kidneys were observed in rats post-streptozotocin treatment. Administration of syringaresinol reduced the lesion degree, with improved pathological morphology in kidney. Syringaresinol administration significantly attenuated streptozotocin-increased levels of BUN, SCR, 24-h urine protein, and blood glucose ($p < 0.01$). Streptozotocin-induced oxidative stress, shown by enhanced MDA level and reduced levels of SOD, CAT, and GSH-PX, was reversed in rat kidneys following syringaresinol administration. However, the expression levels of nuclear factor erythropoietin-2-related factor 2 (Nrf2) and heme oxygenase 1 (HO-1) proteins decreased, while transforming growth factor-beta 1 (TGF-β1) and signal transducer and transcriptional modulator (Smad) 2/3/7 proteins increased in rats post-streptozotocin treatment. Syringaresinol administration reversed the effects of streptozotocin on protein expression of Nrf2, HO-1, TGF-β1, and Smad 2/3/7.*
Conclusion: *Syringaresinol exerted a protective effect against DN through activation of Nrf2 and inactivation of TGF-β1/Smad pathways. Thus, the compound can potentially be developed for management of diabetic nephropathy.*

Keywords: *Syringaresinol, Streptozotocin, Diabetic nephropathy, TGF-β1/Smad, Oxidative stress, Nrf2/HO-1*

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INTRODUCTION

Diabetes is a metabolic disease that causes the blood sugar to rise above normal levels, and results in cardiovascular or renal complications [1]. Diabetic nephropathy (DN), caused by microvascular lesions of renal glomeruli, is one of the most common and serious complications of diabetes [1]. Proteinuria, hypertension, edema, and other symptoms are the main clinical manifestations of DN [1]. Increasing evidence has shown that DN is related to oxidative stress and changes in glomerular hemodynamics [2]. Therefore, suppression of oxidative stress ameliorates DN [2].

Syringaresinol, a phenolic compound, is widely found in cereals and medicinal plants [3]. Syringaresinol functioned as an androgen receptor antagonist to impede prostate cancer progression [3]. UV radiation-induced oxidative stress and inflammatory responses in skin cells were suppressed by syringaresinol [4]. Syringaresinol alleviated oxidative stress and inflammatory responses to attenuate diabetic cardiomyopathy [5]. Therefore, syringaresinol has been hypothesized to attenuate DN through suppression of oxidative stress.

Antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-PX), have free radical scavenging activity and protective ability against DN [6]. Nuclear factor erythropoietin-2-related factor 2 (Nrf2) interacts with anti-oxidative response elements of antioxidant genes, such as heme oxygenase 1 (*HO-1*) and *SOD*, and functions as a cytoprotective regulator to maintain the redox homeostasis [7]. Activation of the Nrf2/anti-oxidative response element signaling pathway ameliorated DN [7]. Since syringaresinol has been shown to promote activation of Nrf2 to alleviate oxidative stress in diabetic cardiomyopathy [5], the present study investigated whether Nrf2/HO-1 was implicated in the protection against DN by syringaresinol.

EXPERIMENTAL

Diabetic rat model

Protocols for the use of animals were approved by the Ethics Committee of Chongqing General Hospital (Approval no. 2017013), and conducted in accordance with the National Institutes of Health Laboratory Animal Care and Use Guidelines [8]. A total of thirty male Sprague-Dawley rats (200 - 220 g weight, 7 - 8 weeks old) were purchased from Vital River Laboratory Animal Technology (Beijing, China), and randomized into five groups: control, streptozotocin (STZ), STZ with 10 mg/kg syringaresinol, STZ with 20 mg/kg syringaresinol, and STZ with 40 mg/kg syringaresinol. For establishment of the diabetic rat model, rats were injected with a single intraperitoneal dose of 55 mg/kg STZ (Sigma-Aldrich, St. Louis, MO, USA) dissolved in 0.1 M citrate buffer. Fasting blood glucose levels of rats were determined using a glucometer (Arkray, Shanghai, China), and rats with blood glucose over 12.0 mM and persistent hyperglycemia over 16.7 mM were regarded as diabetic. Rats in the control group were injected with the same volume of citrate buffer alone. Syringaresinol was administered orally to diabetic rats, post-STZ treatment, at 10, 20, or 40 mg/kg every other day for 10 weeks. Blood glucose was recorded every week from 4 to 10 weeks.

Sample preparation and histological examination

At the end of 10 weeks, rats were sacrificed using carbon dioxide asphyxiation. Blood samples were collected using aortic puncture, and the plasma was collected after centrifugation at 12000 × *g* for 10 min. Renal tissues were isolated and used for histological examination. Following fixation in 4 % formalin and embedding in paraffin, renal tissues were dissected into 4 µm sections, and then deparaffinized in xylene and rehydrated with a graded ethanol series. Sections were stained with eosin (Solarbio, Beijing, China) after staining with hematoxylin (Solarbio). Histopathological changes in kidneys were observed under an optical microscope (Zeiss, Oberkochen, Germany).

Biochemical analyses

At the end of 10 weeks, albuminuria was measured in rats after 24-h urine collection in a metabolic cage. Levels of protein in 24-h urine were measured with the Urine Protein Test Kit (Jiancheng Bioengineering Institute, Nanjing, China). Levels of BUN and SCR were determined using the Urea Assay Kit and Creatinine Assay Kit (Jiancheng Bioengineering Institute). For the determination of MDA, SOD, CAT, and GSH-PX, renal tissues were homogenized in normal saline, and the supernatants were harvested after centrifugation at 1000 × *g* for 20 min. The protein concentration was measured with a bicinchoninic acid protein assay kit (Beyotime Institute of Biotechnology, Shanghai, China), and the levels of MDA, SOD, CAT, and GSH-PX were determined using commercial assay kits (Sigma-Aldrich).

Western blot

Renal tissues were lysed in Cell Lysis Buffer (Sigma-Aldrich), and the protein concentration was determined via the bicinchoninic acid protein assay kit. Proteins were separated using gel electrophoresis and transferred onto nitrocellulose membranes. The membranes were blocked with 5 % skim milk and incubated with primary antibodies against Nrf2 and HO-1 (1:2000; Abcam, Cambridge, MA, USA), TGF-β1 (1:2500; Cell Signaling Technology, Danvers, MA, USA), Smad2/3/7 (1:3000, Cell Signaling Technology) and β-actin (1:4000; Abcam). Following incubation with horseradish peroxidase-linked secondary antibody (1:5000; Cell Signaling Technology), target proteins were detected using the enhanced chemiluminescence detection kit (GE Healthcare, Chicago, IL, USA).

Statistical analyses

Data are expressed as mean ± standard error of the mean. Statistical differences were determined using Student's *t*-test or one-way analysis of variance, with $p < 0.05$ considered statistically significant.

RESULTS

Syringaresinol mitigated renal morphology in diabetic rats

Rats were injected intraperitoneally with streptozotocin to establish the *in vivo* diabetic model. Streptozotocin induced a series of degenerative changes in the kidneys, with renal tissue necrosis, renal tubule atrophy, increased mesangial matrix, thickened glomerular basement membrane, and inflammatory cell infiltration (Figure 1). However, orally administered syringaresinol alleviated the histopathological changes in kidney tissue in a dose-dependent manner (Figure 1).

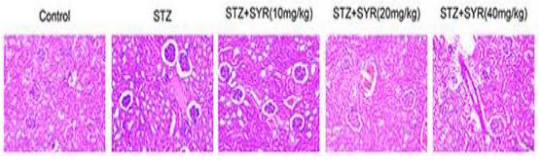

Figure 1: Syringaresinol mitigates renal morphology in diabetic rats. Intraperitoneal injection of streptozotocin induced a series degenerative changes in the kidneys, while oral administration of syringaresinol alleviated the histopathological changes in the kidneys, in a dose-dependent manner

Syringaresinol mitigated renal function in diabetic rats

Intraperitoneal injection with streptozotocin destroyed renal function in rats, as evidenced by elevated BUN (Figure 2 A), SCR (Figure 2 B) and 24-h urine protein (Figure 2 C). Administration of syringaresinol dose-dependently reduced the indicators of impaired kidney function, including BUN (Figure 2 A), SCR (Figure 2 B), and 24-h urine protein (Figure 2 C), demonstrating that syringaresinol mitigated the loss of renal function in diabetic rats.

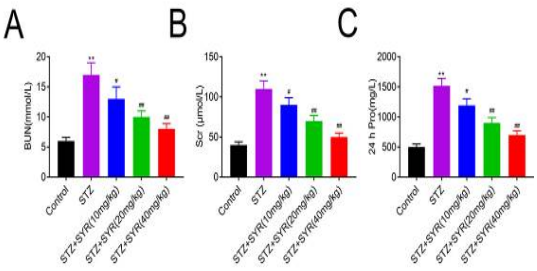

Figure 2: Syringaresinol mitigates loss of renal function in diabetic rats. (A) Administration of syringaresinol dose-dependently attenuated the streptozotocin-induced increase of BUN. (B) Administration of syringaresinol dose-dependently attenuated the streptozotocin-induced increase of SCR. (C) Administration of syringaresinol dose-dependently attenuated the streptozotocin-induced increase of 24-h urine protein; # $p < 0.05$, **, ## $p < 0.01$

Syringaresinol mitigated oxidative stress in diabetic rats

The level of blood glucose in diabetic rats was significantly increased when compared to the control group (Figure 3 A), while syringaresinol administration dose-dependently decreased the level of blood glucose (Figure 3 A). MDA, the indicator of lipid peroxidation, was elevated in rats post-streptozotocin injection (Figure 3 B). However, syringaresinol administration dose-dependently reduced the level of MDA (Figure 3 B).

Moreover, rats post-streptozotocin injection showed a significant decrease of oxidative stress indicators, including SOD (Figure 3 C), CAT (Figure 3 D), and GSH-PX (Figure 3 E) compared to the control group. Syringaresinol mitigated oxidative stress in diabetic rats, resulting in reduced MDA (Figure 3 B), and enhanced SOD (Figure 3 C), CAT (Figure 3 D), and GSH-PX (Figure 3 E).

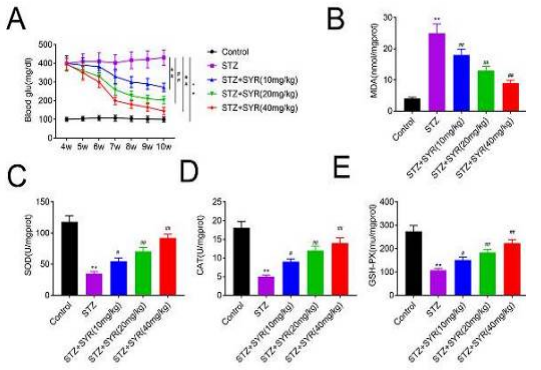

Figure 3: Syringaresinol mitigates oxidative stress in diabetic rats. (A) Administration of syringaresinol dose-dependently attenuated the streptozotocin-induced increase of blood glucose. (B) Administration of syringaresinol dose-dependently attenuated the streptozotocin-induced increase of MDA. (C) Administration of syringaresinol dose-dependently attenuated the streptozotocin-induced decrease of SOD. (D) Administration of syringaresinol dose-dependently attenuated the streptozotocin-induced decrease of CAT. (E) Administration of syringaresinol dose-dependently attenuated the streptozotocin-induced decrease of GSH-PX; #p < 0.05, **, ##p < 0.01

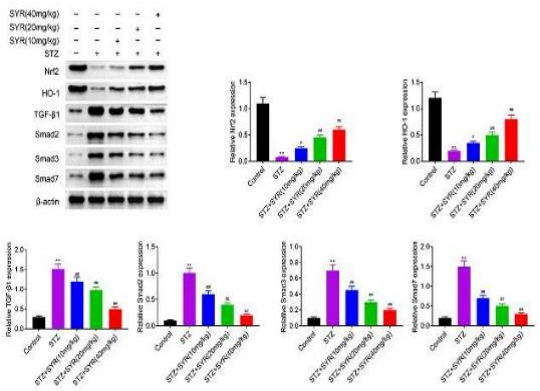

Figure 4: Syringaresinol modulates Nrf2 and TGF-β pathways in diabetic rats. Administration of syringaresinol dose-dependently attenuated the streptozotocin-induced decreases in Nrf2 and HO-1, as well as the streptozotocin-induced increases in TGF-β1 and Smad2/3/7; #p < 0.05, **, ##p < 0.01

Syringaresinol modulated Nrf2 and TGF-β pathways in diabetic rats

Nuclear factor erythropoietin-2-related factor 2 (Nrf2) and HO-1 were reduced in the kidneys of rats post-streptozotocin injection (Figure 4), while syringaresinol administration dose-dependently enhanced protein expression of Nrf2 and HO-1 (Figure 4), suggesting that syringaresinol promoted activation of the Nrf2 pathway in diabetic rats. Moreover, TGF-β1 and Smad2/3/7 proteins were increased in the kidneys of rats post-streptozotocin injection (Figure 4), while syringaresinol administration dose-dependently decreased protein expression of TGF-β1 and Smad2/3/7 (Figure 4), suggesting that syringaresinol promoted inactivation of the TGF-β/Smad pathway in diabetic rats.

DISCUSSION

Diabetes increases accumulation of reactive oxygen species through auto-oxidation of glucose and formation of glycation end products, or suppresses antioxidant defenses through formation of superoxide [9]. Accumulation of reactive oxygen species results in renal fibrosis, which is the main pathological change in DN [10]. Moreover, research has shown that antioxidant therapies suppress DN in rodents [11]. The role of syringaresinol on oxidative stress in DN was investigated in this study.

Rodents given streptozotocin injection demonstrate pancreatic injury and renal injury similar to human DN [12]. In line with a previous study showing that streptozotocin injection induced morphological changes in kidneys, with glomerular basement membrane thickening, mesangial expansion, glomerular hypertrophy, and interstitial fibrosis [12], data in this study identified a series of degenerative changes in the kidneys of rats post-streptozotocin injection. Moreover, streptozotocin injection also induces progressive loss of renal function [12]. Further, syringaresinol ameliorated morphological changes in kidneys and alleviated progressive loss of renal function, as evidenced by decreases in BUN, SCRs and 24-h protein.

Dihydroethidium staining showed that streptozotocin injection promotes the generation of reactive oxygen species in the myocardia of diabetic mice, and this generation of reactive oxygen species was reduced in mice following syringaresinol administration [5]. Syringaresinol-di-O-β-D-glucoside, a phenolic compound from *Polygonatum sibericum*, showed antioxidative and antidiabetic effects against streptozotocin-induced diabetes in mice [13]. Reduction of elevated MDA and restoration of antioxidant enzymes, including SOD, CAT, and GSH-PX, by syringaresinol administration were also validated in the present study, suggesting that syringaresinol may alleviate DN through suppression of oxidative stress.

Nuclear factor erythropoietin-2-related factor 2 (Nrf2) transcriptionally modulates the expression of target genes, including *HO-1*, *GPX*, and *SOD*, through binding to the antioxidative-response elements in the promoters, and the dysregulation

of Nrf2 results in toxic metabolite accumulation and oxidative stress during the pathogenesis of DN [14]. Western blot analysis revealed that syringaresinol administration increased protein expression of Nrf2 and HO-1, suggesting it has an antioxidative effect against DN. Progressive accumulation of extracellular matrix components in tubular interstitium and in glomerular mesangium was reported to result in proteinuria and renal failure during development of DN [15]. TGF-β1 promotes the accumulation of extracellular matrix components through activation of Smad2 and Smad3, or inactivation of Smad7. Blockade of the TGF-β/Smad pathway provides effective prevention of DN progression [12]. This study also indicated that syringaresinol administration attenuates streptozotocin-induced increases in TGF-β1 and Smad2/3/7 protein expression in diabetic rats [12]. Therefore, syringaresinol might demonstrate antioxidative and antidiabetic effects against DN through Nrf2-mediated oxidative stress and Nrf2-extracellular matrix production through the TGF-β/Smad pathway. In addition, hyperglycemia promotes metabolic change in diabetes, with the accumulation of oxidative stress, while accumulation of reactive oxygen species promotes secretion of cytokines during development of DN [16]. Smads interact with NF-κB or MAPK pathways to regulate renal inflammation and fibrosis in DN [17]. Therefore, whether syringaresinol may mediate inflammatory responses to ameliorate DN progression needs further investigation.

CONCLUSION

This study suggests that syringaresinol exerts protective effects against DN via antioxidative effects. Inactivation of the TGF-β/Smad extracellular matrix production pathway and activation of the Nrf2/HO-1 antioxidative pathway were regarded as the mechanisms underlying the antidiabetic effect of syringaresinol against DN. Therefore, these results provide evidence for the potential use of syringaresinol in preventing DN.

DECLARATIONS

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Lei Ji and Xue Zhong designed the study and supervised the data collection, Xingxing Xia analyzed and interpreted the data, Wei Yu and Yuping Qin prepared the manuscript for publication and reviewed the draft of the manuscript. All authors have read and approved the manuscript.

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