Chinese Journal of Physiology 61(6): 349-359, 2018
DOI: 10.4077/CJP.2018.BAH637

Xueshuantong for Injection Ameliorates Diabetic Nephropathy in a Rat Model of Streptozotocin-Induced Diabetes

Jinxin Wang[1, #], Ruilin Li[1, #], Zhiyuan Deng[4], Zuoyan Sun[1], Lijuan Chai[1], Hong Guo[1], Hong Wang[2], Lu Chen[1], Limin Hu[1], and Shaoxia Wang[2, 3]

[1]Tianjin State Key Laboratory of Modern Chinese Medicine, Tianjin University of Traditional Chinese Medicine, Tianjin 300193,
[2]School of Integrative Medicine, Tianjin University of Traditional Chinese Medicine, Tianjin 300193,
[3]Key Laboratory of Pharmacology of Traditional Chinese Medical Formula, Ministry of Education, Tianjin University of Traditional Chinese Medicine, Tianjin 300193
and
[4]Wuzhou Pharmaceutical Group Co., Ltd., Wuzhou 543002, People's Republic of China

Abstract

Diabetic nephropathy (DN) is a major complication of diabetes and becomes the chief cause of end-stage renal disease. Our study was undertaken to investigate the ameliorative effect and underlying mechanism of Xueshuantong for Injection (XST) on DN in streptozotocin (STZ)-induced rats. Effect of XST treatment (XST, 50 mg/kg/day, i.p.) lasting 60 days after STZ-induced (60 mg/kg, i.p.) diabetes was investigated. Blood sugar levels and body weight were recorded every week of the experiment. At the 28th and 56th days after injection urine glucose and 24 h urine protein excretion were determined. Apoptosis related factors such as cleaved caspase-3, Bcl-2, Bax and inflammation related factors, including tumor necrosis factor-α (TNF-α), interleukin 6 (IL-6), IL-1β, inducible nitric oxide synthase (iNOS) and intercellular adhesion molecule-1 (ICAM-1) were detected by PCR or western blot. The expression levels of fibronectin, Collagen I, α-smooth muscle actin (α-SMA) and proliferating cell nuclear antigen (PCNA), TGF-β-Smad2/3 signaling pathway, and receptor for advanced glycation end products (RAGE) was investigated. Our results showed that XST treatment did not affect levels of body weight, blood glucose and urine glucose levels. Our analysis revealed that XST inhibited cell apoptosis and suppressed the properties of RAGE in the kidney. XST treatment could also significantly suppress the overexpression of pro-inflammatory mediators in kidney and prevent renal fibrosis by blocking the TGF-β/Smad2/3 signaling pathway. In conclusion, our findings suggested that XST could provide protection against DN through reduction of RAGE accumulation, decreasing inflammation, inhibition of renal fibrosis, and blocking the TGF-β/Smad2/3 signaling pathway.

Key Words: diabetic nephropathy, panax notoginseng saponins, TGF-β/Smad2/3 signaling pathway, Xueshuantong

Corresponding Author: Dr. Shaoxia Wang, School of Integrative Medicine, Key Laboratory of Pharmacology of Traditional Chinese Medical Formula, Ministry of Education, Tianjin University of Traditional Chinese Medicine, 312 Anshanxi Road, Nankai District, Tianjin 300193, People's Republic of China, E-mail: wangsx@tjutcm.edu.cn #These authors contributed equally to this work.
Received: September 5, 2018; Revised (Final Version): December 11, 2018; Accepted: December 14, 2018.
 ISSN : 0304-4920. http://www.cps.org.tw

Introduction

Diabetes is characterized by elevated blood glucose levels and the number of diabetic patients is expected to be more than 500 million in 2030 globally (17). Nowadays, the chronic complications of diabetes are the major causes of morbidity and mortality of the patients. It is well known that as much as 60-70% of costs related to diabetes are currently attributable to chronic complications (33). It is recognized that hyperglycemia has a pathogenic role in micro- and macro-vascular complications (12). Despite adjustment of blood glucose levels, diabetic complications develop frequently. Correspondingly, the development of new drugs for reducing the risk of development of diabetic complications is an urgent priority.

Diabetic nephropathy (DN) is one of the most frequent chronic microvascular complications of diabetes mellitus, and one of the leading causes of end-stage renal disease (17). More and more evidence suggests that fibrosis plays an irreplaceable role in DN, which could be a consequence of TGF-β1-Smad2/3 signaling pathway activation. Transforming growth factor-β1 (TGF-β1), α-smooth muscle actin (α-SMA), Smad2/3, fibronectin, Collagen I and other numerous factors contribute to the fibrosis of kidneys. The advanced glycation end products (AGE) accumulate in diabetes and activate receptor for AGE (RAGE), which induces inflammation in the kidney and contribute to DN (10). Although some drug treatment had shown that partial therapeutic effect on DN, the current treatment for DN is far from satisfactory. However, the study of the above mechanisms of DN provides a direction for drug development.

Xueshuantong for Injection (Lyophilized) (XST) is a standardized product extracted from San-chi, the root of *Panax notoginseng (BurkillF. H.Chen)*. It is recorded by the People's Republic of China Pharmacopoeia for treatment of stroke and diabetic retinopathy. Many previous researches have demonstrated that its ingredients, which are several kinds of *Panax notoginseng* saponins, are definite and clear (7, 21). In the present study, we found that XST could provide protection against DN through reduction of RAGE accumulation, decreasing inflammation, inhibition of renal fibrosis, and blocking the TGF-β/Smad2/3 signaling pathway.

Materials and Methods

Drug

XST was provided by Wuzhou Pharmaceutical Co., Ltd. (Guangxi province, China), consisting of total saponins from *Panax notoginseng* (Sanqi). The quality standard is consistent with requirement of related part of Chinese Pharmacopeia. In Ch.P (2015), the method of reflux extraction and concentration of 70% alcohol was used to extract crude notoginsenosides from coarse powder of *Panax notoginseng,* a series of complicated special processes are required to obtain XST. Many articles indicated that the composition analysis of XST by high performance liquid chromatography (HPLC) showed that it contains ginsenoside Rg1 (48.1%), ginsenoside Rb1 (26.17%-29.60%), notoginsenoside R1 (11.1%), ginsenoside Re (5.5%) and ginsenoside Rd (1.3%) (7, 34). XST was freshly prepared in 0.9% normal saline before use in our study.

Animals

All animal studies were approved by the Animal Care and Use Committee at Tianjin University of Traditional Chinese Medicine. SPF (Specific pathogen free) male healthy Sprague-Dawley (SD) rats (280 ± 20 g, 6-7 weeks) were purchased from Vital River Laboratory Animal Technology Co. Ltd. (Beijing, PRC). The animals were housed under light-controlled conditions 12h light/12h dark cycle and at room temperature of 22°C with free access to food and water.

Induction of Type I Diabetes in Rats and Treatment

As shown in Fig. 1A, diabetes mellitus was induced by high-dose of streptozotocin (STZ) (Sigma, St. Louis, MO, USA) through single intraperitoneal injection in SD rats (60 mg/kg dissolved in citrate buffer solution, 0.1 mM, pH 4.5) (8), 58 in total. The fasting blood glucose level from a tail vein sample was tested by using a glucose analyzer (ONETOUCH Ultra System; Johnson & Johnson, USA) in 72 hours (n = 40). Diabetes was defined by fasting blood glucose >15 mmol/L (n = 36). The experimental procedure was shown in Fig 1A. The diabetes mellitus (DM) rats were randomly divided into the following 2 groups: DM group and Xueshuantong-treatment (50 mg/kg) group (XST50). There were 19 rats in the model group and 15 survived at the end of the experiment, DM (n = 15). There were 17 rats in the XST50 group and 16 survived at the end of the experiment, XST50 (n = 16). XST was dissolved in normal saline, and was administrated (50 mg/kg, i.p.) once a day continuously for 60 days. The control group (n = 18) and DM group were treated with isodose saline.

Biochemical Examination

Blood glucose levels and body weight were

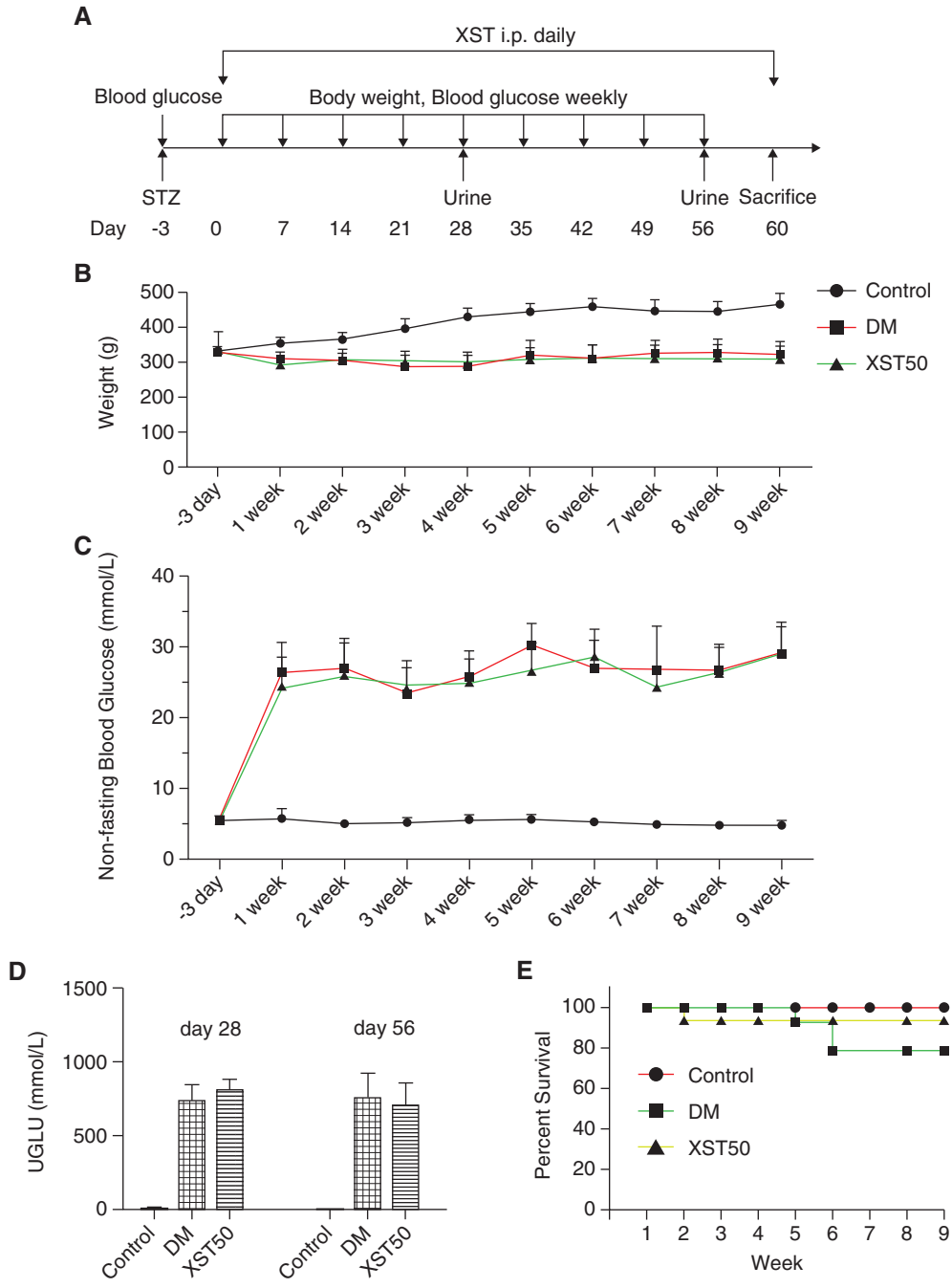

Fig. 1. Effect of XST on body weight, blood glucose levels and urine glucose in DM rats. (A) The experimental procedure to test XST effect on body weight and blood glucose in rats is depicted. (B, C) Compared with control group, the body weight was significantly down-regulated and blood glucose was markedly enhanced in DM group. However, XST treatment did not affect the levels of body weight and blood glucose. (D, E) no significant differences were observed in urine glucose levels and survival rate between DM and XST50 groups. (A) Control group, n = 18; DM group, n = 15; XST50, n = 16. (B, C) n = 4, (D) n = 5.

recorded every week of the experiment. At the 28th and 56th days after injection urine glucose (UGLU) and 24 hours urine protein excretion was determined.

Western Blot Analyses

Sixty days after XST treatment, kidney tissues were harvested and frozen immediately in liquid

Table 1. Primers of real-time RT–PCR

Gene	Primer Pair(5'-3') F, forward; R, reverse	Ref.
Bax	F 5'-CCAAGAAGCTGAGCGAGTGTCTC-3' R 5'- AGTTGCCATCAGCAAACATGTCA-3'	(30)
Bcl-2	F 5'-GGAGCGTCAACAGGGAGATG-3' R 5'-GATGCCGGTTCAGGTACTCAG-3'	(30)
RAGE	F 5'-ACAGAAACCGGTGATGAAGG-3' R 5'-ATTCAGCTCTGCACGTTCCCT-3'	(20)
TNF-α	F 5'- TCTTCTC ATTCC TGC TCGTGG-3' R 5'- GGTC TGGGCCATGGAAC TGA-3'	(28)
ICAM-1	F 5'-GTTCGTTCTCTTCTTTTCCTT-3' R 5'-ATTATCCACATTTACCTGCGG-3'	(27)
β-actin	F 3'-GTAAAGACCTCTATGCCAACA-5' R 3'-GGACTCATCGTACTCCTGCT-5'	(27)
IL-6	F 5'-ACAGCGATGATGCACTGTCA-3' R 5'-AGCACACTAGGTTTGCCGAG-3'	(14)
iNOS	F 5'- AAAATGGTTTCCCCCAGTTC-3' R 5'- GTCGATGGAGTC ACATGC AG-3	(28)
IL-1β	F 5'- GAAGTCAAGACCAAAGTGG-3' R 5'- TGAAGTCAACTATGTCCCG-3'	(28)

nitrogen until homogenization. Total proteins of tissues were extracted using Protein Extraction Reagent Kit according to the manufacturer's protocol. The same amounts of proteins were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Then, the proteins were transferred onto PVDF membranes (Millipore, Germany). After 1 h blocking at room temperature in 5% fat-free dry milk, the membranes were incubated overnight at 4°C with anti-cleaved caspase-3 (1:1000, CST, #9664), anti-RAGE (1:1000, Abcam, ab3611), anti-Collagen I (1:2000, Abcam, ab34170), anti-Fibronectin (1:3000, Abcam, ab2413), anti-α-SMA (1:5000, Abcam, ab5694), anti-PCNA (1:1000, CST, #13110), anti-TGF-β1 (1:1000, Abcam, ab66043), anti-CTGF (1:1000, Abcam, ab6992), anti-p-Smad2/3 (1:1000, Abcam, ab63399), anti-β-actin (1:1000, Abcam, ab8226) antibodies. After washing, the membranes were probed with horseradish peroxidase-labeled secondary antibodies (1:10000). Finally, immune complexes were visualized with chemiluminescent (ECL) detection.

Quantitative Real-Time Polymerase Chain Reaction

Total RNA was extracted from kidney using Trizol reagent according to the manufacturer's instructions. First-strand cDNA was synthesized 1 μg of total RNA with oligo dT primers using the High-Capacity cDNA Reverse Transcription kit (Applied Biosystems). The mRNA expression levels was performed using SYBR Green qPCR Select Master Mix Kit (ABI) on an ABI prism 7500 sequence detector system (Applied Biosystems, Foster City, CA, USA). The specific primer pairs are listed in Table 1. The relative expression level was calculated using the $2^{-\Delta\Delta CT}$ method.

Statistical Analysis

Data are presented as the mean ± standard deviation of the mean. Group means were compared by a one-way analysis of variance (ANOVA) using the statistical software program SPSS19.0 for Windows (Chicago, IL, USA). P values < 0.05 were considered statistically significant in all cases.

Results

Effect of XST on the Biochemical Criterion in DM Rats

During XST treatment, the body weight, blood glucose levels and urine glucose levels of rats were routinely checked (Fig. 1, B and C). As indicated in Fig. 1C, body weight continuously increased in control group. However, in the DM group, body weight increased more slowly than control group. Compared with control group, the blood glucose and urine glucose levels in DM rats were increased after STZ administration (Fig. 1, C and D). In XST50 group, no significant effects on the body weight, blood glucose levels, urine glucose levels

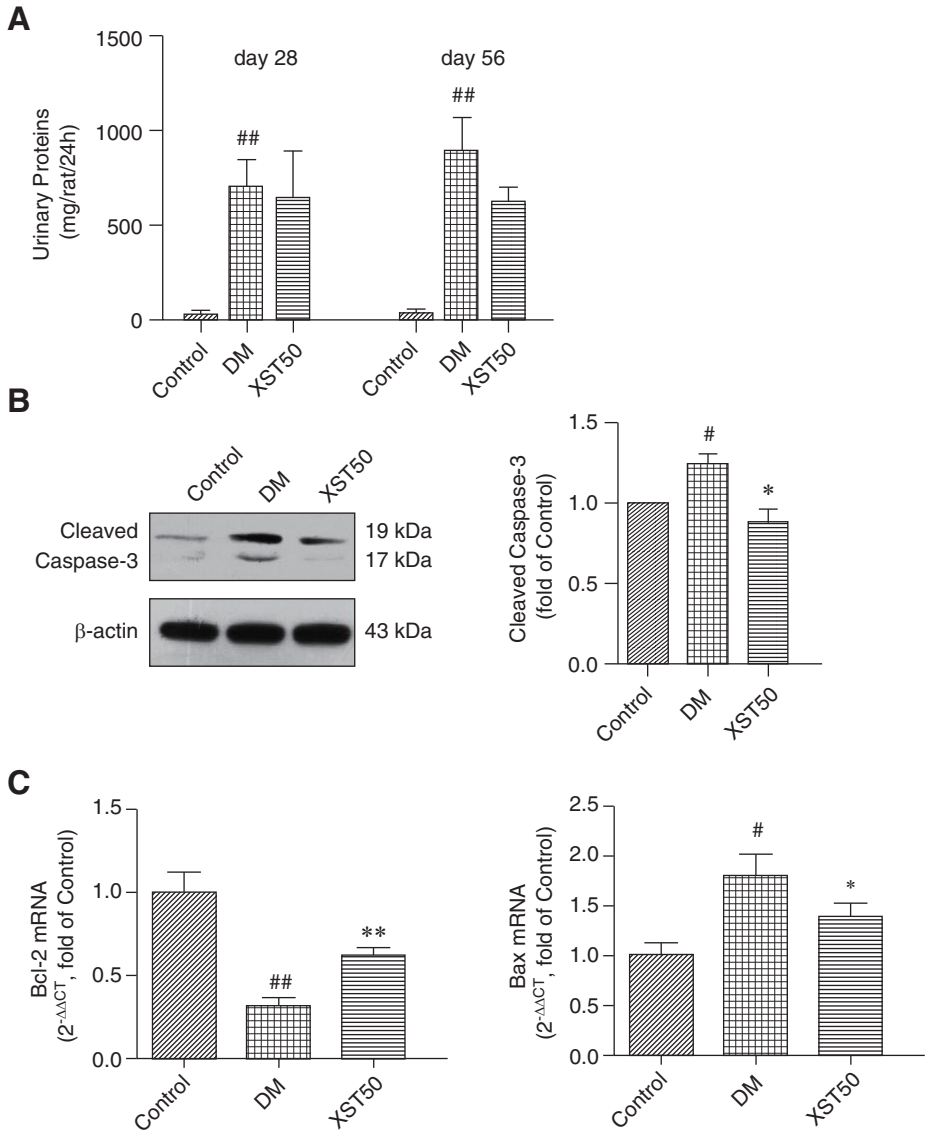

Fig. 2. XST protected against DN. (A) A higher level of excretion of urinary protein was detected in DM group. Treatment with XST reduced the excretion of proteins significantly at the 56th day. (B) Results of western blot suggested that the expression of cleaved Caspase-3 was increased in DM group, and XST treatment significantly lowered cleaved Caspase-3 level. (C) XST treatment could regulate the expression of apoptosis-related genes Bax, Bcl-2. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, DM *vs.* Control; $^{*}P < 0.05$, $^{**}P < 0.01$, XST50 *vs*. DM. (A) Control group, n = 18; DM group, n = 15; XST50, n = 16, (B) n = 4, (C) n = 5.

and survival rate were observed compared with the DM group (Fig. 1, B-E).

XST Protected against DN

To determine if XST can protect DM rats against DN, urine samples for a 24-h duration at the 28th day and 56th day after XST treatment were collected and urinary proteins were determined. XST treatment significantly reduced urinary proteins at the 56th day (Fig. 2A).

High expression of cleaved caspase-3 can make contributions to the development of DN. XST treatment decreased the level of cleaved caspase-3 protein in rats' kidneys compared with DM group (Fig. 2B). Meanwhile, XST treatment could regulate the expressions of apoptosis-related genes Bax, Bcl (Fig. 2C).

XST Treatment Inhibited the Accumulation of Advanced Glycation End Products in DM Rats.

RAGE is an important risk factor contributing

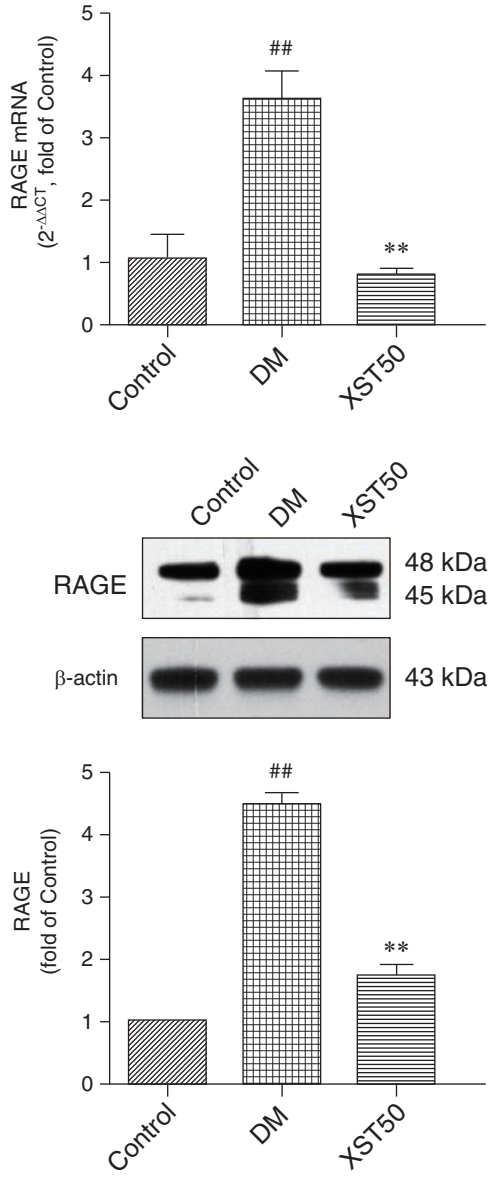

Fig. 3. XST treatment inhibited the accumulation of RAGE in DM Rats. Results of western blot and PCR indicated that RAGE protein expressions were significantly increased in DM group compared to control rats. After treatment with XST, the accumulation of RAGE protein in diabetic rat kidney was markedly less than DM group. $^{\#\#}P < 0.01$, DM *vs*. Control; $^{**}P < 0.01$, XST50 *vs.* DM, n = 4.

to the development of DN. To further elucidate the mechanism of the protective effects of XST against DN, the effect of XST on the expression of RAGE in DM rat kidney was explored. As shown in Fig. 3, DM induced RAGE accumulation in kidney compared with control rats. However, XST administration blocked RAGE accumulation as compared to the DM group.

XST Inhibited mRNA Expressions of Inflammatory Cytokines in DM Rats

Changes in mRNA levels of intercellular adhesion molecule-1 (ICAM-1), interleukin 6 (IL-6), IL-1β, tumor necrosis factor-α (TNF-α) and inducible nitric oxide synthase (iNOS) in kidney were observed by real-time PCR analysis. As shown in Fig.4, the results showed that DM significantly upregulated the mRNA levels of ICAM-1, IL-6, IL-1β, TNF-α and iNOS, respectively. XST treatment reversed the increase of mRNA levels of these cytokines.

XST Inhibited the Fibrosis of Kidney in DM Rats

Fibrosis is the final common pathway to DN (7). To determine the effect of XST treatment on fibrosis of kidney, the level of fibrosis protein in the kidney was measured using western blot. Fig. 5 showed that DM induced fibronectin, Collagen I, α-SMA and proliferating cell nuclear antigen (PCNA) accumulation in kidney compared with control group. XST significantly inhibited the expression of fibronectin, Collagen I, α-SMA and PCNA compared with DM group.

XST Treatment Inhibited the Activation of TGFβ-Smad2/3 Signaling Pathway in DM Rats

As shown in Fig 6, the expression levels of TGF-β1, connective tissue growth factor (CTGF) and phosphorylation of Smad2/3 (p-Smad2/3) were markedly increased in the DM group compared with those in the control group. While XST treatment could decrease the expression levels of TGF-β1, CTGF and p-Smad2/3 compared with DM group.

Discussion

DN is one of the most common and severe chronic complications of DM, the leading cause of chronic renal disease, and a major cause of cardiovascular mortality in the patients (2, 31). It is frequently observed in the patients with a long history of diabetes (9). Despite the constant progress in the treatment of DM, there still lack of effective means to control DM and the complications. XST is a natural mixture, extracted from San-chi. In this study, we evaluated the protective effect of XST against DN, and found its protective effect were related with reduction of RAGE accumulation, decreasing inflammation, inhibition of renal fibrosis, and blocking the TGF-β/Smad2/3 signaling pathway.

Based on whether XST could improve DN, the pathological changes in the kidney were due to XST

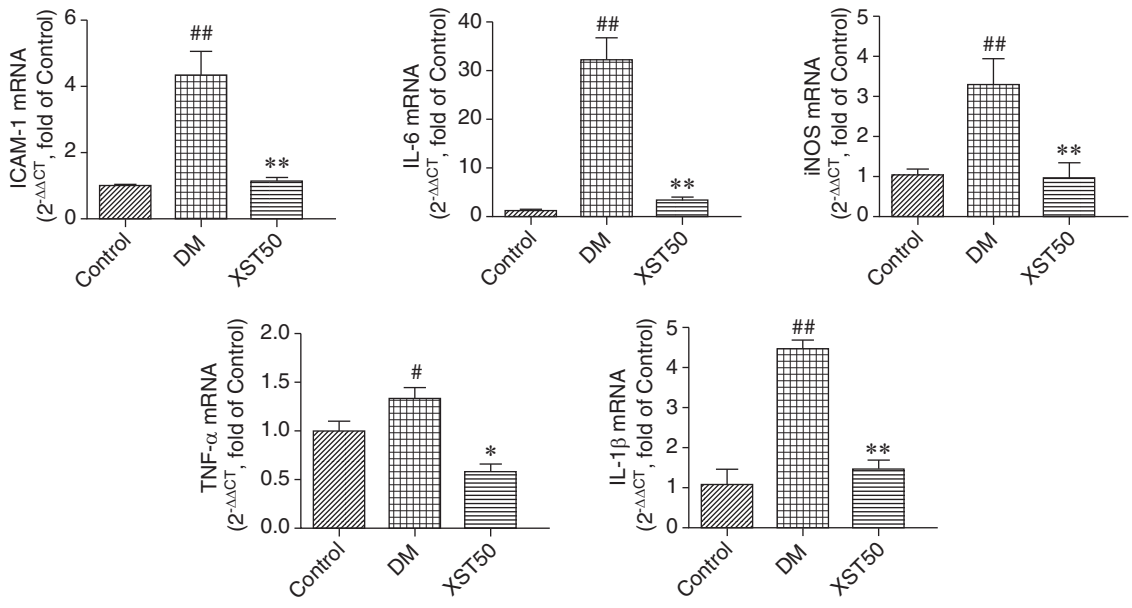

Fig. 4. XST inhibited mRNA expressions of inflammatory cytokines (ICAM-1, IL-6, iNOS, TNF-α, IL-1β) in DM rats. Inflammatory cytokines expressions were determined by PCR. XST treatment reversed the increase of mRNA levels of these cytokines. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, DM *vs*. Control; $^{*}P < 0.05$, $^{**}P < 0.01$, XST50 *vs*. DM, n = 5.

treatment or because of lower blood glucose levels, further research is needed. So we have conducted a series of studies. In our study, no significant differences were observed between the blood glucose of XST50 group and DM group. In fact, many drugs have a therapeutic effect on diabetes without affecting blood glucose levels. Farhad Ghadiri Soufi *et al*. revealed that resveratrol can inhibit the enhancement of retinal apoptosis and pro-inflammatory mediators such as TNF-α, IL-6, and reduced the retinal nuclear factor-keppa B (NF-κB) activity in diabetic rats. However, resveratrol administration did not significantly change the blood glucose concentration (8). Hydrogen sulfide could improve STZ-induced diabetes by regulating extracellular-signal-regulated kinase 1/2 (ERK1/2)/cyclooxygenase-2 (COX-2)/prostaglandin E2 (PGE2) pathway without affecting blood glucose levels (13). Our experiment did not reveal any significant change in blood glucose between XST50 group and DM group. This result might be attributable to the different extraction methods and animal models used in each experiment. The DN is initially characterized by thickening glomerular and tubular basal membrane with progressive mesangial expansion which ultimately leads to glomerulosclerosis and loss of renal functions. This progress causes DN, generally accompanies with adding of urine protein and deteriorating of kidney function (32). In fact, patients with diabetes have been shown to have higher urine protein excretion for kidney injury when compared to health people. This has led to the routine evaluation of the urine protein as a screening tool for detecting the presence of DN in patients.

Cell apoptosis plays an important role in the onset and development of DN. Hyperglycemia is suggested as a strong sensitizer to renal apoptosis (18) and renal apoptosis may mediate loss of renal cells with subsequent gradual renal dysfunction (7, 16). Activation of cleaved caspase-3 is critical for apoptosis. It can control the release and the activation of downstream protease and mediate cell apoptosis and it has been recognized as an important indicator and marker of cell apoptosis (21, 25). Bcl-2 and Bax are also the most characteristic of the regulators of apoptosis. In this study, we demonstrated that XST substantially suppress the apoptosis signaling pathway, implying the protective effects of XST against DN.

Heightened expression of RAGE, a signal transduction receptor of the immunoglobulin superfamily, contributes to development of systemic diabetic complications. In diabetes, activated RAGE could induce inflammation in the kidney and then contribute to DN (23). Previous research showed that RAGE can activate the pro-inflammatory transcription factor NF-κB and subsequent expression of genes regulated by NF-kB. What's more, the

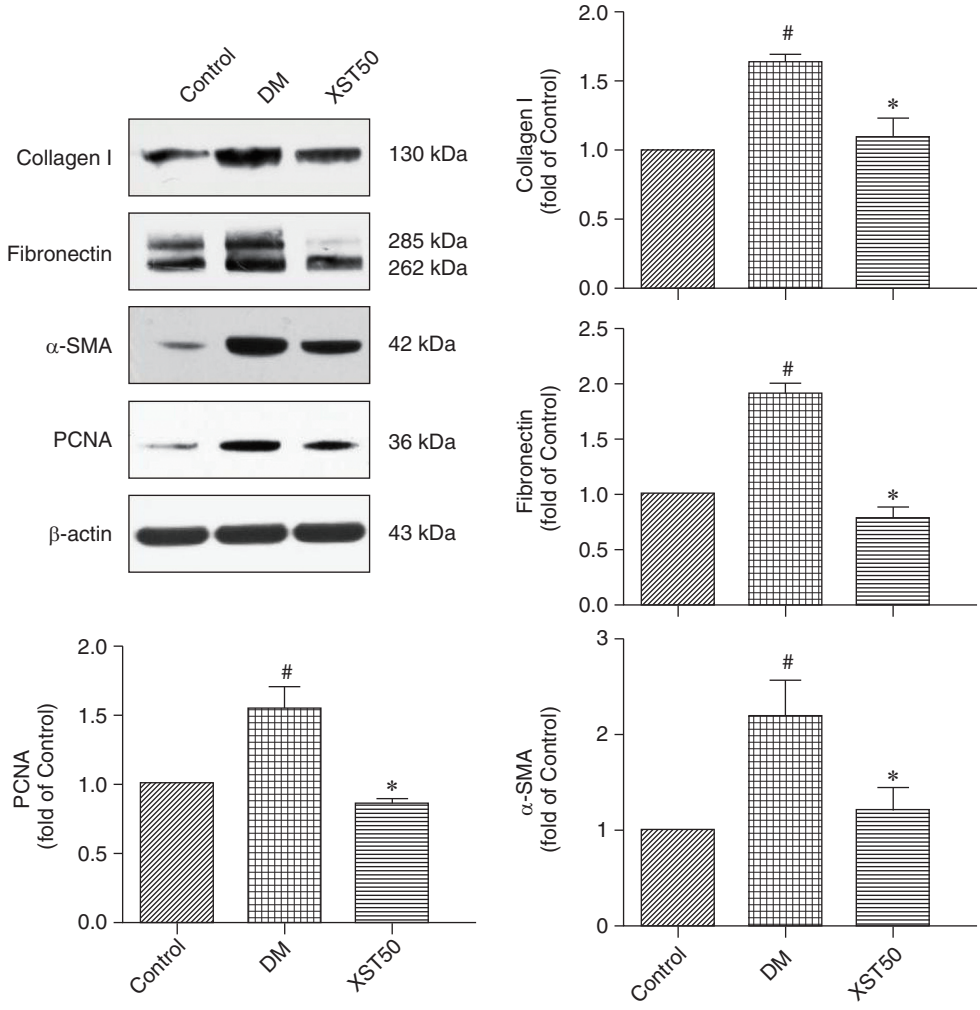

Fig. 5. XST inhibited the fibrosis of kidney in DM Rats. The western blot analysis showed that the expressions of fibronectin, Collagen I, α-SMA and PCNA in diabetic rats were significantly raised in DM group than that of control group. Treatment with XST effectively suppressed the expression of fibronectin, Collagen I, α-SMA and PCNA. $^{\#}P < 0.05$, DM *vs*. Control; $^{*}P < 0.05$, XST50 *vs*. DM, n = 4.

kidneys of RAGE knockout mice with STZ-induced diabetes, compared with normal diabetic mice, were shown to develop less glomerular matrix expansion and basement membrane thickening (1). In the current study, the expression of RAGE was higher in DM group than in the control group. Treatment with XST significantly reduced RAGE expression, which suggested that the protective effect of XST may be related with down-regulation of RAGE. Our experiment did not reveal any significant change in blood glucose between XST treatment groups and DM group and the mechanism remains to be further studied. A variety of compounds have been proved that they can prevent *in vivo* accumulation of AGE/RAGE without altering blood glucose levels in STZ-induced diabetic rats. For example, Eucommia ulmoides extract treatment did not change blood glucose but could ameliorate the renal damage in diabetic mice by inhibiting AGEs formation and RAGE expression and reducing oxidative stress, through the Glo1 and Nrf2 pathways in STZ-induced diabetic mice (6). Ellagic acid could prevent *in vivo* accumulation of AGE and to ameliorate retinal changes, without altering blood glucose levels in STZ-induced diabetic rats (24).

It was the important pathological basis of DN that extracellular matrix gradually accumulation and fibrosis (22). Patients with DN eventually develop kidney fibrosis (11). In our study, we demonstrate that XST decreased fibronectin, Collagen I, α-SMA and PCNA expression in kidney of DM rats. TGF-β1-Smad2/3 signal pathway has been considered to play a key role in the fibrosis of tissues and organs (31). Clinical researches have demonstrated

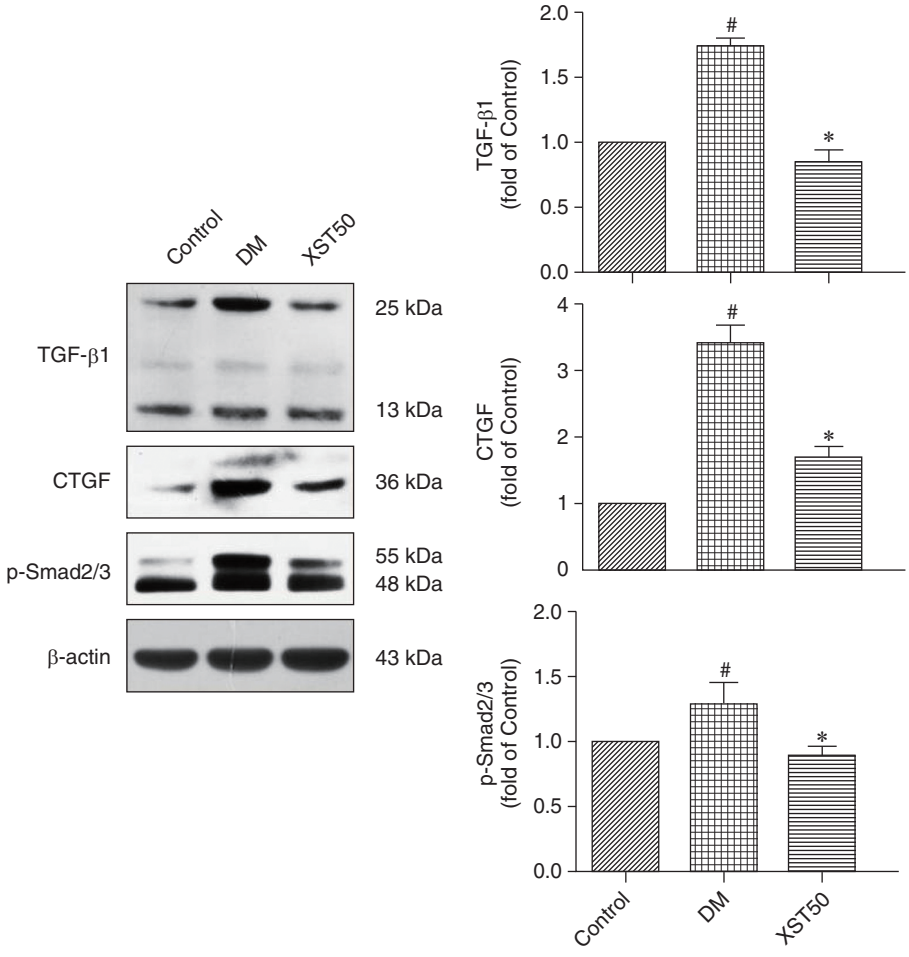

Fig. 6. XST treatment inhibited the activation of TGFβ-Smad2/3 signaling pathway in DM Rats. Western blot analysis was performed to detect the relative levels of TGF-β1, CTGF protein and phosphorylation of Smad2/3 (p-Smad2/3) in rat kidney tissue. XST treatment significantly suppressed TGF-β1 and CTGF protein expression and inhibited p-Smad2/3 compared with those of DM group. $^{\#}P < 0.05$, DM *vs*. Control; $^{*}P < 0.05$, XST50 *vs*. DM, n = 4.

that high glucose could stimulate the production of TGF-β1 (5), promote fibrosis and thereby contributing to kidney functional loss. In the current study, the amount of TGF-β1 expression was higher in the DM group than in the control group, which was in accord with previously reported findings (15). Additionally, XST significantly decreased the expression of TGF-β1 and inhibited the activation of TGF-β1-Smad2/3 signal pathway in diabetic rats.

Nowadays, the average drug discovery and development process costs billions of dollars and spends several years. Traditional herbs and botanical extracts already become important sources of discovering and developing therapies for diseases. XST is a standardized product extracted from *Panax notoginseng* (Burkill F.H.Chen) (San-chi), one of the most famous traditional Chinese medicinal herbs. The composition analysis of XST by HPLC showed that it contains ginsenoside Rg1 (48.1%), ginsenoside Rb1 (26.17%-29.60%), notoginsenoside R1 (11.1%), ginsenoside Re (5.5%) and ginsenoside Rd (1.3%). XST is a standard extract, but it is a mixture. This is doomed that its pharmacological effects may not be single, with multi-component and multi-target characteristics. In fact, for all Chinese medicine compounds, because of the complex composition, there is a still problem that it's difficult to determine the target cells/sites, and we need further research. For active ingredients, recent studies showed a wide range of pharmacological applications of these ginsenosides or notoginsenosides. For example, previous study has demonstrated that ginsenoside Rg1 can provide an anti-inflammatory effect by the inhibition of the c-Jun N-terminal kinase (JNK) signaling pathway (26). Another in vivo experiment has showed that treatment of Rg1

to diabetic rats was related to decrease oxidative stress and attenuated myocardial apoptosis (3). Intraperitoneal injection of ginsenoside Rb1 could suppress the level of pro-inflammatory cytokines, including TNF-α, IL-6 and NF-κB pathway molecules (29). In rats with unilateral ureteral obstruction, ginsenoside Rb1 treatment was able to prevent oxidative damage and renal interstitial fibrosis (19). These suggested that the active compounds may contribute to the protective effect of XST against DN.

Conclusions

In conclusion, we found out that XST had protective effect on DN with possible mechanisms of suppressing the accumulation of RAGE in diabetic rats, inhibiting the inflammation and apoptosis. In addition, XST could block the TGF-β/Smad2/3 signaling pathway and thus exert its anti-fibrosis effect. The current studies provide experimental evidence of the potential clinical application of XST as an anti-DN drug.

Acknowledgments

This work was supported in part by funds from the Ministry of Science and Technology of the People's Republic of China, Tianjin 131 innovative team project, Tianjin Science and Technology Committee and Guangxi Provincial Department of Science and Technology (81573644, 81573733, 2012ZX09101201-004, 16PTSYJC00120, 14JCYBJC28900, 2015DFA30430, 16ICZDJC36300, TD13-5050 and 14125008-2-5).

Conflict of Interest

The authors have nothing to declare.

References

1. Abdel-Wahab, A.F., Bamagous, G.A., Al-Harizy, R.M., ElSawy, N.A., Shahzad, N., Ibrahim, I.A. and Ghamdi, S.S.A. Renal protective effect of SGLT2 inhibitor dapagliflozin alone and in combination with irbesartan in a rat model of diabetic nephropathy. *Biomed. Pharmacother*. 103: 59-66, 2018.
2. Abrao, L., Chagas, J.K. and Schmid, H. Periodontal disease and risk for neuropathic foot ulceration in type 2 diabetes. *Diabetes Res. Clin. Pract*. 90: 34-39, 2010.
3. Bai, L., Gao, J., Wei, F., Zhao, J., Wang, D. and Wei, J. Therapeutic potential of Ginsenosides as an adjuvant treatment for diabetes. *Front. Pharmacol*. 9: 423, 2018.
4. Chao, P.C., Li, Y., Chang, C.H., Shieh, J.P., Cheng, J.T. and Cheng, K.C. Investigation of insulin resistance in the popularly used four rat models of type-2 diabetes. *Biomed. Pharmacother*. 101: 155-161, 2018.
5. Ding, H., Bai, F., Cao, H., Xu, J., Fang, L., Wu, J., Yuan, Q., Zhou, Y., Sun, Q., He, W., Dai, C., Zen, K., Jiang, L. and Yang, J. PDE/cAMP/Epac/C/EBP-β signaling cascade regulates mitochondria biogenesis of tubular epithelial cells in renal fibrosis. *Antioxid. Redox. Signal*. 29: 637-652, 2018.
6. Do, M.H., Hur, J., Choi, J., Kim, M., Kim, M.J., Kim, Y. and Ha, S.K. *Eucommia ulmoides* ameliorates glucotoxicity by suppressing advanced glycation end-products in diabetic mice kidney. *Nutrients* 10: 265, 2018.
7. Dongjie, L. HPLC fingerprints of freeze- dried Xueshuantong for Injection. *China Pharm*. 20: 2606-2608, 2009.
8. Ghadiri Soufi, F., Arbabi-Aval, E., Rezaei Kanavi, M. and Ahmadieh, H. Anti-inflammatory properties of resveratrol in the retinas of type 2 diabetic rats. *Clin. Exp. Pharmacol. Physiol*. 42: 63-68, 2015.
9. Ghosh, P., Sahoo, R., Vaidya, A., Chorev, M. and Halperin, J.A. Role of complement and complement regulatory proteins in the complications of diabetes. *Endocr Rev*. 36: 272-288, 2015.
10. Green, F.C., Levison, R., Newton, D.J., Littleford, R., Stonebridge, P.A. and Belch, J.J. Detecting diabetes and impaired glucose tolerance in patients with atherosclerotic peripheral arterial disease. *Int. Angiol*. 31: 125-128, 2012.
11. Karihaloo, A. Anti-fibrosis therapy and diabetic nephropathy. *Curr. Diab. Rep*. 12: 414-422, 2012.
12. Kumar Pasupulati, A., Chitra, P.S. and Reddy, G.B. Advanced glycation end products mediated cellular and molecular events in the pathology of diabetic nephropathy. *Biomol. Concepts* 7: 293-309, 2016.
13. Li, T., Hu, J., Du, S., Chen, Y., Wang, S. and Wu, Q. ERK1/2/COX-2/PGE2 signaling pathway mediates GPR91-dependent VEGF release in streptozotocin-induced diabetes. *Mol. Vis*. 20: 1109-1121, 2014.
14. Li, Y., Ren, Q., Zhu, L., Li, Y., Li, J., Zhang, Y., Zheng, G., Han, T., Sun, S. and Feng, F. Involvement of methylation of MicroRNA-122, -125b and -106b in regulation of Cyclin G1, CAT-1 and STAT3 target genes in isoniazid-induced liver injury. *BMC Pharmacol. Toxicol*. 19: 11, 2018.
15. Lin, S.H., Chen, I.J., Chuang, C.T., Ho, W.T., Chuang, L.Y. and Guh, J.Y. KMUP-1 attenuates high glucose and transforming growth factor-beta1-induced pro-fibrotic proteins in mesangial cells. *Mol. Med. Rep*. 15: 4199-4206, 2017.
16. Lin, Y., Sheng, M., Ding, Y., Zhang, N., Song, Y., Du, H., Lu, N. and Yu, W. Berberine protects renal tubular cells against hypoxia/reoxygenation injury via the Sirt1/p53 pathway. *J. Nat. Med*. 72: 715-723, 2018.
17. Maniruzzaman, M., Kumar, N., Menhazul Abedin, M., Shaykhul Islam, M., Suri, H.S., El-Baz, A.S. and Suri, J.S. Comparative approaches for classification of diabetes mellitus data: Machine learning paradigm. *Comput. Methods Programs Biomed*. 152: 23-34, 2017.
18. Ng, T.B. Pharmacological activity of sanchi ginseng (*Panax notoginseng*). *J. Pharm. Pharmacol*. 58: 1007-1019, 2006.
19. Park, M.J., Bae, C.S., Lim, S.K., Kim, D.I., Lim, J.C., Kim, J.C., Han, H.J., Moon, J.H., Kim, K.Y., Yoon, K.C. and Park, S.H. Effect of protopanaxadiol derivatives in high glucose-induced fibronectin expression in primary cultured rat mesangial cells: role of mitogen-activated protein kinases and Akt. *Arch. Pharm. Res.* 33: 151-157, 2010.
20. Perrone, L., Devi, T.S., Hosoya, K., Terasaki, T. and Singh, L.P. Thioredoxin interacting protein (TXNIP) induces inflammation through chromatin modification in retinal capillary endothelial cells under diabetic conditions. *J. Cell. Physiol*. 221: 262-272, 2009.
21. Ping, F. and Zhongyi, L. Determ ination of the effecient elem ents of Xuesaitong for injection, Xueshuantong injection and Xuemaitong for injection by fingerprint. *West China J. Pharm. Sci*. 22: 76-78, 2007.
22. Placier, S., Boffa, J.J., Dussaule, J.C. and Chatziantoniou, C. Re-

versal of renal lesions following interruption of nitric oxide synthesis inhibition in transgenic mice. *Nephrol. Dial. Transplant*. 21: 881-888, 2006.

23. Prasad, K., Dhar, I., Zhou, Q., Elmoselhi, H., Shoker, M. and Shoker, A. AGEs/sRAGE, a novel risk factor in the pathogenesis of end-stage renal disease. *Mol. Cell. Biochem*. 423: 105-114, 2016.
24. Raghu, G., Akileshwari, C., Reddy, V.S. and Reddy, G.B. Attenuation of diabetic retinopathy in rats by ellagic acid through inhibition of AGE formation. *J. Food Sci. Technol*. 54: 2411-2421, 2017.
25. Shukla, R., Banerjee, S. and Tripathi, Y.B. Antioxidant and Antiapoptotic effect of aqueous extract of *Pueraria tuberosa* (Roxb. Ex Willd.) DC. On streptozotocin-induced diabetic nephropathy in rats. *BMC Complement. Altern. Med*. 18: 156, 2018.
26. Sun, X.C., Ren, X.F., Chen, L., Gao, X.Q., Xie, J.X. and Chen, W.F. Glucocorticoid receptor is involved in the neuroprotective effect of ginsenoside Rg1 against inflammation-induced dopaminergic neuronal degeneration in substantia nigra. *J. Steroid Biochem. Mol. Biol*. 155: 94-103, 2016.
27. Wang, F., Sun, Z., Li, R., Hu, L., Chai, L., Wang, S., Guo, H. and Zhang, Y. Protection of Salvianolate Lyophilized Injection combined with Xueshuantong Injection (Lyophilized) against focal cerebral ischemia/reperfusion injury in rats through suppression of inflammatory response. *J. Chinese Herbal Med.* 10: 46-53, 2018.
28. Wang, X., Wang, S., Wang, J., Guo, H., Dong, Z., Chai, L., Hu, L., Zhang, Y., Wang, H. and Chen, L. Neuroprotective effect of Xueshuantong for Injection (Lyophilized) in transient and permanent rat cerebral ischemia model. *Evid. Based Complement. Alternat. Med*. 2015: 134685, 2015.
29. Wu, Y., Yu, Y., Szabo, A., Han, M. and Huang, X.F. Central inflammation and leptin resistance are attenuated by ginsenoside Rb1 treatment in obese mice fed a high-fat diet. *PLoS One* 9: e92618, 2014.
30. Xie, J., Li, R., Fan, R. and Meng, Z. Effects of sulfur dioxide on expressions of p53, bax and bcl-2 in lungs of asthmatic rats. *Inhal. Toxicol*. 21: 952-957, 2009.
31. Yoon, J.J., Lee, Y.J., Namgung, S., Han, B.H., Choi, E.S., Kang, D.G. and Lee, H.S. Samchuleum attenuates diabetic renal injury through the regulation of TGFbeta/Smad signaling in human renal mesangial cells. *Mol. Med. Rep*. 17: 3099-3108, 2018.
32. Yuan, D., Liu, X.M., Fang, Z., Du, L.L., Chang, J. and Lin, S.H. Protective effect of resveratrol on kidney in rats with diabetic nephropathy and its effect on endoplasmic reticulum stress. *Eur. Rev. Med. Pharmacol. Sci*. 22: 1485-1493, 2018.
33. Zhang, Z., Wu, F., Zheng, F. and Li, H. Adenovirus-mediated decorin gene transfection has therapeutic effects in a streptozocin-induced diabetic rat model. *Nephron Exp. Nephrol*. 116: e11-e21, 2010.
34. Zou, C., Xie, R., Bao, Y., Liu, X., Sui, M., M, S., Li, S. and Yin, H. Iron chelator alleviates tubulointerstitial fibrosis in diabetic nephropathy rats by inhibiting the expression of tenascinC and other correlation factors. *Endocrine* 44: 666-674, 2013.