Syringaresinol-di-*O*-β-*D*-glucoside, a phenolic compound from *Polygonatum sibiricum*, exhibits an antidiabetic and antioxidative effect on a streptozotocin-induced mouse model of diabetes

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Abstract. Syringaresinol-di-*O*-β-*D*-glucoside (SOG) is a phenolic compound extracted from *Polygonatum sibiricum*. The present study aimed to investigate the antidiabetic effect of SOG on streptozocin (STZ)-induced diabetic mice and determine the potential underlying mechanisms. In the present study, fasting blood glucose and organ indexes of mice were analyzed. Body weight, water intake and food intake were also recorded. Furthermore, serum fasting insulin, pancreatic insulin and pancreatic interleukin-6 levels of mice were determined using ELISA kits to investigate the effect of SOG on the levels of insulin. Levels of total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol, low-density lipoprotein cholesterol (LDL-C), very low-density lipoprotein cholesterol (VLDL-C) and free fatty acid (FFA) in the serum of mice, and levels of TC, TG and total protein in the kidney, were also determined to investigate the effects of SOG on lipid and protein metabolism in mice. Furthermore, malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) levels, as well as total antioxidant capacity (T-AOC), in the kidneys of mice were determined to investigate the effect of SOG on oxidative stress. Western blotting was also performed to determine the expression of proteins associated with oxidative stress. The results demonstrated that SOG (25, 50 and 75 mg/kg) induced a significant antidiabetic effect in mice. Treatment with SOG promoted insulin secretion and decreased TC, TG, LDL-C, VLDL-C, FFA, MDA, SOD, CAT, AST, ALT and ALP levels in the kidneys of mice, as well as kidney TC and TG levels, but increased the levels of kidney total protein and the T-AOC in kidneys. Furthermore, SOG treatment could significantly downregulate the expressions of nitrotyrosine and transforming growth factor-β1 in diabetic mice. Therefore, the present study indicated that SOG may exert an antidiabetic effect on STZ-induced diabetic mice and that the mechanism of SOG may be associated with its antioxidative activity.

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Key words: syringaresinol-di-O-β-D-glucoside, antidiabetic activity, streptozocin-induced diabetic mice, oxidative stress

Introduction

Currently, diabetes is regarded as an epidemic disease with an increasing incidence (1) and it has become the third major disease after cardiovascular and cerebrovascular diseases and cancer (2). Diabetes is divided into type 1 and type 2, with type 2 diabetes accounting for 90% of all cases, and caused a heavy burden on public health and socioeconomic development of all nations. (3,4). Diabetes is a chronic metabolic disease characterized by hyperglycemia caused by a lack of insulin or impaired insulin sensitivity (5). It has been reported that the pathogenesis of diabetes may be associated with an imbalance between the production of reactive oxygen species (ROS) and antioxidant defense systems (6). Oxidative stress refers to the imbalance between the production of ROS and reactive nitrogen species and the clearance of antioxidant defense systems in the body, which resulted in biological macromolecular damage including tissue cells, proteins and nucleic acids (7). Currently, the main therapy for diabetes aims to lower blood glucose with physical activities, nutritional intervention, oral hypoglycemic agents, insulin and glucagonlike peptide-1 receptor agonists (8). In addition, a number of studies have revealed that 30-40% of patients with diabetes have kidney damage, and there is no effective method for the prevention and treatment of diabetic nephropathy (1,4,9).

Traditional Chinese Medicine (TCM) has been used to treat various diseases in China for centuries, and natural products extracted from herbs or plants are crucial resources for finding novel candidate drugs with reliable effects and fewer side effects (10,11). TCM has commonly been used to treat diabetes since ancient times and Chinese medicine formulas with antidiabetic activities are well developed (12). Rhizoma of *Polygonatum kingianum* Coll. et Hemsl or *Polygonatum sibiricum* Red (*Polygonati* rhizoma), a

well-known herbal medicine in China, has been used for the treatment of diabetes, cough, anorexia, spleen deficiency and stomach diseases (13). In addition, according to various Chinese traditional prescriptions, *Polygonati* rhizoma has been widely used to treat diabetes (14). Syringaresinol-di-*O*-β-*D*-glucoside (SOG), whose chemical structure presented in Fig. 1, is a phenolic compound isolated from *Polygonati* rhizome. The activities of this compound have not been extensively investigated (15). Therefore, the present study aimed to elucidate the antidiabetic effect of SOG on streptozocin (STZ)-induced experimental diabetic mice and determine the potential underlying mechanisms.

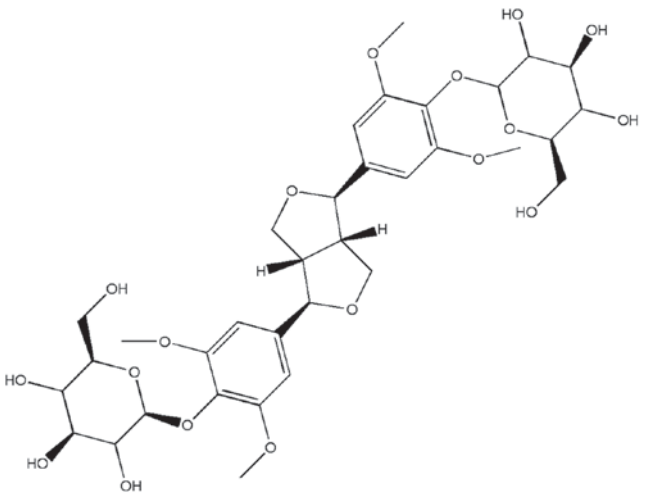

Figure 1. Chemical structure of syringaresinol-di-O-β-D-glucoside.

Materials and methods

Chemicals. Syringaresinol-di-*O*-β-*D*-glucoside (SOG; cat. no. E-0542; http://www.tautobiotech.com/BRM/ProductShow.asp?ArticleID=542) was purchased from Shanghai TAUTO BIOTECH Co., Ltd. (Shanghai, China). STZ (cat. no. S0130) and dimethyl sulfoxide (DMSO) were obtained from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany). Nitrotyrosine (NT) antibody (cat. no. DMABT-Z60522) was purchased from Creative Diagnostics, Shirley, NY, USA. Insulin (cat. no. ab100578) and interleukin-6 (IL-6; cat. no. ab100712) ELISA kits, and transforming growth factor-β1 (TGF-β1; cat. no. ab9758) and GAPDH (cat. no. ab8245) antibodies, were purchased from Abcam (Cambridge, MA, USA). Total cholesterol (TC; cat. no. CY81061), triglyceride (TG; cat. no. CY81057), high-density lipoprotein cholesterol (HDL-C; cat. no. HZL2329), total protein (cat. no. HZL2206), low-density lipoprotein cholesterol (LDL-C; cat. no. HZL2321), free fatty acid (FFA; cat. no. CY81055), malondialdehyde (MDA; cat. no. EY2), superoxide dismutase (SOD; cat. no. FY1), catalase (CAT; cat. no. HZL2444), total antioxidant capacity (T-AOC; cat. no. FG5), aspartate transaminase (AST; cat. no. HZL2382), alanine transaminase (ALT; cat. no. SEA207Ra) and alkaline phosphatase (ALP; cat. no. HZL2351) kits were produced by Shanghai Chaoyan Biological Technology Co., Ltd., (Shanghai, China; http://biosis.biogo.net/sell/typeid-5638.shtml). Bicinchoninic acid (BCA) protein assay reagent was purchased from Beyotime Institute of Biotechnology (Haimen, China). All other chemicals used in the present study were of analytical reagent grade.

Animals. A total of 50 male specific pathogen-free mice (6-8 weeks old; weight, 18-23 g) were purchased from the Laboratory Animal Center of Fujian Medical University (Fuzhou, China). The animals were maintained under controlled conditions (22±2˚C, 30-70% humidity and <0.03% CO_2) with a 12-h light/dark cycle and free access to food and water. All animal treatments were strictly in accordance with international ethical guidelines and the National Institutes of Health Guide concerning the Care and Use of Laboratory Animals (16), and the experiments were performed with the approval of the Animal Experimentation Ethics Committee of the Affiliated Hospital of Nanjing University of Chinese Medicine (Nanjing, China).

Animal model establishment and grouping. A diabetic animal model was established according to the previously reported method with minor modifications (17). A total 50 male mice were divided into five groups (n=10/group): Normal group, model group, 25 mg/kg SOG-treated group, 50 mg/kg SOG-treated group and 75 mg/kg SOG-treated group. All mice excluding the normal group were injected with STZ dissolved in 0.1 mol/l citric acid buffer solution (Chengdu Chemical Co., Ltd., Chengdu, China; 100 mg/kg) intravenously. Mice in the normal group were treated with an equal volume of 0.01 mol/l citric acid buffer solution. After 3 days, the fasting blood glucose (FBG) of mice treated with STZ was measured using an Accu-Chek Performa monitor. STZ-treated mice with FBG >11.1 mmol/l were considered to be diabetic. Following successful establishment of the diabetic model, mice in the normal and the model group were treated with normal saline (containing 0.5% DMSO) and mice in SOG-treated groups were treated with SOG at 25, 50 or 75 mg/kg by means of intragastric administration daily for 2 weeks. SOG was dissolved in normal saline (containing 0.5% DMSO).

Effects of SOG on FBG, body weight, water intake, food intake and organ indexes (kidney, pancreas, spleen and liver) of diabetic mice induced by STZ. During administration of SOG for 2 weeks, the water and food intake of mice were recorded each day. On days 0 (a day prior to the administration of the first dose of SOG) and 15, the body weight of mice was measured and the weight gain was calculated. On days 0, 7 and 15, the FBG of mice was determined using glucose testing strips. Blood samples were collected from the caudal vein of mice. Following the final administration of SOG, mice were sacrificed the next day by cervical dislocation. The organs were separated and the organ index was calculated according to the following formula: Organ index (g/100 g)=W_{Organ}/W_{Body}, where W stands for weight.

Effect of SOG on serum fasting insulin, pancreatic insulin and pancreatic IL-6 levels in diabetic mice induced by STZ. Following treatment with SOG for 2 weeks, the mice were fasted for 12 h with free access to water. A total of 50 μl serum was obtained from whole blood by centrifugation for 5 min at 10,000 x g at 4˚C and serum fasting insulin of mice was measured using a commercial insulin ELISA kit (Abcam; cat. no. ab100578), according to manufacturer's protocol. In

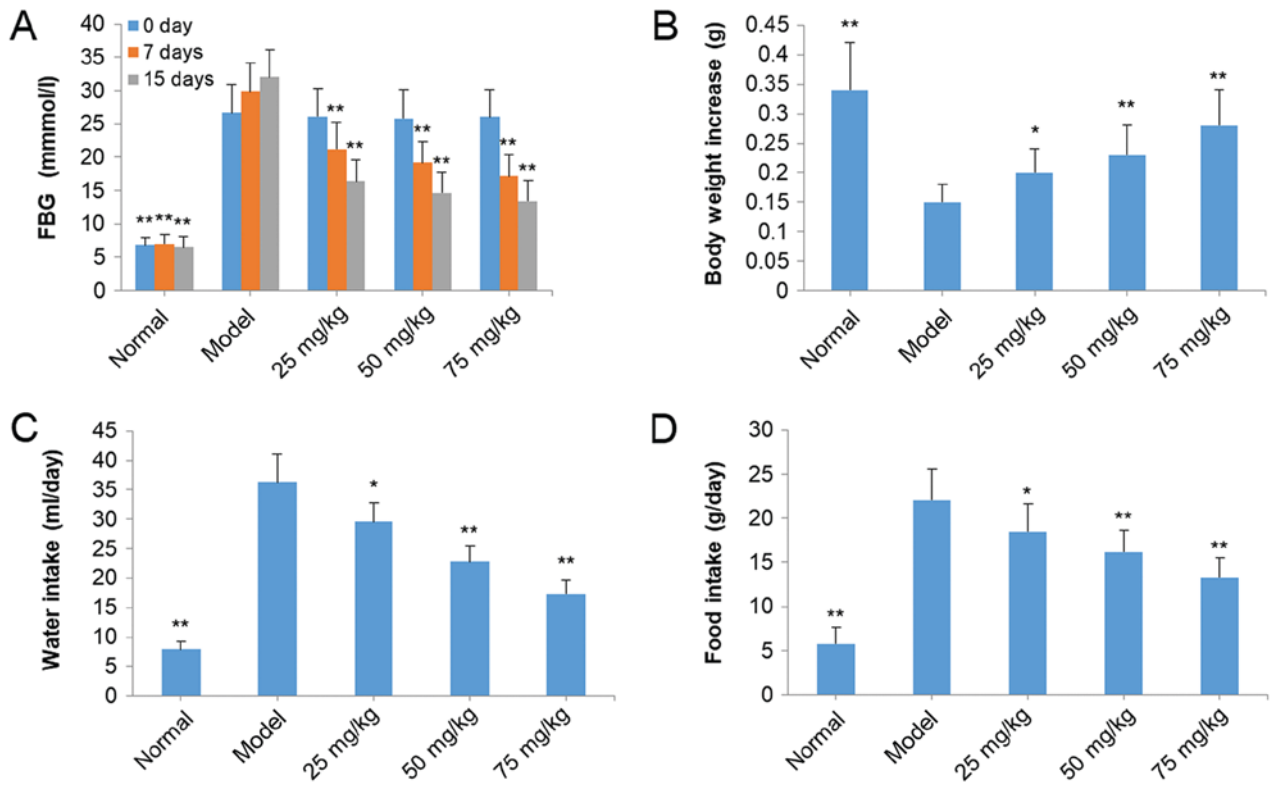

Figure 2. Effects of SOG on (A) FBG, (B) body weight, and (C) water and (D) food intake of experimental diabetic mice induced by STZ. The normal group represents mice without STZ injection and the model group represents mice with the induction of diabetes by STZ injection. Data are presented as the mean ± standard deviation (n=10 mice). *P<0.05 and **P<0.01 vs. model group. SOG, syringaresinol-di-O-β-D-glucoside; FBG, fasting blood glucose; STZ, streptozocin; 25 mg/kg, 25 mg/kg SOG; 50 mg/kg, 50 mg/kg SOG; 75 mg/kg, 75 mg/kg SOG.

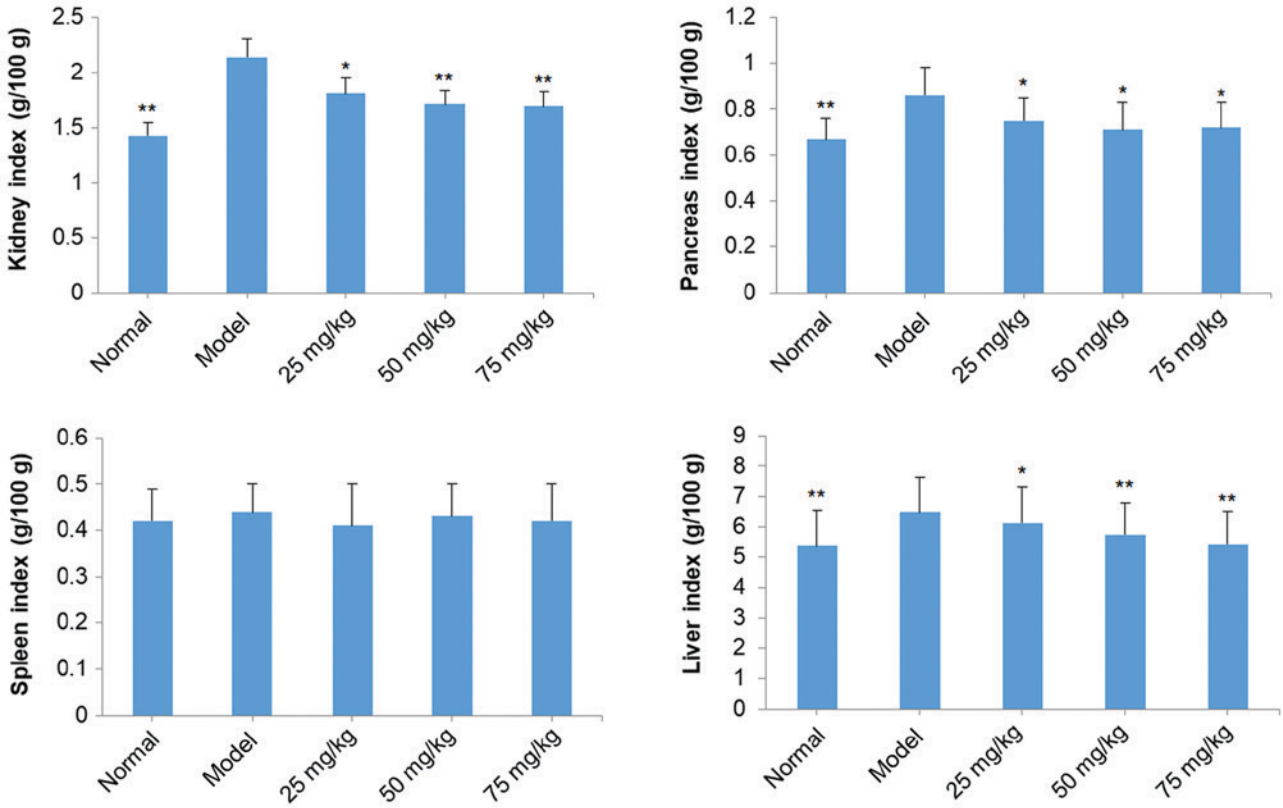

Figure 3. Effect of SOG on organ indexes of experimental diabetic mice induced by STZ. The normal group represents mice without STZ injection and the model group represents mice with the induction of diabetes by STZ injection. Data are presented as the mean ± standard deviation (n=10 mice). *P<0.05 and **P<0.01 vs. model group. SOG, syringaresinol-di-O-β-D-glucoside; STZ, streptozocin; 25 mg/kg, 25 mg/kg SOG; 50 mg/kg, 50 mg/kg SOG; 75 mg/kg, 75 mg/kg SOG.

addition, the pancreas were treated with lysis buffer (Abcam; cat. no. ab156035) for 10 min and centrifuged at 4˚C for 5 min (10,000 x g) and then the pancreatic levels of insulin and IL-6 in were also detected using insulin and IL-6 ELISA kits (Abcam; cat. no. ab100712), respectively.

Effect of SOG on TC, TG, HDL-C, LDL-C, very low-density lipoprotein cholesterol (VLDL-C) and FFA in the serum of diabetic mice induced by STZ. The levels of TC, TG, HDL-C, LDL-C and FFA in the serum of STZ-induced diabetic mice after 12 h of fasting were measured using TC, TG, HDL-C, LDL-C and FFA kits, respectively. The value of VLDL-C in the serum was calculated according to the Friedewald formula reported by previous study (17): VLDL-C=TG/5.

Effect of SOG on kidney TC, TG and total protein levels in diabetic mice induced by STZ. Kidneys were treated with lysis buffer (Abcam; cat. no. ab156035) for 10 min and centrifuged at 4˚C for 5 min (10,000 x g) and then the levels of kidney TC and TG in STZ-induced diabetic mice were determined using TC and TG kits, according to the manufacturer's protocols. The content of kidney protein in STZ-induced diabetic mice was measured using a BCA protein assay reagent.

Effect of SOG on MDA, SOD, CAT, T-AOC, AST, ALT and ALP in the kidneys of diabetic mice induced by STZ. Following the final administration of SOG, mice were fasted for 12 h and the blood samples were collected from the eyeballs and centrifuged for 10 min (3,500 x g) at 4˚C to obtain the serum. The levels of MDA, SOD, CAT, T-AOC, AST, ALT and ALP in the serum of STZ-induced diabetic mice were measured using commercial ELISA kits for MDA (cat. no. EY2), SOD (cat. no. FY1), CAT (cat. no. HZL2444), T-AOC (cat. no. FG5), AST (cat. no. HZL2382), ALT (cat. no. SEA207Ra) and ALP (cat. no. HZL2351) kits, respectively following the manufacturers' protocol.

Western blot analysis. The kidney tissues of mice were cut into small pieces and treated with RIPA lysis buffer (Abcam; cat. no. ab156035). The supernatant was obtained by centrifugation at 12,000 x g for 15 min at 4˚C. Protein concentration was determined using BCA protein assay reagent (Beyotime Institute of Biotechnology; cat. no. P0010). Subsequently, 35 μg proteins were separated by 12% SDS-PAGE and transferred to polyvinylidene difluoride membranes. Membranes were blocked with 5% fat-free dry milk in 1X TBS-Tween-20 (containing 0.1% Tween-20) for 2 h at room temperature. Membranes were subsequently incubated with primary antibodies specific to NT (1:1,000), TGF-β1 (1:1,000) and GAPDH (1:2,000) at 4˚C overnight. Membranes were further incubated with horseradish peroxidase-conjugated secondary antibodies (1:2,000; Beyotime Institute of Biotechnology; cat. no. A0286) at room temperature for 1 h. The proteins were detected using the Beyo ECL Star reagents (Beyotime Institute of Biotechnology; cat. no. P0018A). Signals were quantified using ImageQuant LAS 4000 Imaging system (GE Healthcare Bio-Sciences, Pittsburgh, PA, USA). To normalize for protein loading, an antibody against GAPDH was used and protein expression levels were expressed relative to GAPDH.

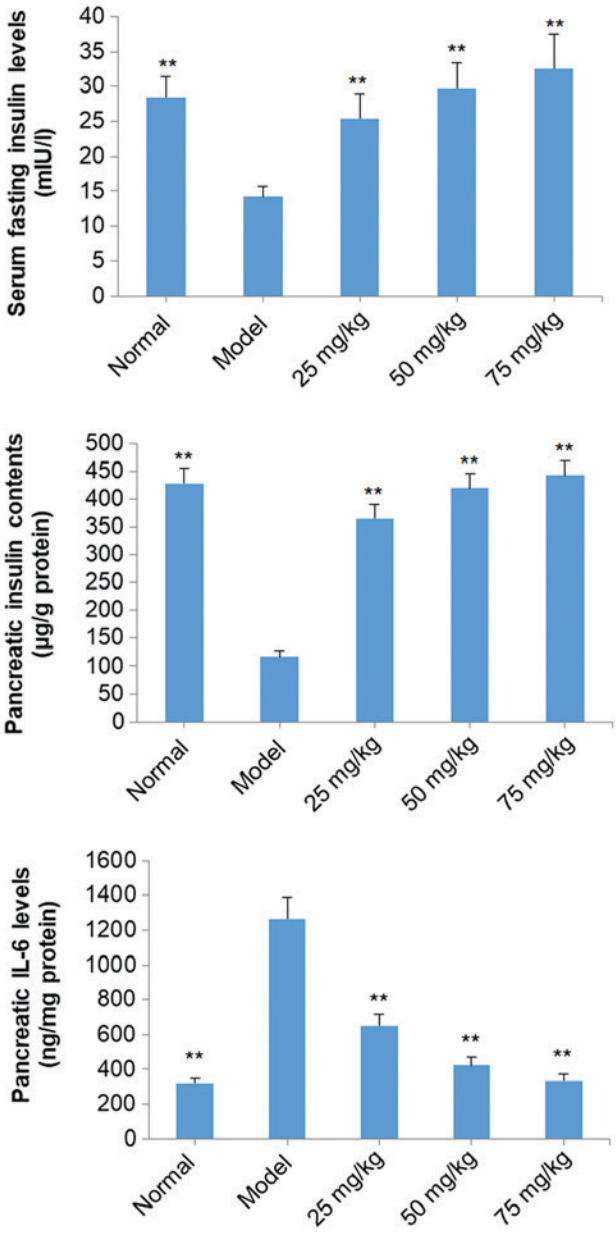

Figure 4. Effect of SOG on serum fasting insulin, pancreatic insulin and pancreatic IL-6 levels in experimental diabetic mice induced by STZ. The normal group represents mice without STZ injection and the model group represents mice with the induction of diabetes by STZ injection. Data are presented as the mean ± standard deviation (n=10 mice). **P<0.01 vs. model group. SOG, syring aresinol-di-O-β-D-glucoside; IL-6, interleukin-6; STZ, streptozocin; 25 mg/kg, 25 mg/kg SOG; 50 mg/kg, 50 mg/kg SOG; 75 mg/kg, 75 mg/kg SOG.

Statistical analysis. All tests were conducted in triplicate and all data are presented as the mean ± standard deviation. The significance of differences between groups was analyzed by one-way analysis of variance followed by a Dunnett's multiple comparisons post-hoc test using SPSS software (SPSS for Windows version 19.0; IBM Corp., Armonk, NY, USA). P<0.05 was considered to indicate a statistically significant difference.

Results

Effects of SOG on FBG, body weight, water intake, food intake and organ indexes of diabetic mice induced by STZ. The

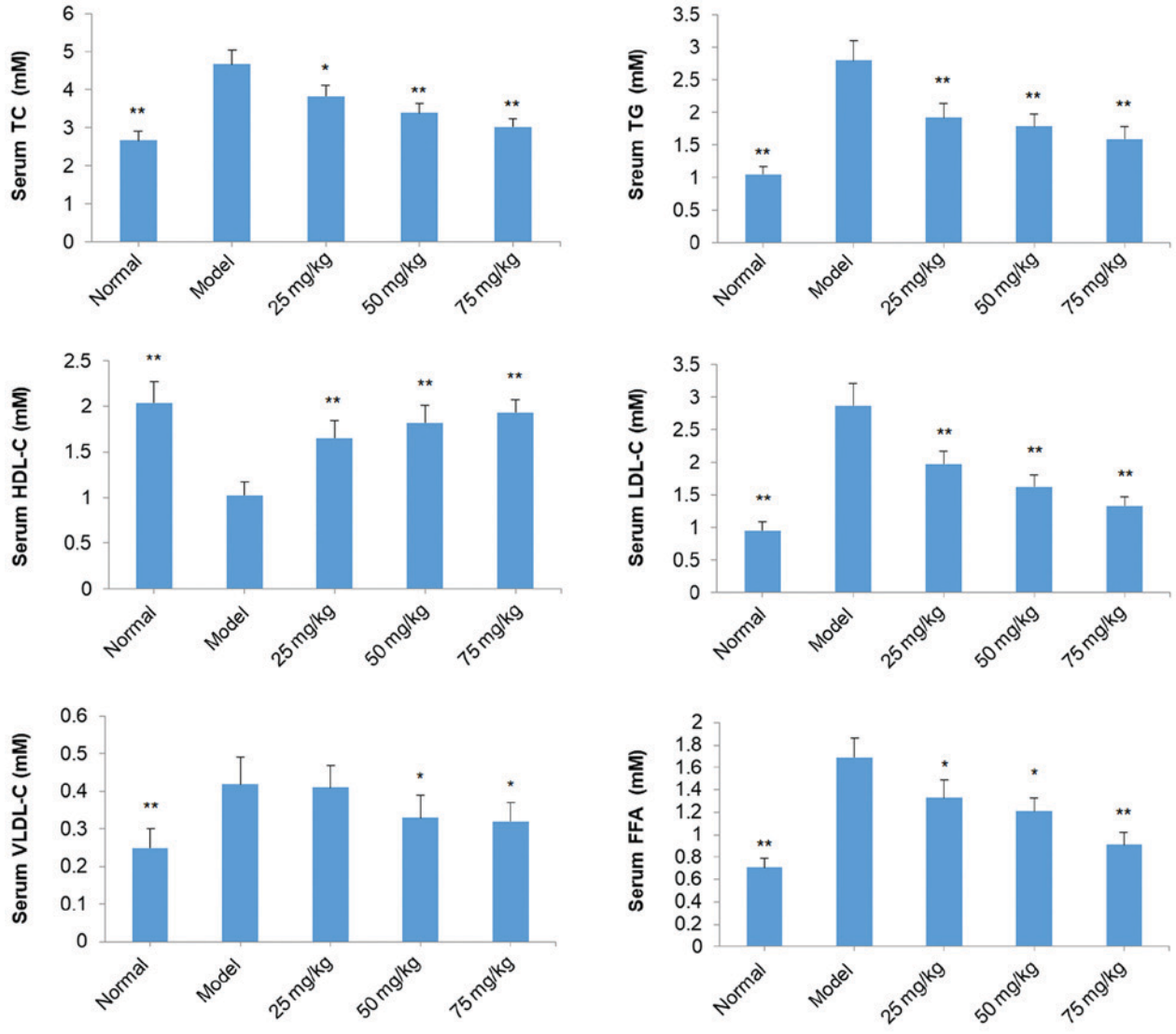

Figure 5. Effect of SOG on the levels of TC, TG, HDL-C, LDL-C, VLDL-C and FFA in the serum of experimental diabetic mice induced by STZ. The normal group represents mice without STZ injection and the model group represents mice with the induction of diabetes by STZ injection. Data are presented as the mean ± standard deviation (n=10 mice). *P<0.05 and **P<0.01 vs. model group. SOG, syringaresinol-di-O-β-D-glucoside; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; VLDL-C, very low-density lipoprotein cholesterol; FFA, free fatty acid; STZ, streptozocin; 25 mg/kg, 25 mg/kg SOG; 50 mg/kg, 50 mg/kg SOG; 75 mg/kg, 75 mg/kg SOG.

FBG levels in model mice were higher compared with mice in other groups, indicating that the diabetic animal model was established successfully (Fig. 2A). However, treatment with SOG at 25, 50 and 75 mg/kg significantly decreased the levels of FBG in STZ-induced diabetic mice in a dose-dependent and time-dependent manner compared with the model group (P<0.01; Fig. 2A). In addition, the body weight of the model significantly decreased compared with the normal mice (P<0.01; Fig. 2B). The body weight of mice in SOG-treated groups (25, 50 and 75 mg/kg) was significantly increased compared with model group mice (P<0.05; Fig. 2B). Overeating is a primary characteristic of mice with diabetes (16). Water and food intake of model mice with diabetes was increased compared with normal mice (Fig. 2C and D). Following treatment with SOG (25, 50 and 75 mg/kg), the water and food intake of mice with diabetes was significantly decreased compared with the model group (P<0.05; Fig. 2C and D). The effect of treatment with SOG on organ indexes (kidney, pancreas, spleen and liver) of STZ-induced diabetic mice are presented in Fig. 3. Kidney, pancreas and liver indexes of diabetic mice induced by STZ were increased compared with mice in the normal group. SOG treatment (25, 50 and 75 mg/kg) significantly reduced the kidney, pancreas and liver indexes, compared with the model group, to levels similar to that in the normal group. However, no significant effects of diabetes induction or SOG treatment were observed on the spleen index.

Effects of SOG on serum fasting insulin, pancreatic insulin and pancreatic IL-6 of diabetic mice induced by STZ. Serum fasting insulin and pancreatic insulin levels of STZ-induced diabetic mice in the model group were significantly decreased compared with normal mice (P<0.01; Fig. 4). However, treatment with SOG (25, 50 and 75 mg/kg) significantly increased serum fasting insulin and pancreatic insulin levels in STZ-induced diabetic model mice dose-dependently (P<0.01; Fig. 4). The pancreatic levels of IL-6 in diabetic mice in the

model group were significantly increased compared with the normal group (P<0.01); however, following treatment with SOG at doses of 25, 50 and 75 mg/kg, the level of pancreatic IL-6 in diabetic mice was significantly reduced compared with mice in the model group (P<0.01; Fig. 4).

Effects of SOG on TC, TG, HDL-C, LDL-C, VLDL-C and FFA in the serum of diabetic mice induced by STZ. TC, TG, LDL-C, VLDL-C and FFA levels in the serum of mice in the model group were significantly increased compared with the normal mice (P<0.01; Fig. 5). However, HDL-C levels in the serum of model mice were decreased compared with normal mice (P<0.01; Fig. 5). In all SOG-treated groups (25, 50 and 75 mg/kg), TC, TG, LDL-C and FFA levels in the serum of mice were significantly decreased compared with model group mice (P<0.05; Fig. 5). Similar effects were observed for VLDL-C levels at 50 and 75 mg/kg SOG; however, 25 mg/kg SOG did not induce a significant reduction in VLDL-C levels in diabetic model mice (Fig. 5). By contrast, all three concentrations of SOG led to significant increases in the HDL-C levels in the serum of diabetic model mice (P<0.01; Fig. 5).

Effects of SOG on kidney TC, TG and total protein levels in diabetic mice induced by STZ. TC and TG levels in the kidneys of model group mice were markedly increased compared with mice in the normal group (P<0.01), while total kidney protein levels were significantly decreased (P<0.01; Fig. 6). SOG (25, 50 and 75 mg/kg) significantly decreased kidney TC and TG levels, and increased levels of total kidney protein, in diabetic mice induced by STZ in a dose-dependent manner (P<0.01; Fig. 6).

Effects of SOG on MDA, SOD, CAT, T-AOC, AST, ALT and ALP in the kidneys of diabetic mice induced by STZ. Levels of MDA, SOD, CAT, AST, ALT and ALP in the kidneys of model group mice were increased significantly, and T-AOC was decreased significantly, compared with normal mice (P<0.01; Fig. 7). Following treatment with SOG (25, 50 and 75 mg/kg), the levels of MDA, SOD, CAT, AST, ALT and ALP in the kidneys of diabetic mice were significantly decreased, and T-AOC was significantly increased, compared with model group mice (P<0.05; Fig. 7).

Effects of SOG on the protein expression of NT and TGF-β1 in the kidneys of diabetic mice induced by STZ. The results of western blot analysis demonstrated that the protein expression of NT and TGF-β1 were significantly increased in diabetic model mice compared with normal mice (P<0.01; Fig. 8). Treatment with SOG at 25, 50 and 75 mg/kg in diabetic model mice resulted in a significant decrease in the protein expression of NT, compared with the model group (P<0.01; Fig. 8). SOG treatment at 50 and 75 mg/kg also significantly decreased the expression levels of TGF-β1 compared with mice in the model group (P<0.05), but no significant reduction in TGF-β1 levels was observed at 25 mg/kg SOG in diabetic model mice (Fig. 8).

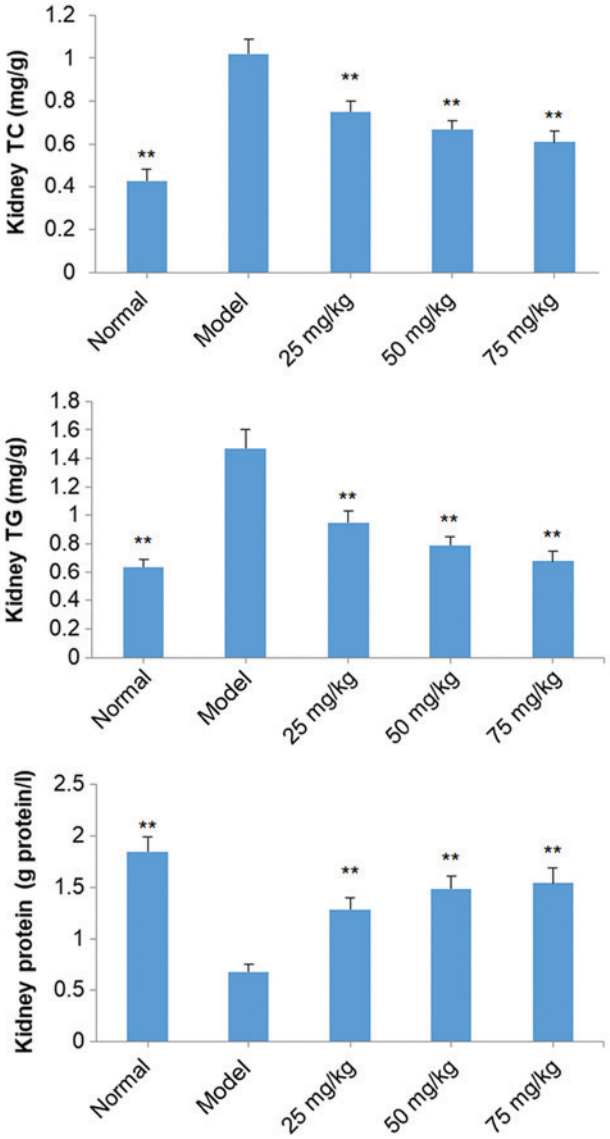

Figure 6. Effect of SOG on TC, TG and total protein levels in the kidneys of experimental diabetic mice induced by STZ. The normal group represents mice without STZ injection and the model group represents mice with the induction of diabetes by STZ injection. Data are presented as the mean ± standard deviation (n=10 mice). **P<0.01 vs. model group. SOG, syringaresinol-di-O-β-D-glucoside; TC, total cholesterol; TG, triglyceride; STZ, streptozocin; 25 mg/kg, 25 mg/kg SOG; 50 mg/kg, 50 mg/kg SOG; 75 mg/kg, 75 mg/kg SOG.

Discussion

To the best of our knowledge, the present study is the first to investigate the antidiabetic effect of SOG in a diabetic mouse model induced by STZ. In the present study, SOG exhibited an antidiabetic effect and its mechanism may be associated with antioxidative effects.

STZ is a widely used antibiotic produced by *Streptomyces achromogenes*. In 1963, Rakieten *et al* (18) demonstrated that rats and dogs exhibited symptoms of diabetes following intravenous injection with STZ. STZ is formed by the coupling of a glucose molecule with a highly active urea side chain, which is considered to contribute to cytotoxicity. Therefore, a STZ-induced diabetic animal model was used in the present study.

The primary symptoms of diabetes include polydipsia, polyuria, emaciation, weakness and hyperglycemia (17). In the present study, FBG, body weight, water intake, food intake and organ indexes (kidney, pancreas, spleen and liver) of diabetic

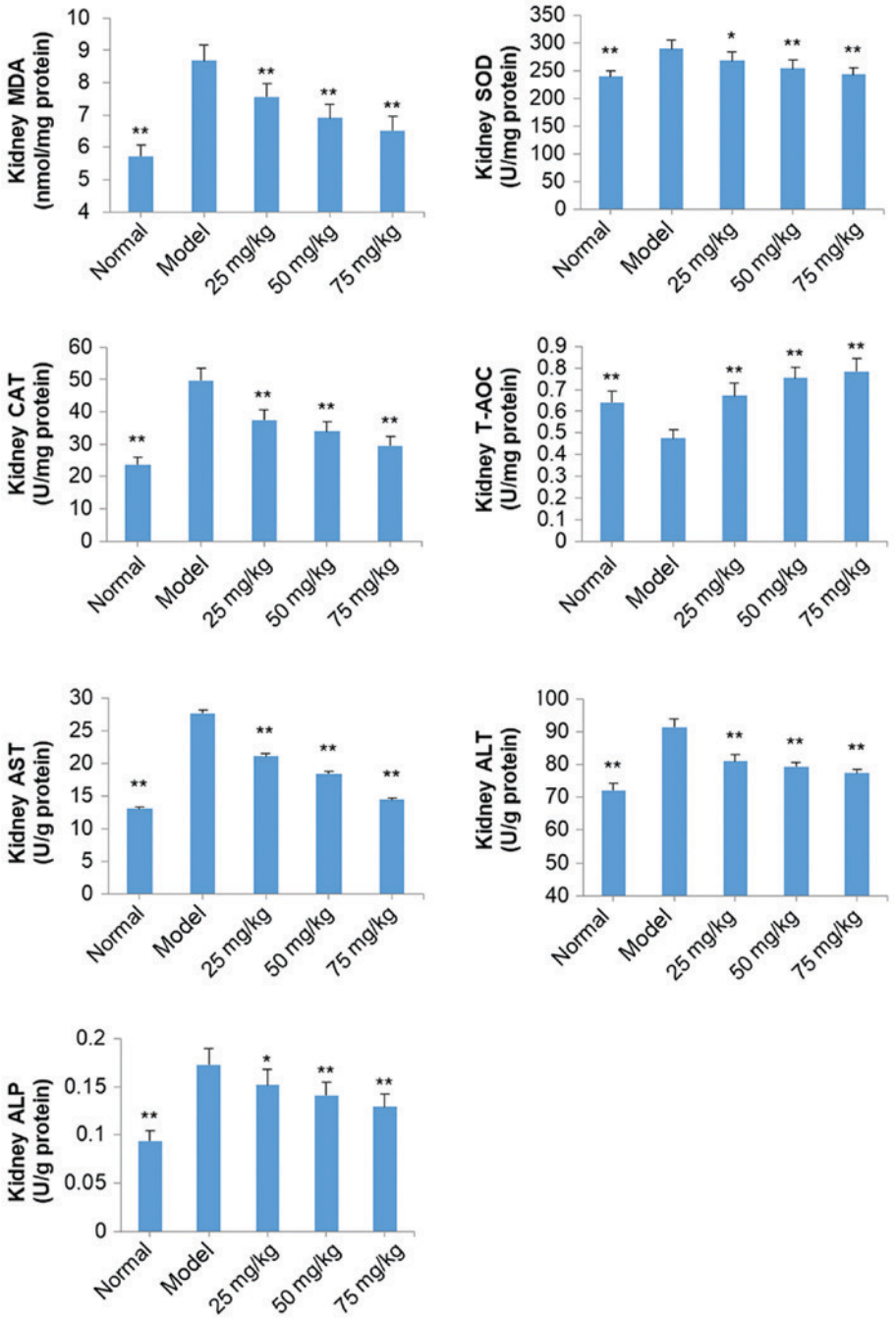

Figure 7. Effect of SOG on MDA, SOD, CAT, T-AOC, AST, ALT and ALP levels in the *kidneys* of experimental diabetic mice induced by STZ. The normal group represents mice without STZ injection and the model group represents mice with the induction of diabetes by STZ injection. Data are presented as the mean ± standard deviation (n=10 mice). *P<0.05 and **P<0.01 vs. model group. SOG, syringaresinol-di-O-β-D-glucoside; MDA, malondialdehyde; SOD, superoxide dismutase; CAT, catalase; T-AOC; total antioxidant capacity, AST; aspartate transaminase, ALT; alanine transaminase, ALP; alkaline phosphatase; STZ, streptozocin; 25 mg/kg, 25 mg/kg SOG; 50 mg/kg, 50 mg/kg SOG; 75 mg/kg, 75 mg/kg SOG.

mice induced by STZ were analyzed, and the results demonstrated that SOG ameliorated symptoms of STZ-induced diabetes. In addition, diabetes primarily results from insulin deficiency or insulin insensitivity (5). Therefore, the levels of serum fasting insulin and pancreatic insulin may reflect the severity of diabetes symptoms. In the process of insulin resistance, a series of reactions occur, including increases in the levels of fatty acids, inflammation and oxidative stress (19). The level of pancreatic IL-6 in experimental mice was determined in the present study. The results indicated that SOG exerted an antidiabetic effect through increasing the serum fasting insulin and pancreatic insulin levels, and decreasing the levels of pancreatic IL-6.

Lipids, including fat and fatty substances in the blood, serve roles in energy balance, the physiological function of reproduction and organs, and cell biology (20). The major components of fatty substances in the blood are fatty acids, cholesterol, triglycerides and phospholipids (21). Additionally, increased concentrations of TC, TG, LDL-C and VLDL-C, and reduced HDL-C levels, are characteristic of hyperlipidemia and hypercholesterolemia (22,23), which are associated with diabetes (21). In the present study, TC, TG, HDL-C, LDL-C,

VLDL-C and FFA in the serum of experimental mice were analyzed. The results demonstrated that SOG significantly decreased the levels of TC, TG, LDL-C, VLDL-C and FFA, and increased the levels of HDL-C, in the serum of STZ-induced diabetic mice, indicating that SOG may increase sensitivity to insulin.

Diabetic nephropathy is the most common chronic complication of diabetes (24). It was reported that 30-40% of diabetic patients suffer from kidney damage (9). Rats with diabetic nephropathy have specific symptoms, including high levels of TC and TG, and low total kidney protein levels (25). In the present study, SOG reduced levels of TC and TG in the kidney, and increased total kidney protein levels, which indicate that SOG may ameliorate diabetic nephropathy of STZ-induced diabetic mice.

Oxidative stress is a major mechanism contributing to the development of diabetes mellitus, which it mediates via alterations in the formation of free radicals (26,27). Oxygen free radicals are associated with the pathogenesis of diabetes mellitus (20). It has been previously demonstrated that an imbalance between ROS and the endogenous protective mechanisms in patients with diabetes is associated with cell damage (25). The level of MDA reflects the extent of lipid peroxidation, with increases in MDA levels indicating an increase in the number of free radicals. SOD catalyzes the dismutation of superoxide anion radicals into hydrogen peroxide. Hydrogen peroxide may be changed into peroxynitrite to weaken the interaction between the superoxide anion and nitric oxide or be hydrolyzed by hydrogen peroxide to water and oxygen (28-30). T-AOC is considered to be the cumulative effect of all antioxidants in the blood and body fluids, which is used to assess alterations in the antioxidant status, particularly in patients with diabetes (31). It was reported that the levels of AST and ALT were increased in the kidneys of STZ-induced diabetic mice compared with normal mice (32). ALP is a membrane-bound glycoprotein enzyme that is used as an indicator of biliary function and cholestasis (33). In the present study, SOG decreased the levels of SOD, CAT, AST, ALT and ALP in the kidney of STZ-induced diabetic mice, and increased levels of T-AOC, indicating that SOG may exhibit antidiabetic effects by regulating oxidative stress.

Excessive amounts of ROS and NO are produced by patients with diabetes, leading to the production of peroxynitrite anions, which is an oxidant and leads to elevated oxidative stress (34). Peroxynitrite anions have been reported to activate nuclear factor-κB to upregulate the expression of TGF-β and eventually promote the development of diabetes. Furthermore, NT in the kidney is a specific marker of peroxynitrite anions (35). The present study investigated the protein expression of NT and TGF-β1. The results demonstrated that SOG downregulated the expression levels of NT and TGF-β1 in the kidneys of STZ-induced diabetic mice in a dose-dependent manner.

In conclusion, the results of the present study demonstrated that SOG may exert notable antidiabetic effects and ameliorate diabetic nephropathy. The mechanism of SOG may be associated with decreasing the levels of oxidative stress through downregulation of the expression of NT and TGF-β1 in kidneys. In addition, the present investigation can provide specific scientific basis for the use of *Polygonati* rhizome. Further studies on structure-bioactivity association of SOG should be performed in the future.

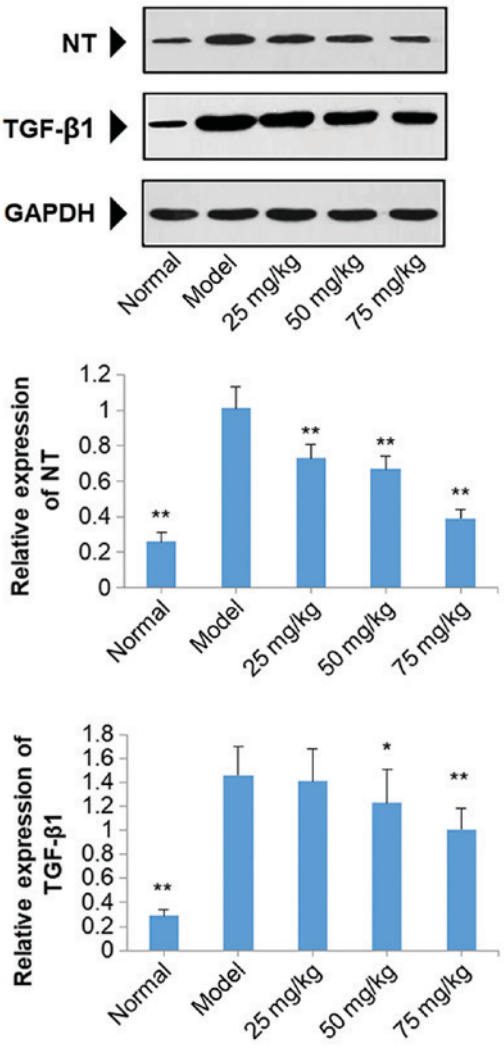

Figure 8. Effect of SOG on the protein expression of NT and TGF-β1 in the kidneys of experimental diabetic mice induced by STZ. The normal group represents mice without STZ injection and the model group represents mice with the induction of diabetes by STZ injection. Data are presented as the mean ± standard deviation (n=10 mice). *P<0.05 and **P<0.01 vs. model group. SOG, syringaresinol-di-O-β-D-glucoside; NT, nitrotyrosine; TGF-β1, transforming growth factor-β1; STZ, streptozocin; 25 mg/kg, 25 mg/kg SOG; 50 mg/kg, 50 mg/kg SOG; 75 mg/kg, 75 mg/kg SOG.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

For preparation of the paper, XW conceived and designed the study; LZ performed the experiments; XW analyzed the data; LZ and XW wrote the manuscript.

Ethics approval and consent to participate

Animal experiments were performed with the approval of the Animal Experimentation Ethics Committee of The Affiliated Hospital of Nanjing University of Chinese Medicine.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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