JDI Journal of Diabetes Investigation Open access
Official Journal of the Asian Association for the Study of Diabetes
ORIGINAL ARTICLE

Polygonatum sibiricum polysaccharide potentially attenuates diabetic retinal injury in a diabetic rat model

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Keywords
Apoptosis, Diabetic retinopathy, *Polygonatum sibiricum* polysaccharide

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J Diabetes Investig 2019; 10: 915–924

doi: 10.1111/jdi.12976

ABSTRACT

Aims/Introduction: To investigate the protective effect of *Polygonatum sibiricum* polysaccharide (PSP) on the retina in diabetic rats.

Materials and Methods: A total of 120 Sprague–Dawley rats were randomly divided into blank control, control model (meaning diabetes mellitus), and diabetes mellitus with PSP intervention of high, medium and low doses groups. The difference of retinal vascularization between groups was evaluated by fluorescein isothiocyanate-dextran perfusion. Terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling staining was used to assess apoptosis in the retinal ganglion cells; reverse transcriptase polymerase chain reaction and western blotting were utilized to evaluate the expression of Bcl2-associated X protein, B-cell lymphoma-2 factor, epidermal growth factor, p38 mitogen-activated protein kinases, transforming growth factor-β and vascular endothelial growth factor at the messenger ribonucleic acid and protein level.

Results: Fluorescein isothiocyanate-dextran perfusion showed retinal vascular anomaly in diabetes mellitus rats, but vascular tortuosity and leakage were relatively alleviated after PSP intervention. Terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling staining showed numerous terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling-positive retinal cells in the diabetes mellitus group, which then were reduced by PSP treatment. Reverse transcriptase polymerase chain reaction showed that PSP intervention decreased Bcl2-associated X protein, epidermal growth factor, p38 mitogen-activated protein kinases, vascular endothelial growth factor and transforming growth factor-β messenger ribonucleic acid expression, but increased B-cell lymphoma-2 factor messenger ribonucleic acid expression. Western blot showed that PSP intervention upregulated the expression of B-cell lymphoma-2 factor, and downregulated the expression of Bcl2-associated X protein, epidermal growth factor, p38 mitogen-activated protein kinases, vascular endothelial growth factor and transforming growth factor-β proteins.

Conclusions: *Polygonatum sibiricum* polysaccharide shows a protective effect against diabetes-induced retinal injury in a dose-dependent manner. The mechanism of action deserves further study and exploration.

INTRODUCTION

Diabetes mellitus is a metabolic disease mainly caused by abnormal insulin secretion and characterized by chronic hyperglycemia. Long-term high blood glucose can cause a variety of complications, including diabetic retinopathy (DR) and blindness, which seriously affect the quality of life in patients[1]. The American Diabetes Association proposed four types of diabetes mellitus: type 1, which is characterized by β-cell destruction, leading to an absolute lack of insulin; type 2, which is mainly characterized by a progressive loss of β-cell insulin secretion

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Received 25 July 2018; revised 28 October 2018; accepted 7 November 2018

usually on the background of insulin resistance; gestational diabetes mellitus; and specific types of diabetes due to other causes[2]. Injection of streptozocin (STZ), an antibiotic produced by *Streptomyces achromogenes*, was found to cause sustained hyperglycemia, accompanied by polydipsia and polyuria characteristics of diabetes. The mechanism is considered to be pancreatic islets disruption and β-cells death by deoxyribonucleic acid (DNA) fragmentation[3]. STZ-induced diabetic rats were commonly used as the DR animal model.

In recent years, traditional Chinese medicine and Chinese herbal medicines have drawn attention because of their unique curative effect on the prevention and treatment of diabetes[4]. *Polygonatum sibiricum* is a kind of tonic-type traditional Chinese medicine, made up of many compositions including polysaccharides, digitalose, steroids saponins and cardiac glycosides[5]. Thereinto, polysaccharide is one of the important effective components with various biological activities and medical effects, such as anti-oxidant, anti-inflammatory and anti-aging. A pharmacological study showed that *P. sibiricum* polysaccharide (PSP) can decrease free radicals and hematic fat, as well as increase radiation resistance and enhance immunity[6]; PSP also can adjust the level of the second messenger cyclic adenosine monophasphate[7]. An experimental study found that PSP can reduce the levels of fasting blood glucose and serum glycosylated hemoglobin of diabetes mellitus rats and might enhance the glucose tolerance[8,9]; thus, PSP plays an important role in regulating glucose metabolism.

In the present study, we used one-time intraperitoneal injection of STZ to establish diabetes mellitus rat models. We studied the intervention effect of three different concentrations of rhizome PSP on STZ-induced diabetes mellitus rats, by counting apoptotic retinal ganglion cells (RGCs) and determining retinal expression of some indicators (Bcl2-associated X protein [Bax], B-cell lymphoma-2 factor [Bcl-2], epidermal growth factor [EGF], transforming growth factor [TGF]-β, p38 mitogen-activated protein kinases [p38] and vascular endothelial growth factor [VEGF]), which had been found expressed in retina tissues and retinal injury induced by diabetes mellitus. The protective effect of PSP on the retina of diabetes mellitus rats was investigated, and underlying mechanisms were discussed.

METHODS

Experimental Animals

Healthy male Sprague–Dawley rats (7-weeks-old, 200 ± 20 g) were purchased from Sibedford-Stuyvesant Section Experimental Animal Technology Co. Ltd. (production license: SCXK, Beijing, China; 2011-0004). The rats were placed in a clean laboratory with an average temperature of 22–24°C and dark–light cycles of 12:12 h. Animals had free access to drinking water and food for 1 week to adapt. Animal welfare and experimental procedures were carried out according to the Guide for the Care and Use of Laboratory Animals of the US National Institutes of Health[10], and the study protocol was approved by the institutional review board and ethics committee of Taishan Medical University (No. 2016047). Every effort was made to reduce the number of animals and minimize their suffering.

Drugs and Reagent

Polygonatum sibiricum polysaccharide (100%) was purchased from ZhongHui Plant Biochemical Co. Ltd. (Tai'an, China). STZ, fluorescein isothiocyanate-dextran (FITC-dextran; Fd-2000S) and other antibodies were purchased from Sigma-Aldrich (St. Louis, MO, USA). Terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) assay kit, PrimeScript RT reagent Kit and fluorescent quantitative kit were all obtained from Takara (Dalian, China). Trizol reagent was purchased from Invitrogen (Carlsbad, CA, USA). Bradford protein assay kit and enhanced chemiluminescence reagent were purchased from Beyotime Biotechnology Co., Ltd (Shanghai, China). Bovine serum albumin was purchased from Roche (Indianapolis, IN, USA). All other regents were purchased from Bioval Biotechnology Co., Ltd (Shanghai, China). Kodak BT X-OMAT medical X-ray film (XBT-1) was purchased from Kodak (Rochester, NY, USA).

Modeling and Irrigation

The protocol used to generate STZ-induced diabetes and following PSP intervention was reported in our previously study[9]. Fast abdominal cavity injection of STZ (60 mg/kg, with 2% sodium citrate buffer solution, pH 4.44) was carried out in 30 min for modeling. The fasting blood glucose was measured before STZ injection, 72 h after STZ injection, the end of each week and 24 h after completion of the study in week 12. The diabetes mellitus rats were randomly divided into four subgroups: control model, meaning the diabetes mellitus group (diabetes mellitus), normal diet with intragastric administration of 2 mL saline; diabetes mellitus with low-dose PSP group (L), intragastric administration of 2 mL PSP sterile saline solution (200 mg/kg,); diabetes mellitus with medium-dose PSP group (M), 2 mL PSP (400 mg/kg); and diabetes mellitus with high-dose PSP group (H), 2 mL PSP (800 mg/kg). Another 24 rats were only injected with 2% sodium citrate buffer solution and used as the blank control group (BC). Three rats were used for each group for each time.

FITC-Dextran Retinal Vascular Perfusion

FITC-dextran perfusion was carried out at 4, 8 and 12 weeks, respectively. The rats were anesthetized using intraperitoneal injection of ketamine combined with chlorpromazine (0.01 mL/g bodyweight). Neck skin incision was carried out to search for the superior vena cava, where FITC-dextran Fd-2000s was injected with 30-G needle. After 5 min, the eyeballs were collected entirely, and then enucleated and fixed in 4% paraformaldehyde for 15 min. After the eyes were cut behind the ora serrata 0.5 mm, the anterior segment and vitreous were gently removed. Retinal tissues were obtained and then placed

on the glass slide with radial incision centered on the optic disc. The samples were observed under a fluorescence microscope Olympus (Tokyo, Japan) to compare the difference of retinal vascularization between groups.

TUNEL Assay

TUNEL assay was carried out at 4, 8 and 12 weeks, respectively. The eyeball samples were fixed with paraformaldehyde, dehydrated and sectioned, and then, cell apoptosis was analyzed according to the TUNEL assay kit instructions. These sections were stained with diaminobenzidine and counterstained with hematoxylin. Apoptotic cells were identified when brown-yellow granules were found in the nucleus of RGCs for further quantitative analysis. The processed sections were observed and photographed under a fluorescence microscope. The Apoptotic Index (AI) was calculated by counting the number of TUNEL-positive cell nuclei/total number of cell nuclei per field. Five randomly microscopic fields per slide and 10 slides per animal were studied.

Total Ribonucleic Acid Extraction and Reverse Transcription Polymerase Chain Reaction

At the end of the 12-week treatment, retinas from the right and left eyes of one rat were pooled to generate each sample, which were then utilized to extract total ribonucleic acid (RNA) using Trizol reagent according to the manufacturer's instructions, followed by reverse transcription with Primescript RT reagent Kit (Takara, Kyoto, Japan) and complementary DNA amplification through an SYBR Premix Ex Taq kit (Takara). The expressions of target genes were normalized to against β-actin expression, and the data were calculated using the $2^{-\Delta\Delta Ct}$ method. Amplification and detection were carried out using a real-time polymerase chain reaction system ABi7500 (Applied Biosystems, Foster, CA, USA). The polymerase chain reaction amplification conditions were as follows: 94°C for 5 min; 94°C for 30 s, 55°C for 30 s, 72°C for 30 s, 28 cycles; and 72°C for 5 min. The sequences of primers are shown in Table 1.

Western Blotting

At the end of 12 weeks, two retinas from both eyes of one rat were pooled as one sample, which were then lysed in radioimmunoprecipitation assay solution on ice for 2 h. The lysates were collected and centrifuged at 11,000 *g* for 20 min. The supernatants were obtained, and the protein concentration was determined. The protein samples were suspended in 5× loading buffer, denatured for 5 min at 100 °C, loaded with 40 μg of protein lysate, separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis, and transferred onto nitrocellulose membranes by electroelution. The blots were blocked with 5% bovine serum albumin in Tris-buffered saline buffer at 37°C for 2 h. The membranes were then incubated overnight with primary antibodies (Bax, Bcl-2, EGF, p38, TGF-β and VEGF) at 4°C and secondary antibodies for 1 h at room temperature. Target proteins on the membranes were visualized using the enhanced chemiluminescence reagents (Beyotime Biotechnology) and the Bio-Rad ChemiDoc XRS+ System (Hercules, CA, USA). β-Actin was used as the positive control.

Statistical Analysis

All data in the present study were expressed as the mean value and standard deviation for each group. Student's *t*-test was carried out to compare two experimental groups. ANOVA was used to compare more than two groups and supplemented by the Student–Newman–Keuls test for pairwise comparisons. The statistical analysis was carried out using SPSS 20.0 software (SPSS Inc., Chicago, IL, USA). Statistical significance was considered at $P < 0.05$.

RESULTS

General Situation and Blood Glucose Level

A total of 120 rats were included in final analysis. Rats in the BC group ($n = 24$) were lively and reactive, with symmetric body and healthy hair, as well as shiny and transparent cornea, and clear dioptric media, all without exception. Blood glucose levels in the BC group remained stable. STZ-treated rats ($n = 96$) met the standard of fasting blood

Table 1 | Primer pairs used for polymerase chain reaction amplification of Bcl2-associated X protein, B-cell lymphoma-2 factor, epidermal growth factor, p38 mitogen-activated protein kinases, transforming growth factor-β and vascular endothelial growth factor complementary deoxyribonucleic acids

Gene	Forward primer	Reverse primer
β-Actin	5′-TGACGTGGACATCCGCAAAG-3′	5′-CTGGAAGGTGGACAGCGAGG-3′
Bax	5′-AAAGTAGAAGAGGGCAACCAC-3	5′-AACCACCTGCGTAGGACC-3′
Bcl-2	5′-CTACCGTCGTGACTTCGC-3	5′-CCTATTGCCTCCGACCCT-3′
EGF	5′-CCCTAAGTCGAGACCGGAAGT-3′	5′-ACATTGCGTGGACAGGAAACA-3′
p38	5′-TGTCTGTCTTTGTGGGAGGGTA-3	5′-TGGGATGTTGTCAAGTCTACGC-3′
TGF-β	5′-ATGTCACCGGAGTTGTGCG-3′	5′-GGTCCTTGCGGAAGTCAATGTA-3′
VEGF	5′-GCTACTGCCATCCAATCGAGA-3′	5′-TGTGCTGTAGGAAGCTCATCTCTC-3

Bax, Bcl2-associated X protein; Bcl-2, B-cell lymphoma-2 factor; EGF, epidermal growth factor; p38, p38 mitogen-activated protein kinases; TGF-β, transforming growth factor; VEGF, vascular endothelial growth factor.

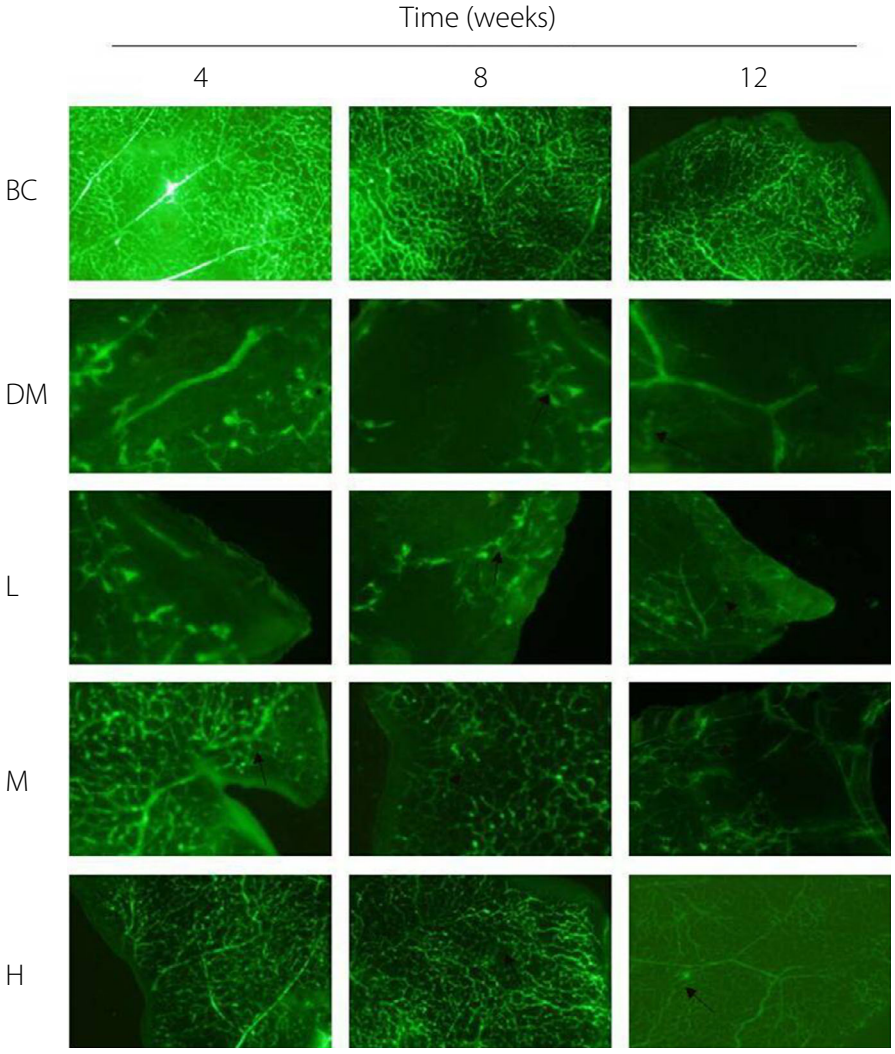

Figure 1 | Pathological observation of retinal vascular spread in fluorescein isothiocyanate-dextran perfusion. BC, blank control group; DM, diabetes mellitus group; H, diabetes mellitus with high-dose *Polygonatum sibiricum* polysaccharide group; L, diabetes mellitus with low-dose *Polygonatum sibiricum* polysaccharide group, M, diabetes mellitus with medium-dose *Polygonatum sibiricum* polysaccharide group.

glucose >13.9 mmol/L, were considered diabetic and were included in the study. After 3 days of successful modeling, the visible symptoms of typical polyphagia, polydipsia, polyuria and weight loss were observed, with sustained and stable hyperglycemia. These rats gradually showed the signs of emaciation, hair withering and weakness, especially in the diabetes mellitus group and L group, with their urine volume increased and bodyweight decreased significantly, as well as yellow fur and white lens. However, fasting blood glucose and glycosylated hemoglobin levels in the L, M and H groups ($n = 24$, respectively) gradually decreased with PSP dosage increase; that is, in a dose-dependent manner. There were significant differences in fasting blood glucose and glycosylated hemoglobin level ($P < 0.05$) between groups (Table S1).

FITC-Dextran Perfusion of Retinal Vascular Patch

In the BC group, the retinas were completely covered with normal blood vessels, which were distributed evenly and naturally without obvious leakage. We could clearly observe that there were three layers of retinal vessels, and four to six arterial branches accompanying venous radiating from the optic papilla. In the diabetes mellitus group, blood vessels were tortuous and dilated, with disordered distribution, serious leakage and arteriovenous communication. The large unvascularized areas were shown in the posterior pole retina of the diabetes mellitus group, but also the H group. However, in the H group, the retinal vascular morphology was more normal, and vascular tortuosity and leakage were relatively less severe. For the M and L groups, the retinal vascular conditions were situated between the BC group and diabetes mellitus group. Given the

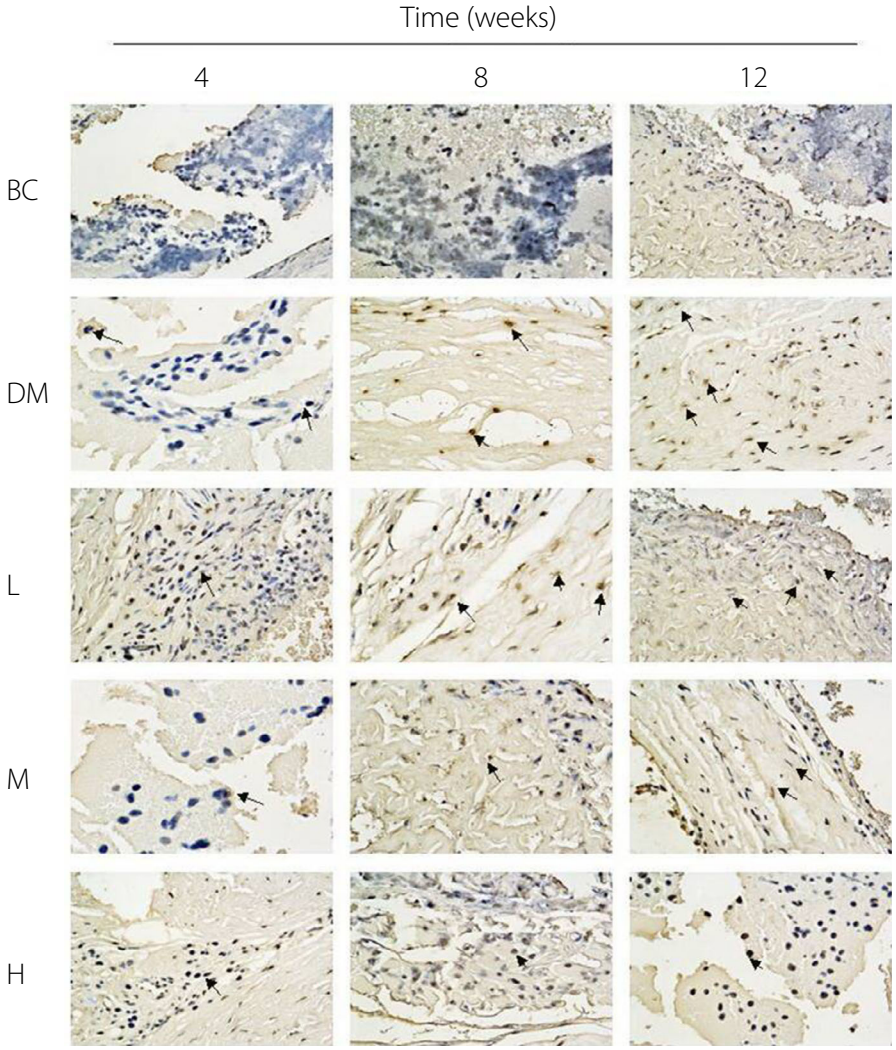

Figure 2 | Apoptosis staining of retinal ganglion cells by *in situ* terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling assay. Arrows indicate labeled terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling-positive cells. BC, blank control group; DM, diabetes mellitus group; H, diabetes mellitus with high-dose *Polygonatum sibiricum* polysaccharide group; L, diabetes mellitus with low-dose *Polygonatum sibiricum* polysaccharide group, M, diabetes mellitus with medium-dose *Polygonatum sibiricum* polysaccharide group.

aforementioned feature, the present study showed the time- and dose-dependent relationships for PSP to intervene in DR (Figure 1).

TUNEL Method for the Detection of RGCs Apoptosis

The AI of RGCs in the diabetes mellitus group was significantly higher than that in the BC group ($P < 0.05$), where RGCs occasionally showed signs of apoptosis characterized by nuclear dark brown staining. However, the AI of RGCs decreased after using PSP intervention of high, medium and low doses. Among them, the H group showed the most obvious signs, with cell nuclear light brown staining. RGCs in the BC group had normal volume and cellar structure, but the diabetes mellitus group showed cellar membrane wrinkling, cytoplasm concentration, nucleus shrinkage, chromatin margination phenomenon and mitochondrial swelling, meaning that cells tended to apoptosis. Whereas RGCs had no obvious changes in the H group, except that mitochondria became mildly swollen and chromatin were slightly loose (Figure 2). There also existed time- and dose-dependent relationships for PSP to intervene in DR (Table S2).

Expression of retinal Bax, Bcl-2, EGF, p38, TGF-β and VEGF Messenger RNA was Detected by reverse Transcription Polymerase Chain Reaction

The levels of retinal Bax, Bcl-2, EGF, p38, TGF-β and VEGF messenger (mRNA) expression were analyzed by the $2^{-\Delta\Delta\ CT}$ method (Figure 3). All of them were expressed in each group. The mRNA expressions of Bax, EGF, p38, TGF-β and VEGF in the retinal tissue of the diabetes mellitus group of rats were

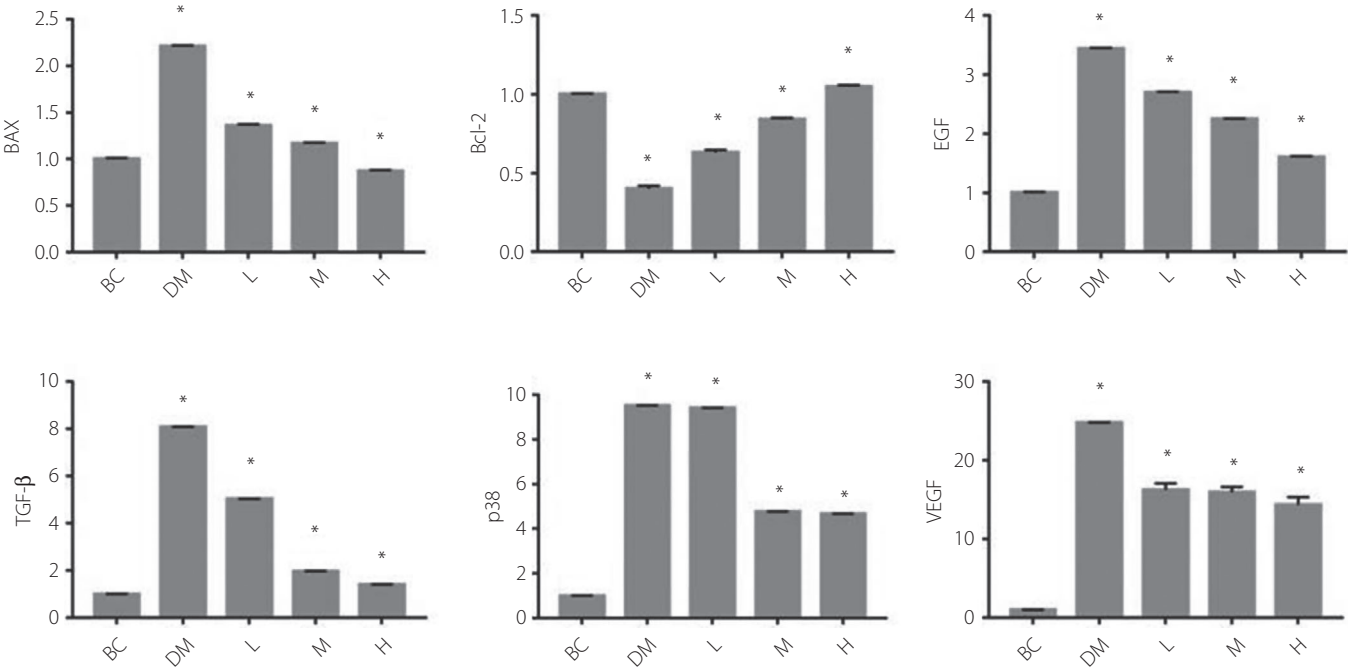

Figure 3 | Evaluation of messenger ribonucleic acid expression levels of Bcl2-associated X protein (Bax), B-cell lymphoma-2 factor (Bcl-2), epidermal growth factor (EGF), p38, transforming growth factor (TGF-β) and vascular endothelial growth factor (VEGF) in rat retinas by reverse transcription polymerase chain reactions analysis. *$P < 0.05$ compared with the blank control (BC) group. DM, diabetes mellitus group; H, diabetes mellitus with high-dose *Polygonatum sibiricum* polysaccharide group; L, diabetes mellitus with low-dose *Polygonatum sibiricum* polysaccharide group, M, diabetes mellitus with medium-dose *Polygonatum sibiricum* polysaccharide group.

significantly higher than those of the L, M and H groups ($P < 0.05$). Furthermore, they presented a decreasing trend with the increase of the PSP injection. The Bcl-2 mRNA expression in the retinal tissue of the rats in the diabetes mellitus group was obviously lower than those of the L, M and H groups ($P < 0.05$). In addition, Bcl-2 mRNA expression presented an increasing trend with the increase of PSP dosages. These suggested that PSP has a protective effect against diabetes-induced retinal injury in a dose-dependent manner. Significant differences were found ($P < 0.05$) among the five groups (Table S3).

Western Blot was Used to Detect the Contents of Retinal Bax, Bcl-2, EGF, p38, TGF-β and VEGF Proteins

Bax, Bcl-2, EGF, p38, TGF-β and VEGF proteins were all expressed in the retinas of rates in the BC, diabetes mellitus, L, M and H groups. The protein expression of Bax, EGF, p38, TGF-β and VEGF in the retinal tissue of the rates in the diabetes mellitus group was obviously higher than those of the PSP intervention groups ($P < 0.01$), where the protein levels decreased with the increase of PSP dosages. However, it was also observed that Bcl-2 protein expression in the retinal tissue was significantly downregulated in the rats in the diabetes mellitus group than those in the L, M and H groups ($P < 0.01$). Bcl-2 protein increased with the increase of PSP dosage (Figure 4). This observation further confirmed that PSP prevented and delayed the occurrence and development of DR.

DISCUSSION

Based on our previous studies, PSP restrained DR progression and improved visual function in experimental diabetic rats[9]. In the present study, we further investigated the protective effect of PSP against diabetes-induced retinal injury and its possible underlying mechanisms. The present results showed that PSP intervention improved blood glucose homeostasis and retinal microvascular complications, inhibited apoptosis in the retinal ganglion cells, and downregulated expression of Bax, EGF, p38, VEGF and TGF-β accompanied by upregulation of Bcl-2 in STZ-diabetic rats in a dose-dependent manner.

The hallmark of DR is progressive microvascular disease, in which abnormal proliferation of retinal angiogenesis in DR progression is an important pathological change[11]. Retinal blood vessels normally do not penetrate through the inner limiting membrane, so those vessels that break through the inner membrane can be considered as newly generated vessels of the retina[12]. In the present study, the vascular endothelial cell nucleus through the inner limiting membrane was visible in the diabetes mellitus group and increased in numbers as diabetes progressed. Retinal inner limiting membrane cell proliferation and disordered arrangement were observed. Also, new blood vessels could be seen as sprouts, as well as red blood cells seen in some capillary lumen[13]. The results showed that DR was aggravated and vascular proliferation

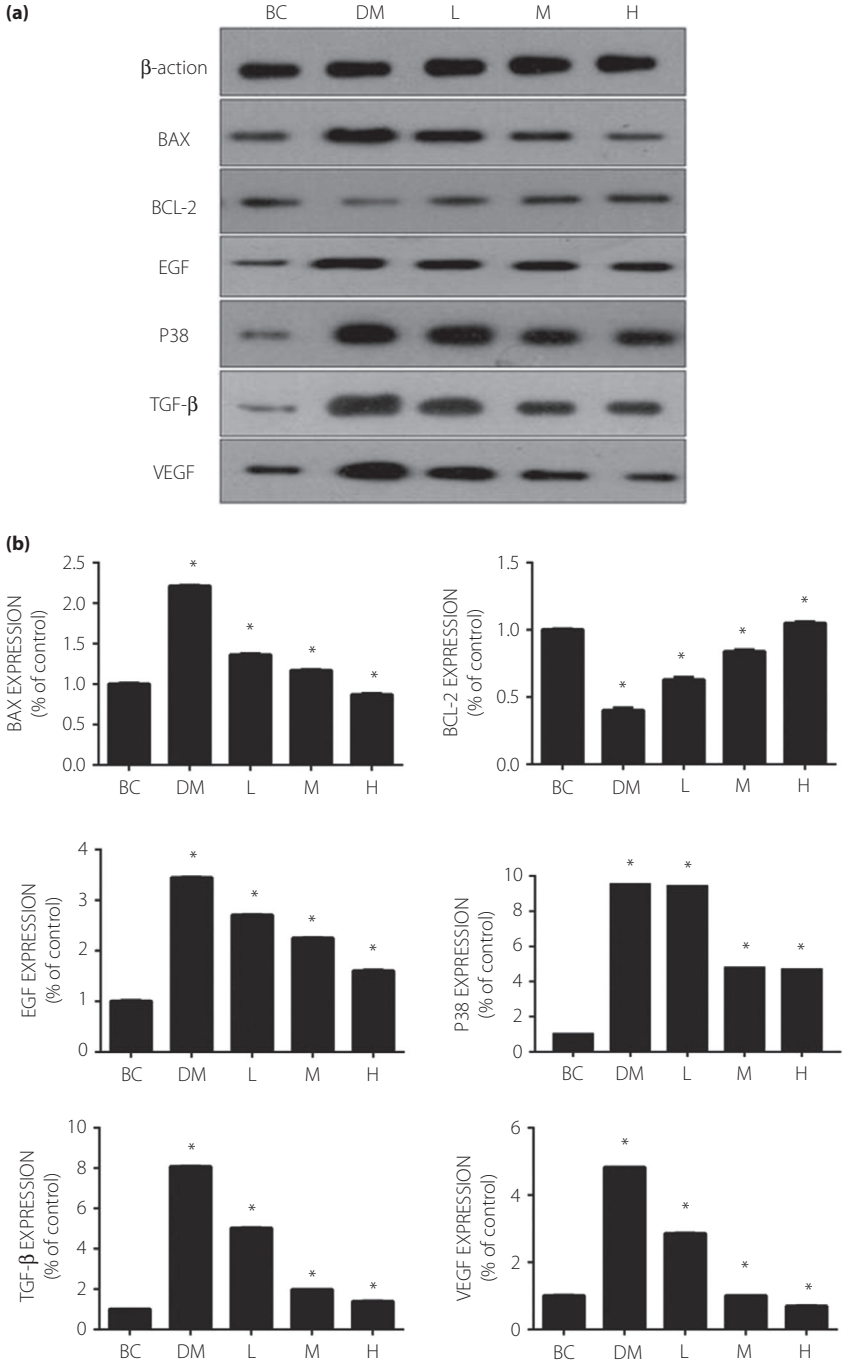

Figure 4 | Evaluation of protein expression levels of Bcl2-associated X protein (Bax), B-cell lymphoma-2 factor (Bcl-2), epidermal growth factor (EGF), p38 mitogen-activated protein kinases (p38), transforming growth factor (TGF-β) and vascular endothelial growth factor (VEGF) in rat retinas by western blot analysis. (a) Western blot results showed a band of protein expression. β-Actin was used as the control. (b) A histogram of the relative protein expression is corrected as was shown. *$P < 0.05$ compared with the blank control (BC) group. DM, diabetes mellitus group; H, diabetes mellitus with high-dose *Polygonatum sibiricum* polysaccharide group; L, diabetes mellitus with low-dose *Polygonatum sibiricum* polysaccharide group, M, diabetes mellitus with medium-dose *Polygonatum sibiricum* polysaccharide group.

became more obvious with the course of diabetes mellitus [14]. Hyperglycemia in diabetic rats will result in the reduction of retinal capillary pericytes, proliferation of endothelial cell and thickening of basement membrane, vascular occlusion and perfusion loss, and eventually lead to retinal ischemia and hypoxia injury[15].

Currently, the pathogenesis of diabetes-induced retinopathy remains to be completely clarified. It is commonly accepted that oxidative stress plays a pivotal role therein. Oxidative stress can produce reactive oxygen species, thereby destroying the balance between oxidation and anti-oxidation, activating downstream signal transduction pathways, generating membrane protein damage factors, and ultimately leading to retinal vascular lesions[16]. In early diabetic animal models, it can be observed that inflammation reaction activated leukocytes adhere to the retinal vessels, resulting in an increase in inflammatory cytokines and retinal capillary pathological changes[17]. In the present study, the retinal vasculopathy of rats in the H group was less severe than that in the diabetes mellitus, L and M groups, showing that administration of high-dosage PSP could significantly alleviate the vessel injury in STZ-induced DR. The retinal vascular pathological changes of rats in the three intervention groups were presented in a dose- and time-dependent manner, indicating that PSP might play an important protective role against STZ-induced diabetic retinal injury.

In the cellular response to genotoxic factors, the cell cycle limit point (cell cycle restrictive point) maintains the main part of cell genome integrity and p53 is involved[18]. P53 can activate p21 and p27 genes, and induce the cell stagnation in the G1/S limit, so that sufficient time is provided for the damaged DNA to be repaired[19]. Nevertheless, expression of the *BCL2* gene can prevent p53-mediated apoptosis[20]. P53 can be downregulated when Bcl-2 is activated and its expression is increased[21]. To increase p53 and decrease Bcl-2, expression was observed in the pathogenesis of DR[22]. We believe that hyperglycaemia can induce DNA damage in vascular endothelial cells and therefore upregulate p53 expression, to exert its effect on repairing DNA damage and avoiding cell death[23]. In this process, the role of Bcl-2 might be to prevent retinal vascular endothelial cells from apoptosis and assist p53 in DNA repair. The two synergistically resist the damage of high blood glucose and maintain cells' relative stability. The *Bax* gene belongs to the Bcl-2 family, which is associated with the regulation of apoptosis[24]. When the amount of Bax was higher than Bcl-2, apoptosis was induced; otherwise cells survived. Mitogen-activated protein kinase p38 is an important regulator of cell apoptosis. Recent studies showed that inhibition of mitogen-activated protein kinase p38 significantly improved recovery and attenuated apoptosis ascribed to retinal ischemia of rats[25]. The present results showed increased Bcl-2 expression, and decreased both Bax and p38 expression in the PSP treatment group in STZ-induced DR rats. We speculate that the high expression level of Bcl-2 might function as a protective factor by repairing DNA damage and maintaining cell survival, therefore reducing retinal ganglion cell death; the expression inhibition of Bax and mitogen-activated protein kinase p38 also reduces their function, therefore lessening the effect of Bax and p38on cell death.

VEGF is an important angiogenic growth factor that stimulates the proliferation of vascular endothelial cells and maintains the survival of cells[26]. VEGF promotes angiogenesis through cell proliferation and migration, and capillary formation[27]. In addition, many growth factors stimulate the proliferation of retinal cells. One of them is EGF, which plays a pivotal role in the proliferation of retinal capillary endothelial cells[28]. TGF-β works on cell differentiation and proliferation, and inhibits the DNA synthesis of vascular endothelial cells[29]. Also, the inhibition of the TGF-β signaling pathway promotes retinal regeneration in a zebrafish model[30]. In the present study, the mRNA and protein expression levels of VEGF, EGF and TGF-β in the retina were significantly increased with the aggravation lesions of DR, but PSP intervention can significantly reduce VEGF, EGF and TGF-β expression. We reasoned that the downregulated expression of VEGF, EGF and TGF-β under the PSP treatment condition might inhibit retinal vascular regeneration and reduce retinal cell damage in STZ-induced diabetic rats. In addition, it is also possible that the reduced expression of VEGF, EGF and TGF-β might show synergistic effect to suppress the retinal injury in DR.

Overall, the present findings showed that PSP has a protective effect on diabetic retinal injury with possible underlying mechanisms involved in lowering blood glucose, limiting pathological angiogenesis and inhibiting cellular apoptosis through downregulated signaling of Bax, EGF, p38, VEGF and TGF-β, and upregulation of Bcl-2. The present study suggested that PSP supplementation might be considered as an alternative choice based on Chinese herbal medicines for the prevention and management of diabetic retinal vascular diseases. Further studies are required to illuminate the intrinsic characteristics and biological activities of PSP, the interrelation and interaction between signaling molecules, and its underlying mechanisms of action.

ACKNOWLEDGMENTS

This study was financially supported by Tai'an Science Technology Plan development program (2015NS1119).

DISCLOSURE

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1 | Fasting blood glucose and glycosylated hemoglobin values at different time points in each group (mmol/L) ($\bar{x} \pm s$)
Table S2 | Terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling positive rate expression results (%) ($\bar{x} \pm s$)
Table S3 | Expression of Bcl2-associated X protein, B-cell lymphoma-2 factor, epidermal growth factor, p38 mitogen-activated protein kinases, transforming growth factor-β and vascular endothelial growth factor messenger ribonucleic acid on the retina ($\bar{x} \pm s$)