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Ganoderma atrum polysaccharide improves aortic relaxation in diabetic rats via PI3K/Akt pathway

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ABSTRACT

A newly identified polysaccharide (PSG-1) has been purified from *Ganoderma atrum*. The study was to investigate the protective effect of PSG-1 on diabetes-induced endothelial dysfunction in rat aorta. Rats were fed a high fat diet for 8 weeks and then injected with a low dose of streptozotocin to induce type 2 diabetes. The diabetic rats were orally treated with PSG-1 for 4 weeks. It was found that administration of PSG-1 significantly reduced levels of fasting blood glucose, improved endothelium-dependent aortic relaxation, increased levels of phosphoinositide 3-kinase (PI3K), phospho-Akt (p-Akt), endothelial nitric oxide synthase (eNOS) and nitric oxide in the aorta from diabetic rats, compared to un-treated diabetics. These results suggested that the protective effects of PSG-1 against endothelial dysfunction may be related to activation of the PI3K/Akt/eNOS pathway.

1. Introduction

Ganoderma, one of the most popular medicinal fungi, has been used as a folk remedy to promote health and longevity in oriental countries for centuries. The polysaccharides isolated from *Ganoderma* have been reported to exhibit diverse biological properties (Nie, Zhang, Li, & Xie, 2013). Recently, a new bioactive polysaccharide (PSG-1) has been isolated from *Ganoderma atrum* in our laboratory. PSG-1 has been found to exhibit anti-oxidant (Chen, Xie, Nie, Li, & Wang, 2008), anti-tumor (Li et al., 2011), immunomodulatory (Yu et al., 2012), hypoglycemic and hypolipidemic effects (Zhu et al., 2013). PSG-1 was also reported to protect cardiomyocytes from injury induced by anoxia/reoxygenation (Li, Nie, Yan, Zhu, & Xie, 2009).

Cardiovascular complications are the major causes of mortality and morbidity in patients with diabetes (He, Naruse, & King, 2005). While, endothelial dysfunction which is characterized by reduced activity of endothelial nitric oxide synthase (eNOS) and bioavailability of nitric oxide (NO) (Gupta, Toruner, Chung, & Groszmann, 2003; Kim et al., 2002), plays a key role in the pathogenesis of diabetic cardiovascular complications (Kobayashi, Oishi, Hayashi, Matsumoto, & Kamata, 2006). Previous studies have indicated that the endothelium-dependent relaxation to acetylcholine (Ach) was weaker in the aortic rings from streptozotocin (STZ)-induced diabetic rats (Topal et al., 2012).

Apoptosis of endothelial cells is reported to be a primary event in the process of diabetes-associated macrovascular and microvascular diseases (Xu, Zhong, Zeng, & Ge, 2008). The apoptosis of endothelial cells is related to hyperglycemia, hyperlipidemia and insulin resistance in type 2 diabetes (Adler et al., 2002). Hyperglycemia accelerates apoptosis of endothelial cells by multiple proteins inside the cells, including the pro-apoptotic (Bax) and anti-apoptotic (Bcl-2) proteins of Bcl-2 family (Lian, Ren, & Gao, 2011). To our knowledge, there have been few reports about the protective effects of polysaccharides on diabetes-induced endothelial dysfunction. Therefore, the aim of this study was to investigate the effect of PSG-1 on endothelium-dependent relaxation of the aorta from high fat diet and STZ-induced diabetic rats, an animal model of type 2 diabetes in humans.

2. Materials and methods

2.1. Chemicals and reagents

STZ, Ach and norepinephrine (NE) were purchased from Sigma Chemical Co. (St Louis, MO, USA). Cyclosporine A (CSA, purity 99.3%) was obtained from Taizhou Creating Chemical Co. *N*-Acetyl-L-cysteine (NAC) was purchased from Beijing Solarbio Science and Technology Co. One touch glucometer (Accu-chek Performa) was

Abbreviations: CSA, cyclosporine A; eNOS, endothelial nitric oxide synthase; NAC, *N*-acetyl-L-cysteine; NO, nitric oxide; PSG-1, polysaccharide from *Ganoderma atrum*; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling.

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obtained from Roche Diagnostics (Mannheim, Germany). All other chemicals and solvents were of analytical grade.

PSG-1 was prepared from the fruiting bodies of *G. atrum* by boiling water according to the method of Chen et al. (2008). The chemical components were analyzed by the phenol-sulphuric acid method. PSG-1 was found to compose of glucose, mannose, galactose and galacturonic acid in a molar ratio of 4.91:1:1.28:0.71, with an average molecular weight of approximately 1013 kDa, and the preliminary structure of PSG-1 was characterized by using methylation analysis and 1D/2D nuclear magnetic resonance (NMR) spectroscopy (Zhang et al., 2012).

2.2. Animals and treatments

Ninety male Wistar rats (180–200 g) were obtained from Shanghai Slaccas Laboratory Animal Company (Certificate Number: SCXK (hu) 2007-0005, Shanghai, China). Before starting the experiments, all the animals were housed at an ambient temperature of 25 ± 2 °C, 12/12 h of light-dark cycle with ad libitum food and water for 1 week. Then, 10 rats were fed a standard normal chow diet consisting of 12% fat, 60% carbohydrate and 28% protein (as a percentage of total kcal), the others were fed with high fat diet consisting of 40% fat, 42% carbohydrate and 18% protein. After 8 weeks of dietary manipulation, rats fed on high fat diet were fasted for 12 h and injected with a single low dose of STZ [30 mg/kg body weight (BW), in 154 mmol/L isotonic saline, pH 7.2] into the tail vein to induce type 2 diabetes, while those fed on standard normal chow diet received an equivalent volume of saline. Hyperglycemia was confirmed by the levels of fasting blood glucose (FBG) higher than 11.1 mmol/L at Day 4 and Day 7 after STZ injection.

Fifty type 2 diabetic rats were randomly divided into the following groups: un-treated diabetic, diabetic treated with PSG-1 (200 and 400 mg/kg BW) (Zhu et al., 2013), diabetic treated with CSA (8 mg/kg BW) (Wakita, Tomimoto, Akiguchi, & Kimura, 1995) and diabetic treated with NAC (100 mg/kg BW) (Karageorgos et al., 2006). Ten rats fed on standard normal chow diet served as a non-diabetic control group, received the same volume of vehicle solution. All treatments were conducted once daily over a 4-week period. FBG levels were measured at the end of experimental period. All animals used in this study were cared for in accordance with the Guidelines for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (NIH Publication 85-23, 1996). All procedures were approved by the Animal Ethnics Committee, Nanchang University.

2.3. Preparation of aortic ring

All animals were anesthetized with 10% chloralic hydras intraperitoneally, then sacrificed by exsanguination after 4 weeks of treatment. The aorta was removed, cleaned of excess fat and connective tissue with extra care to avoid any damage to the endothelium. Aortic rings of approximately 4 mm in length were prepared and suspended in 10-mL organ bath filled with Krebs–Henseleit solution (KHS) [composition (mmol/L): NaCl 118.0, KCl 4.7, $NaHCO_3$ 25.0, $CaCl_2$ 1.8, NaH_2PO_4 1.2, $MgSO_4$ 1.2, and glucose, 11.0, oxygenated with 95% O_2 + 5% CO_2 (pH 7.4, 37 °C)] for measurement of relaxation of the aorta. The remaining portion of the aorta was fixed in 10% neutral buffered formalin and frozen in liquid nitrogen, respectively.

2.4. Endothelium-dependent relaxation of aorta

To determine the vasoactive effects of PSG-1 on endothelium-dependent relaxation, the responses to Ach were examined in aortic rings from diabetic rats. Aortic rings were mounted on stainless steel hooks and suspended in 10-mL organ bath filled with KHS (95% O_2 + 5% CO_2, pH 7.4, 37 °C), then stretched to a 1.5-g initial tension and equilibrated for 60 min. The Krebs buffer was replaced every 15 min, tension being readjusted each time. At the end of the equilibration period, the functional integrity of the endothelium was verified as described by Nguelefack et al. (2009).

After the verification, the aortic rings were washed with KHS to its initial tension, pre-contracted with NE (2×10^{-7} mol/L) and then relaxed with Ach (10^{-8} mol/L to 10^{-5} mol/L). After each addition, the concentration of Ach was maintained for 10 min to allow the tension to develop. The tension was first amplified by a high input impedance amplifier (JZ100, Chengdu Instrument Factory, Chengdu, China), and then processed by multiple channel physiological signal collecting and processing system (RM6240BD, Chengdu Instrument Factory, Chengdu, China). Relaxation (%) = [(Tension Ach − Basal Tension)/(Tension at steady pre-contracted state × Basal Tension)] × 100%.

2.5. Determination of eNOS and NO levels

The eNOS protein concentration in the aorta was determined with an ELISA kit (Nanjing Jiancheng Bioengineering Institute, Jiangsu, China) following the manufacturer's instructions. NO levels were measured as total nitrite with the spectrophotometric Griess reaction (Cortas & Wakid, 1990). Total protein concentration in tissue homogenates of aorta was determined by Bradford method using a commercial kit (Nanjing Jiancheng Bioengineering Institute, Jiangsu, China).

2.6. Histopathological study

The formalin-fixed aortic tissues were dehydrated in ascending grades of ethyl alcohol, cleared in xylene and embedded in paraffin wax. The specimens were then cut into 5 μm-thick sections using a rotary microtome. Sections were stained with hematoxylin and eosin dye, and photomicrographs were obtained under light microscope (Ti series inverted microscope, Nikon, Japan).

2.7. Analysis of apoptosis

The apoptosis of endothelium cells in the aorta was examined with terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL) assay (Gavrieli, Sherman, & Ben-Sasson, 1992). Briefly, 5 μm-thick sections after deparaffinization were treated with 3% H_2O_2 to quench endogenous peroxidase, permeated with 1% Triton X-100 for 10 min, followed by treatment of 10 mg/ml proteinase K at room temperature for 30 min. Sections were then incubated with terminal deoxynucleotidyl transferase (TdT), an enzyme that catalyzes a template-independent addition of fluorescein-dUTP (FITC-12-dUTP) to the free 3′-OH ends resulting from DNA degradation. The sections were observed with a Nikon Ti series inverted microscope equipped with a standard fluorescent filter to view the fluorescence produced by FITC-12-dUTP.

2.8. Immunohistochemical analysis

The aortic tissue samples collected for histology were subjected to immunohistochemical analysis to determine the expression of Bax and Bcl-2 proteins according to our procedures (Zhu et al., 2013).

The positive signals of these proteins were observed with a Nikon Ti series inverted microscope equipped with bright-field high-quality objectives. Images were acquired by a digital camera (Nikon Digital Sight DS-Fi1c) and image acquisition software.

Table 1
Sequences of the primers used for PCR.

Gene	Primer sequence	Cycle	Product size (bp)	Reference
Bcl-2	F: 5′-GTATGATAACCGGGAGATCG-3′; R: 5′-AGCCAGGAGAAATCAAACAG-3′	34	612	Rogério et al. (2006) and Takahashi et al. (2008)
Bax	F: 5′-AAGAAGCTGAGCGAGTGTCT-3′; R: 5′-CAAAGATGGTCACTGTCTGC-3′	29	361	Rogério et al. (2006), Takahashi et al. (2008) and Dong et al. (2012)
Caspase-3	F: 5′-ATGTCGATGCAGCTAACCTCA-3′; R: 5′-TGTCTCAATACCGCAGTCCAG-3′	31	320	Dong et al. (2012) and Dong, Li, Dong, and Zhang (2011)
GAPDH	F: 5′-CGGAGTCAACGGATTTGGTCGTAT-3′; R: 5′-AGCCTTCTCCATGGTGGTGAAGAC-3′	29	306	Rogério et al. (2006) and Takahashi et al. (2008)

The integral optical density (IOD) and area of immunohistochemistry stain was evaluated using Image-Pro Plus 6.0 Software (Media Cybernetics, CA, USA). The mean optical density [MOD = (IOD/area of DAB staining)], which is stoichiometrically related to the cellular concentration of antigen.

2.9. Western blotting

Protein was extracted from aortic tissue samples, and protein concentration was determined by the Bradford assay. The protein samples were mixed with sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) loading buffer, followed by boiling for 5 min. Each sample (50 μg protein/lane) was separated by electrophoresis on 10% SDS-PAGE, electroblotted onto a nitrocellulose membrane, blocked with 5% BSA for 1 h and then incubated overnight with the following antibodies: rabbit monoclonal anti-phosphoinositide 3-kinase p85 (PI3K, 85 kDa), anti-Akt (60 kDa) and anti-phospho-Akt (p-Akt, 60 kDa) (Cell Signaling Technology, Beverly, MA, USA). Anti-β-actin antibody (43 kDa, Beijing Zhong Shan Golden Bridge Biotechnology Co., Ltd., Beijing, China) was used as an internal control. Subsequently, the membranes were incubated with horseradish peroxidase conjugated anti-rabbit or anti-mouse antibody. The resulting signals were visualized by ECL solution (Amersham Biosciences, Uppsala, Sweden) and photographed using Molecular Imager® system (Bio-Rad Laboratories, Hercules, CA, USA).

2.10. RT-PCR analysis

Total RNA was isolated from aortic tissue using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instruction. The ratios of A_{260}/A_{280} in all the RNA samples were in the range of 1.9–2.1, indicating that the RNA prepared by this method was of high quality (Singh, Misra, Sane, & Nath, 2009). Then 2 μg of total RNA was reverse-transcribed to first-strand cDNA using oligo$(dT)_{18}$ primers with a RevertAid First Strand cDNA Synthesis Kit (Fermentas, St. Leonrod, Germany) according to the manufacturer's instruction.

The PCR amplification of different genes was performed in a 50 μL reaction mixture containing 1× Easy Taq PCR SuperMix (TransGen Biotech, Beijing, China), 0.4 μM forward and reverse primers and 5 ng of cDNA. The PCR conditions were based on previous studies (Rogério et al., 2006; Takahashi, Yoshimura, Yamamoto, & Wakita, 2008) with modifications: initial denaturation for 5 min at 94 °C; repeated cycles of denaturation for 1 min at 94 °C, annealing for 1 min at 58 °C (Bcl-2), 59 °C (Bax), 53 °C (Caspase-3) or 63 °C (GAPDH), extension for 1 min at 72 °C; final extension for 10 min at 72 °C. PCR products were separated on 1.5% agarose gels, stained with Goldview (Solarbio, Beijing, China) and photographed under UV light using Molecular Imager® system (Bio-Rad Laboratories, Hercules, CA, USA). The sequences of the primers used for PCR were shown in Table 1.

2.11. Statistical analysis

Data was expressed as the mean ± standard error of the mean (SEM). One-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test was used to assess the differences using SPSS 13.0 software. A value of $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. Effect of PSG-1 on FBG levels in type 2 diabetic rats

As shown in Fig. 1A, the FBG levels increased significantly ($P < 0.05$) in un-treated diabetic rats compared to non-diabetic controls, which were significantly ($P < 0.05$) reduced by oral administration of PSG-1 in a dose-dependent manner.

3.2. Vasoactive effects of PSG-1 on endothelium-dependent relaxation of aorta

Endothelium-dependent relaxation responses induced by Ach were significantly ($P < 0.05$) lower in un-treated diabetic rats compared to non-diabetic rats. The differences among PSG-1 treated and un-treated diabetic rats were significant ($P < 0.05$) at concentrations higher than 10^{-7} mol/L. Relaxation response to Ach in diabetic rats treated with PSG-1 at 200 mg/kg BW was lower than that in diabetic rats treated with PSG-1 at 400 mg/kg BW (Fig. 1B).

3.3. PSG-1 increased NO and eNOS levels in aorta from diabetic rats

As shown in Fig. 1C, NO levels decreased significantly ($P < 0.05$) in un-treated diabetic group compared to the non-diabetic control group. The NO production in diabetic rats was significantly ($P < 0.05$) increased by the PSG-1 treatment. The level of eNOS protein in the aorta of diabetic rats was significantly ($P < 0.05$) higher than that in non-diabetic control group. Interestingly, PSG-1 treatment evoked a significant ($P < 0.05$) increase of eNOS protein level in the aorta of diabetic rats (Fig. 1D).

3.4. PSG-1 improved diabetes-induced impairment in integrity of endothelium

As shown in Fig. 2, the integrity of vascular endothelium was impaired and intimal hyperplasia was found in the aortic tissue of diabetic rats. Interestingly, treatment with PSG-1 markedly improved the diabetes-induced impairment in integrity of endothelium and intimal hyperplasia.

3.5. Effect of PSG-1 on apoptosis of aortic endothelial cells

TUNEL-positive cells were observed in the endothelium of the aorta (Fig. 3). Cell apoptosis in un-treated diabetic rats increased

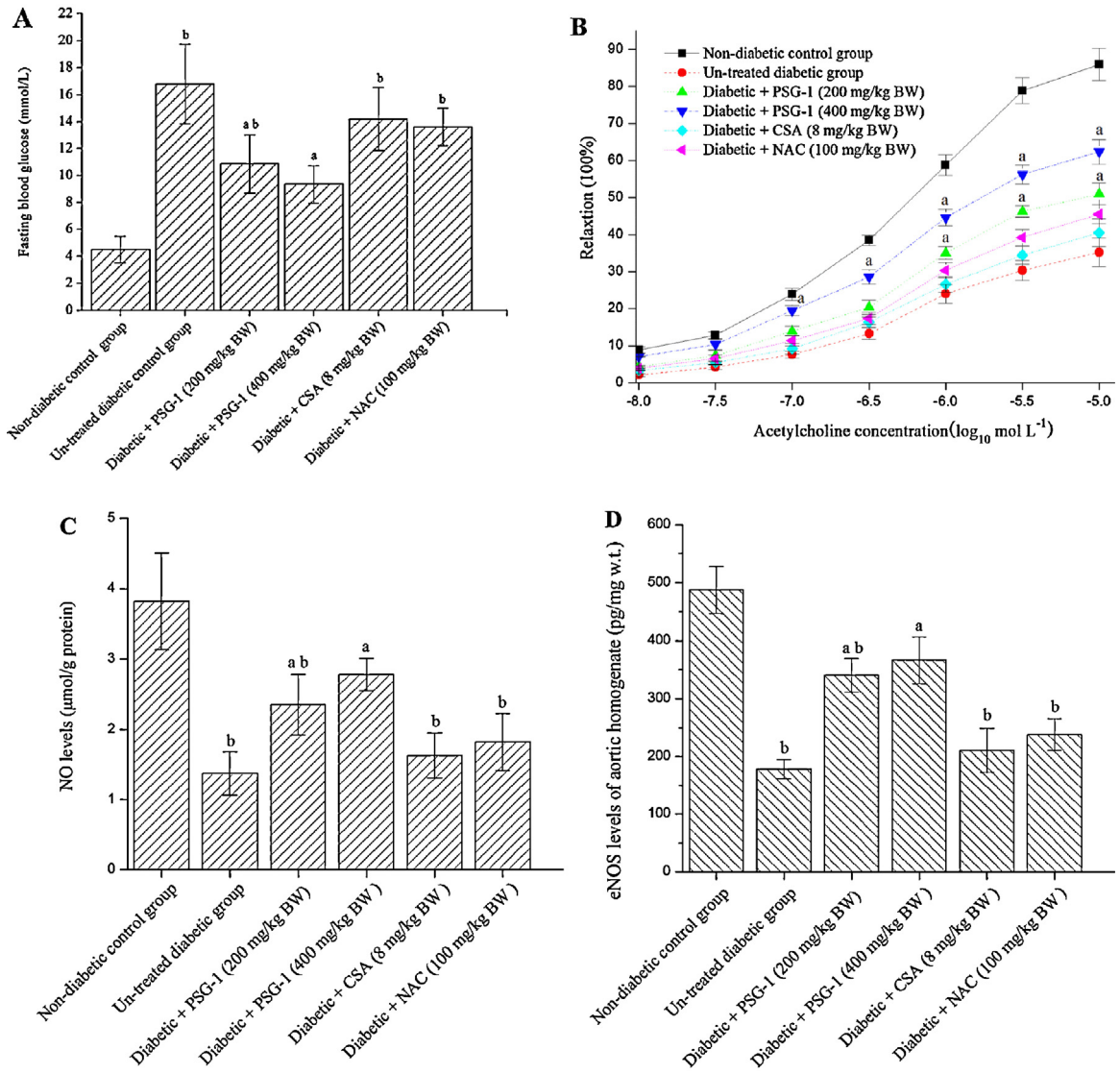

Fig. 1. (A) Effect of PSG-1 on FBG levels in type 2 diabetic rats. (B) Endothelium-dependent relaxations induced by Ach in isolated aorta rings. (C) Effect of PSG-1 on NO levels in aorta from type 2 diabetic rats and (D) effect of PSG-1 on eNOS levels in aorta from type 2 diabetic rats. Values are expressed as means ± SEM ($n = 10$). [a] $P < 0.05$ compared with un-treated diabetic group. [b] $P < 0.05$ compared with non-diabetic control group.

as shown by the higher incidence of TUNEL-positive cells in the endothelium compared with non-diabetic rats. However, the aortic endothelial cells of diabetic rats treated by PSG-1 at either 200 or 400 mg/kg BW exhibited a dramatic decrease in TUNEL-positive cells.

3.6. Effect of PSG-1 on expression of apoptosis related proteins

The expression levels of pro-apoptosis protein Bax in diabetic rats were significantly higher than that in non-diabetic controls (Supplemental Fig. 1A and Table 2). Interestingly, the MOD values of Bax in PSG-1 treated groups were significantly ($P < 0.05$) lower than un-treated diabetic group.

As shown in Supplemental Fig. 1B and Table 2, expression of Bcl-2 was weaker in diabetic rats than that in non-diabetic controls. The granules of yellowish-brown in the cytoplasm increased, and the color was obviously deeper in PSG-1 treated groups. The MOD values of Bcl-2 were also significantly ($P < 0.05$) lower in PSG-1 treated diabetic rats than un-treated diabetics.

The expression levels of PI3K p85 and p-Akt were markedly decreased in the aorta of diabetic rats as compared with the non-diabetic controls (Fig. 4A). Interestingly, PSG-1 treatment was found to increase the expression of PI3K p85 and p-Akt in the aorta of diabetic rats.

3.7. Effect of PSG-1 on mRNA expression of Bax, Bcl-2 and caspase-3

RT-PCR analysis (Fig. 4B) showed that the levels of Bax and caspase-3 mRNA expression were increased, while Bcl-2 mRNA expression level was decreased in diabetic rats compared to the un-treated diabetics. Interestingly, PSG-1 treatment down-regulated the mRNA expression of Bax and caspase-3, and up-regulated mRNA expression of Bcl-2.

4. Discussion

Diabetes substantially impairs the vasodilating properties of the endothelium and leads to endothelial dysfunction (Anderson, 2003). The aorta in diabetic rats was characterized by aortic histologic changes commonly seen in human diabetic arteriosclerosis, including vascular smooth muscle cell proliferation, aortic lumens

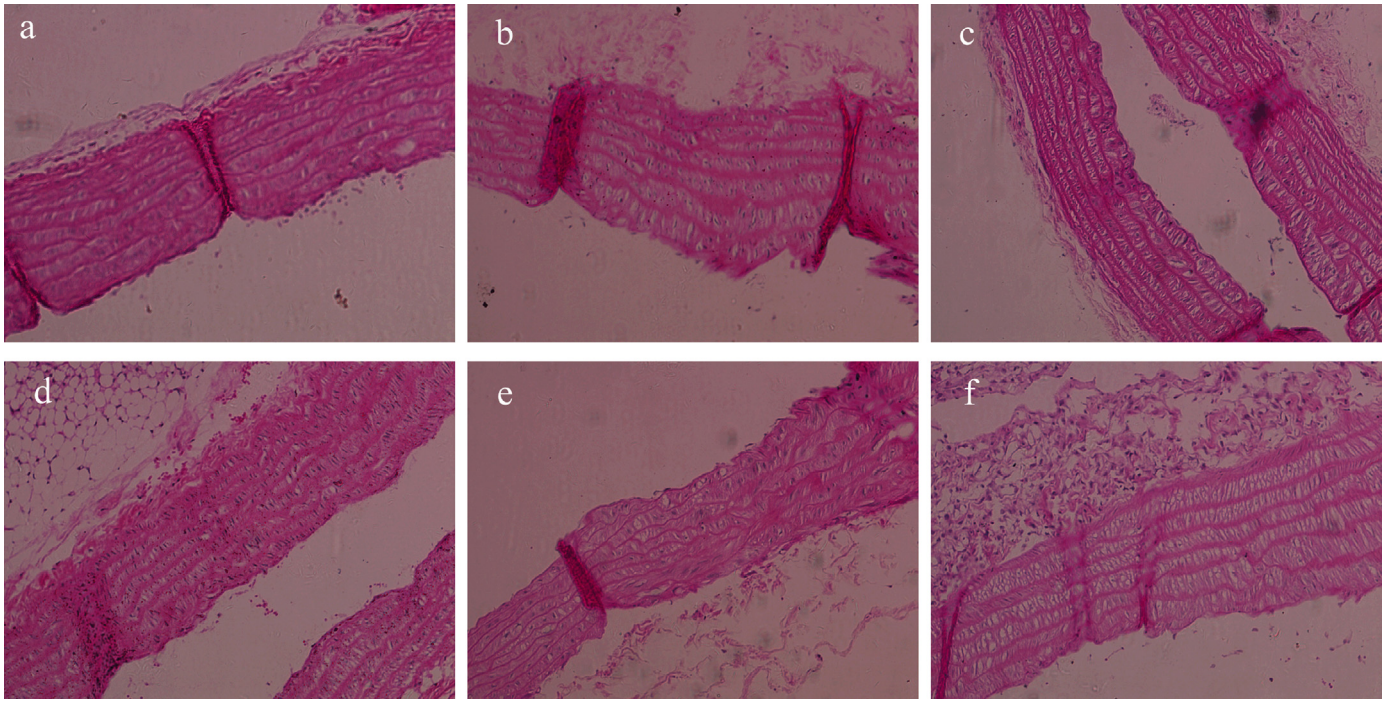

Fig. 2. Photomicrographs of the aorta. (a) Non-diabetic control group, (b) un-treated diabetic group, (c) diabetic + PSG-1 (200 mg/kg BW), (d) diabetic + PSG-1 (400 mg/kg BW), (e) diabetic + CSA (8 mg/kg BW), and (f) diabetic + NAC (100 mg/kg BW), $n = 10$ in all groups. Original magnification 200×.

stenosis and aortic remodeling (Li et al., 2010). Many studies have demonstrated that the endothelium-dependent relaxation in response to Ach was impaired in the aorta of diabetic rats (Wang, Yang, Tian, Yang, & Du, 2009). As expected, diabetes caused tissue damage in the aorta and impaired its relaxation ability. PSG-1 treatment was found to alleviate this functional impairment, possibly by providing protection for aortic tissues under hyperglycemia (Fig. 1B). Our previous study has also found that treatment with PSG-1 produced a significant reduction in serum triglyceride, total cholesterol, low-density lipoprotein cholesterol and free fatty acid levels, and an increase in serum high-density lipoprotein cholesterol in type 2 diabetic rats (Zhu et al., 2013). These results have demonstrated that PSG-1 may exert beneficial effects on diabetic cardiovascular diseases.

Endothelial cells play a major role in maintaining cardiovascular homeostasis in health (Avogaro, Fadini, Gallo, Pagnin, & de

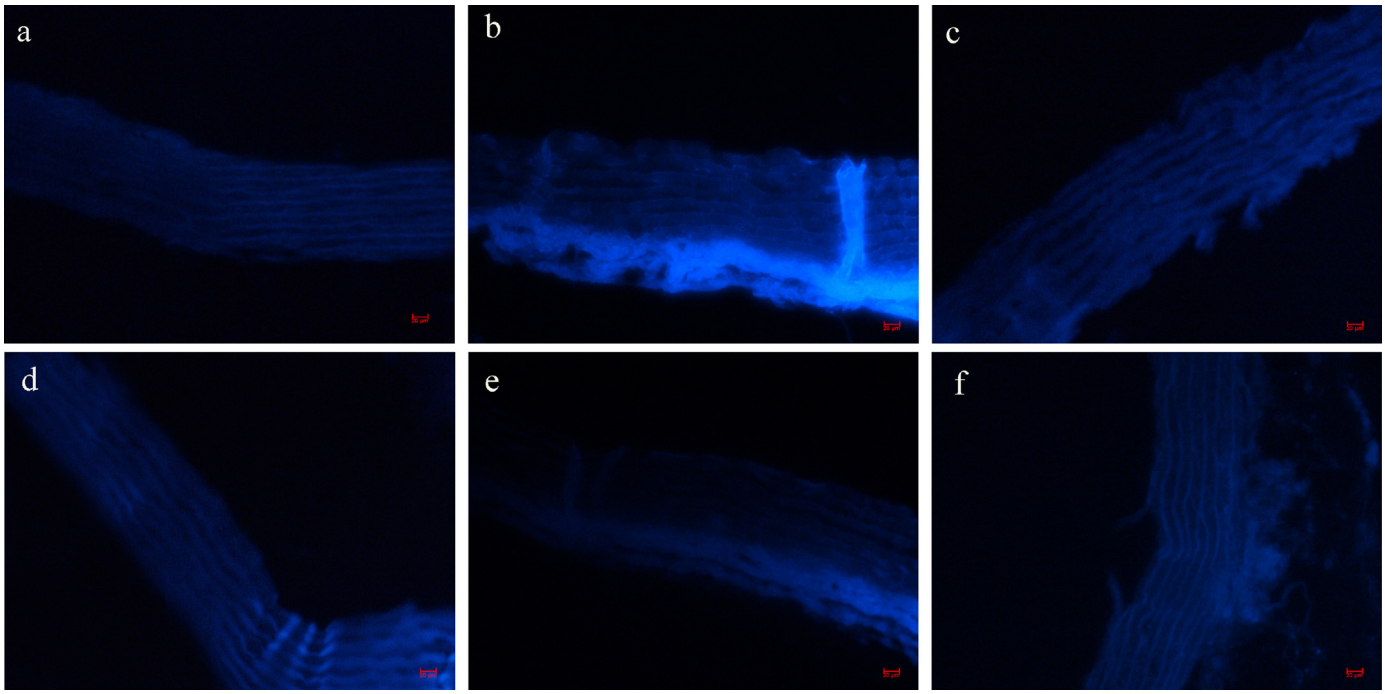

Fig. 3. Effect of PSG-1 on apoptosis of aortic endothelial cells. (a) Non-diabetic control group, (b) un-treated diabetic group, (c) diabetic + PSG-1 (200 mg/kg BW), (d) diabetic + PSG-1 (400 mg/kg BW), (e) diabetic + CSA (8 mg/kg BW), and (f) diabetic + NAC (100 mg/kg BW). $n = 10$ in all groups. Original magnification 200×.

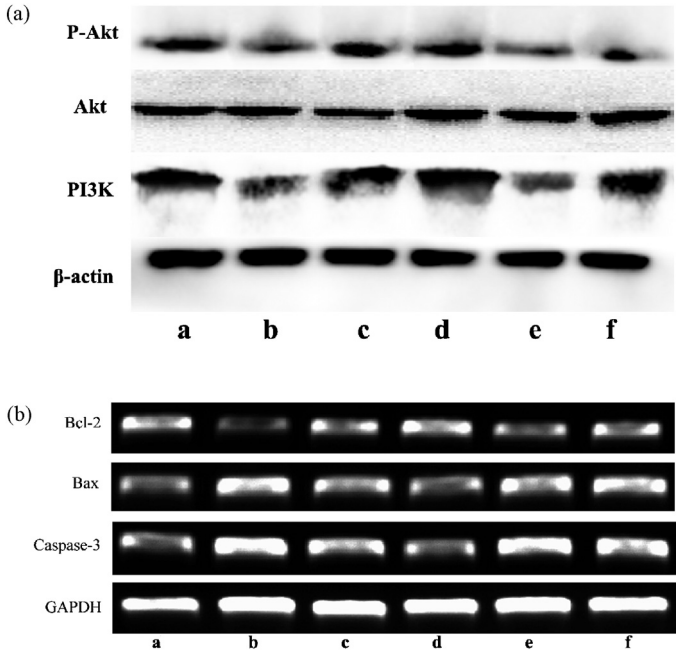

Fig. 4. Effect of PSG-1 on expression of PI3K, p-Akt, Bcl-2, Bax and caspase-3. (A) Western blotting analysis of p-Akt, Akt and PI3K p85 protein expression and (B) RT-PCR analysis of mRNA expression of Bcl-2, Bax and caspase-3. (a) Non-diabetic control group, (b) un-treated diabetic group, (c) diabetic + PSG-1 (200 mg/kg BW), (d) diabetic + PSG-1 (400 mg/kg BW), (e) diabetic + CSA (8 mg/kg BW), and (f) diabetic + NAC (100 mg/kg BW). β-Actin and GAPDH were used as internal controls.

Kreutzenberg, 2006). NO is a major contributor to endothelium-dependent relaxation in conduit arteries, which is produced in the endothelium by eNOS (Laroia, Ganti, Laroia, & Tendulkar, 2003). NO produced by endothelial cells shows a potent vasodilator effect (Oh et al., 2011), and acts as an important endothelium-dependent relaxing factor which mediates vascular relaxation in response to Ach (Keaney Jr et al., 1993). Similarly, some studies have shown that levels of eNOS protein and mRNA expression in human aortic endothelial cells, as well as NO levels were reduced in the aorta of diabetic mice (Srinivasan et al., 2004). Our findings are consistent with previous results showing that the impaired aortic relaxation seen in diabetic rats was due to reduced level of eNOS and a decrease in NO production in endothelial cells. Moreover, our results have clearly demonstrated that PSG-1 exerted beneficial effects on NO release and attenuated the reduced eNOS levels in the aorta of diabetic rats (Fig. 1C and D).

Endothelial cell survival is critical in the maintenance of endothelial function (Huang et al., 2012). Endothelial dysfunction plays a key role in the pathogenesis of atherosclerosis and the related vasculopathy. Excessive apoptosis of endothelial cells induced by hyperglycemia may be one of the reasons for loss of endothelial integrity and dysfunction of vascular endothelium. In the present study, our data have also demonstrated that diabetes contributed to the apoptosis of endothelial cells (Fig. 3). Members of the Bcl-2 family proteins are critical regulators of the apoptotic pathway (Song, Ouyang, & Bao, 2005), the ratio of Bax to Bcl-2 plays an important role in determining whether cells undergo survival or apoptosis. In order to probe the probable mechanism of PSG-1 against diabetes-induced apoptosis, we further examined expression levels of apoptosis related proteins, Bax and Bcl-2. PSG-1 was found to activate the protein expression of Bcl-2 and inhibit the expression of Bax, and significantly decreased the ratio of Bax to Bcl-2.

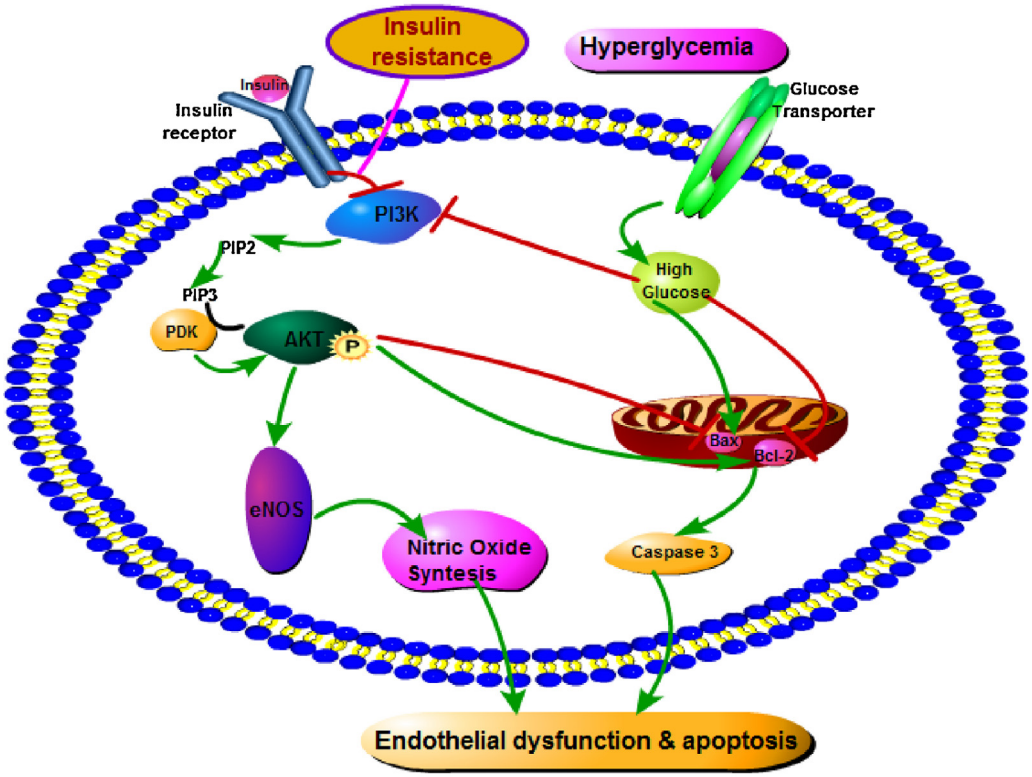

Fig. 5. Proposed effect of PSG-1 on dysfunction and apoptosis of endothelial cells. Hyperglycemia and insulin resistance result in dysfunction and apoptosis of the endothelium via inhibition of PI3K/Akt pathway in diabetes, sequentially reducing levels of eNOS and NO. High glucose induced mitochondria apoptosis through up-regulating the ratio of Bax to Bcl-2 and activating caspase-3 expression. Our results indicate that PSG-1 (or the metabolites of PSG-1) might protect endothelial dysfunction and apoptosis by activating the PI3K/Akt/eNOS pathway and by decreasing the ratio of Bax to Bcl-2.

Table 2
MOD of immunostaining of Bax and Bcl-2 in aorta from type 2 diabetic rats.

Groups	Bax	Bcl-2	Bax/Bcl-2
Non-diabetic control	0.097 ± 0.004	0.067 ± 0.005	1.584 ± 0.376
Un-treated diabetic	0.156 ± 0.035[b]	0.051 ± 0.016[b]	3.035 ± 0.835[b]
Diabetic + PSG-1 (200 mg/kg BW)	0.122 ± 0.027[a,b]	0.060 ± 0.026[a]	2.059 ± 0.628[a]
Diabetic + PSG-1 (400 mg/kg BW)	0.114 ± 0.013[a]	0.065 ± 0.019[a]	1.738 ± 0.416[a]
Diabetic + CSA (8 mg/kg BW)	0.138 ± 0.032[b]	0.055 ± 0.018[b]	2.513 ± 0.620[b]
Diabetic + NAC (100 mg/kg BW)	0.135 ± 0.029[b]	0.059 ± 0.024[b]	2.275 ± 0.655[b]

Values are expressed as means ± SEM ($n = 10$).
[a] $P < 0.05$ compared with un-treated diabetic group.
[b] $P < 0.05$ compared with non-diabetic control group.

PIK/Akt signaling pathway plays a crucial role in endothelial dysfunction and cell apoptosis caused by hyperglycemia (Song et al., 2007). It has been reported that Akt can activate eNOS and increase NO production (Liu, Ma, & Sun, 2012), and PI3K/Akt pathway can modulate Bcl-2 family proteins (Chang et al., 2003). Our findings indicated that PSG-1 might protect endothelial cells against apoptosis by activating the PI3K/Akt/eNOS pathway and by decreasing the ratio of Bax to Bcl-2 (Fig. 4).

It is generally recognized that polysaccharides cannot be absorbed directly into the bloodstream (H.P. Li et al., 2012). Numerous evidence has demonstrated that, β-glucan, a major bioactive ingredients in polysaccharides isolated from fungi sources may be responsible for hypoglycemic (Sun et al., 2008) and anti-atherogenic effects (Stachowiak & Reguła, 2012). Alternatively, some gut microbes may digest polysaccharides into monosaccharides, short chain fatty acids and other substances that exert beneficial effects in the vasculature. Further investigations are needed to identify what components are responsible for the effects of PSG-1 in aortic relaxation in diabetes.

In conclusion, the present study has demonstrated that PSG-1 significantly reduced the FBG levels and improved endothelium-dependent relaxation in the aorta of diabetic rats. The effects of PSG-1 on endothelial dysfunction and apoptosis may be through up-regulating expression of PI3K, p-Akt, eNOS, NO and Bcl-2 levels and down-regulating Bax expression (Fig. 5). Hence, PSG-1 may be a potential therapeutic agent against diabetic arteriosclerosis in humans.

Conflict of interest statement

The authors declare that there is no conflict of interest.

Acknowledgement

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2013.12.080.

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