African Journal of Pharmacy and Pharmacology 6(44), pp. 3067-3074, 29 November, 2012
Available online at http://www.academicjournals.org/AJPP
DOI: 10.5897/AJPP12.853
ISSN 1996-0816 © 2012 Academic Journals

Full Length Research Paper

2,3,4',5-Tetrahydroxystilbene 2-O-β-D-glucoside prevents vascular endothelial dysfunction in a type 2 diabetic rat model

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Accepted 1 October, 2012

The aim of this study was to investigate the effect of 2,3,4',5-tetrahydroxystilbene 2-O-β-D-glucoside (TSG) on vascular endothelial dysfunction in the type 2 diabetes rats, and the possible mechanisms. Sprague-Dawley rats were treated by high-fat diet and intraperitoneal injection of streptozotocin (40 mg/kg) to induce type 2 diabetes. TSG (60 or 120 mg/kg per day) was administered by oral gavage for 6 weeks. The vascular endothelial dysfunction was assessed using isolated aortic ring preparation. Fasting serum insulin (FINS), fasting blood glucose (FBG), malondialdehyde (MDA), superoxide dismutase (SOD), tumor necrosis factor-alpha (TNF-α) and free fatty acids (FFA) in serum were measured. The homeostasis model assessment-insulin resistance (HOMA-IR) was also calculated. The mRNA and protein expression of endothelial nitric oxide synthase (eNOS) and endothelin-1 (ET-1) in the aorta were tested by reverse transcriptase-polymerase chain reaction (RT-PCR) and Western Blot. The results showed that TSG significantly decreased the levels of FBG, HOMA-IR, MDA, TNF-α and FFA and significantly increased the level of SOD. TSG also improved acetylcholine-induced endothelium-dependent relaxation. The expression of eNOS was significantly increased, whereas the expression of ET-1 was significantly decreased in aortas of diabetic rats with TSG treatment. These results demonstrate that TSG prevents vascular endothelial dysfunction in type 2 diabetes rats, and the mechanism may be related to its ability of anti-oxidation, anti-inflammation, improving insulin resistance, up-regulation of eNOS and down-regulation of ET-1.

Key words: 2,3,4',5-Tetrahydroxystilbene2-O-β-D-glucoside, type 2 diabetes, endothelial dysfunction, endothelial nitric oxide synthase, endothelin-1.

INTRODUCTION

Type 2 diabetes is characterized by impaired endothelium-dependent relaxation (EDR), which contributes to the high prevalence of vascular disease in such patients. EDR injury may be intrinsic to the insulin resistance (IR) that commonly precedes type 2 diabetes (Tooke and Hannemann et al., 2000). Insulin plays an important physiological role in keeping the vascular homeostasis under healthy conditions. The balance between nitric oxide (NO)-dependent vasodilator actions and ET-1-dependent vasoconstrictor actions of insulin is regulated by PI3K- and MAPK-dependent signaling in vascular endothelium, respectively (Yang and Li, 2008; Gogg et al., 2009). Under insulin resistance conditions, the balance is broken and the endothelial dysfunction emerges. So, the protection of endothelial function has become the key of treatment type 2 diabetes and the vascular complication of the diabetes.

2,3,4',5-Tetrahydroxystilbene-2-O-β-D-glucoside (TSG) is the water-soluble active component extracted from

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dried tuber root of *Polygonum multiflorum*, which is a traditional Chinese medicinal herb. Our previous results showed that TSG reduced blood lipid content, inhibited oxidative stress and the inflammation process in atherosclerotic rats (Liu et al., 2007; Zhang et al., 2008). The study showed that TSG prevented diabetic nephropathy through the alleviation of oxidative stress injury and down-regulation expression of COX-2 and TGF-β1 partially via activation of SIRT1 in diabetic rats (Li et al., 2010). Lv et al. (2010) also reported that TSG could efficiently inhibit the formation of advanced glycation end products (AGEs) that result in the endothelial dysfunction in a dose-dependent manner. Moreover, our previous study also suggested that TSG improved EDR, which was associated with the prevention of changes in endothelial nitric oxide synthase (eNOS) and induced nitric oxide synthase (iNOS) expression and consequent increase in nitrogen monoxidum (NO) production in atherogenic-diet rats (Zhang et al., 2009).

In this study, we investigated whether TSG could prevent vascular endothelial dysfunction in type 2 diabetic rats induced by high-fat diet and streptozotocin (STZ) and explored the possible mechanism(s).

MATERIALS AND METHODS

Drugs and chemicals

2,3,4',5-Tetrahydroxystilbene2-O-β-D-glucoside (TSG) was purchased from Sikehua Biotechnology Co. Ltd. (purity>98%) (Chengdu, Sichuan, China). Simvastatin was purchased from Hangzhou MSD Pharmaceutical Co. Ltd. (purity>98%) (Hangzhou, Zhejiang, China). The phenylephrine, acetylcholine and streptozotocin (STZ) were purchased from Sigma Co. Ltd. The fasting blood glucose (FBG) was tested by one touch ultra mini blood glucose monitoring system. The fasting serum insulin (FINS) was determined by radioimmunoassay kits. The kits for the assay of superoxide dismutase (SOD), malondialdehyde (MDA), and free fatty acids (FFA) were purchased from Nanjing Jiangcheng Bioengineering Institute (Nanjing, Jiangsu, China). The kit for testing of tumor necrosis factor-alpha (TNF-α) was purchased from Mai Bio Co. Ltd (Shanghai, Shanghai, China). Anti-eNOS and anti-ET-1 antibodies were purchased from Santa Cruz Co. Ltd (Santa Cruz, CA, USA) Trizol reagent and primer sets were purchased from Invitrogen Co. Ltd. (Carlsbad, CA, USA) Reverse-transcript synthesis kit was purchased from TaKaRa Co. Ltd. (Katsushika-Ku, Tokyo, Japan).

Animal treatment

Six-week-old Sprague-Dawley (SD) rats weighing 160 - 180 g, were obtained from the Animal Center of Nantong University. All animal experiments were conducted according to the established protocols governing animal handling and use at Nantong University. The rats were randomly divided into five groups of ten animals each. The rats in the control group were fed with regular diet and those in other groups were fed with a high-fat diet (consisting of 22% fat, 48% carbohydrate, and 20% protein with total caloric value of 44.3 kJ/kg) for 4 weeks, and after 24 h absolute diet, single intraperitoneal injection of streptozotocin (STZ (40 mg/kg) was made to induce the diabetes. STZ was dissolved in citrate buffer solution. The volume of injected citrate buffer solution was 0.5 ml/100g. Equal menstruum (0.5 ml/100 g) was injected in the control group. On the other hand, rats in the simvastatin group (2 mg/kg per day), low dose TSG (TSG-L) group (60 mg/kg per day) and high dose TSG (TSG-H) group (120 mg/kg per day) were administered by oral gavage. SV and TSG were dissolved in triply distilled water. The volume of orally administered drugs was 1 ml/100 g. Equal distilled water (1 ml/100 g) was administered by oral gavage in the control group. The doses of TSG and simvastatin were corrected once a week according to body weight.

After 6 weeks, rats were anesthetized with sodium pentobarbital (40 mg/kg, i.p.). Subsequently, the blood was collected by carotid puncture and the aorta was removed. The fasting serum insulin (FINS) and fasting blood glucose (FBG) were measured. Homeostasis model assessment-insulin resistance (HOMA-IR) was calculated (Ble-Castillo et al., 2012) as:

$$\text{HOMA-IR} = (\text{FBG} \times \text{FINS}) / 22.5.$$

Assays for MDA, SOD, TNF-α and FFA in serum

All biomarkers were tested following the instructions on the kits. MDA in serum was determined by the thiobarbituric acid method, while the assay for SOD was done using xanthine oxidase (XO) method. FFA was measured using a copper colorimetric kit. Serum TNF-α was assayed using a sandwich ELISA. A standard curve was generated using a linear curve-fit. The insulin plasma concentration was measured by radioimmunoassay.

Vascular reactivity study

The aorta was removed rapidly, freed of connective tissue, cut into rings about 3 mm in length, and put in an organ bath gassed with 95% O_2/5% CO_2 containing Krebs-Henseleit solution. The strip was stretched to a resting tension of 2 g and then contracted with phenylephrine (PE, 10^{-6} mol/L). After a stable contractions was reached (considered 100%), they were exposed to the cumulative dose responses of acetylcholine (10^{-10} to 10^{-4} mol/L), in order to test EDR.

Reverse transcriptase-polymerase chain reaction (RT‑PCR) analysis for eNOS and ET-1

Total RNA was extracted from aorta using Trizol reagent. The cDNA was prepared using reverse-transcript synthesis kit according to the manufacturer's protocol, and the reverse-transcribed cDNA was amplified by PCR under standard conditions with specific primers. The primer of the eNOS and ET-1 were of the following sequences: sense of eNOS, 5'-AACATGTGTCCTTGCTCGAGGCA-3', antisense of eNOS 5'-TTCCGGCGTCCACCTGATCCTAA-3' (340bp), sense of ET-1, 5'-AACCATGGATTATTTGCTCATG-3', antisense of ET-1, 5'-AGTGTTGACCCAAATGATGTC-3' (230bp). As a reference, β-actin primers were: sense of β-actin, 5'-GTGGGCCGCTCTAGGCACCAA-3', antisense of β-actin, 5'-CTCTTTGATGTCACGCACGATTTC-3'(540 bp). The amplification profile involved denaturation at 94°C for 2 min. For detection of ET-1 mRNA expression, 28 thermal cycles were performed (94°C for 30 s, 55°C for 30 s, and 72°C for 2 min), followed by a final 10 min incubation at 72°C to extend incomplete products. For detection of eNOS mRNA expression, 30 thermal cycles were performed (94°C for 30 s, 60°C for 30 s, and 72°C for 60 s), followed by a final incubation of 10 min at 72°C to extend incomplete products. Finally, the PCR products were resolved on a 1.5% agarose gel stained. The density of each band was measured by auto gel analysis system (Labworks) (Shanghai, Shanghai, China).

Table 1. Effect of 2,3,4',5-tetrahydroxystilbene-2-O-beta-D-glucoside (TSG) on the fasting serum insulin (FINS), fasting blood glucose (FBG) and homeostasis model assessment-insulin resistance (HOMA-IR) in each group.

	FINS (mIU/L)	FBG (mmol/L)	HOMA-IR
NC	16.21 ± 2.41	4.4 ± 0.8	4.81 ± 0.17
DM	32.31 ± 3.32$^{\#\#}$	14.6 ± 1.9$^{\#\#}$	13.23 ± 2.61$^{\#\#}$
SV	16.87 ± 3.24**	13.7 ± 2.0	8.43 ± 1.47*
TSG-L	23.63 ± 3.43*	10.1 ± 1.6*	10.26 ± 1.44*
TSG-H	20.56 ± 2.11*	9.2 ± 1.4*	8.56 ± 1.23*

NC: Control group; DM: diabetic group; SV: simvastatin group; TSG-L: low dose TSG group (60 mg/kg per day); TSG-H: high dose TSG group (120 mg/kg per day). Data are the mean ± SEM (n = 10). $^{\#\#}P$<0.01 vs. NC group; *P<0.05, **P<0.01 vs. DM group.

Western blot analysis for eNOS and ET-1

eNOS and ET-1 protein samples were denatured by boiling for 5 min and electrophoretically separated on 10% sodium dodecyl sulphate (SDS)-polyacrylamide gels. It was then electroblotted at 4°C and transferred onto polyvinylidene difluoride membranes. Nonspecific binding was blocked by incubation with 5% non-fat dried milk for 2 h at room temperature. The blots were then probed with primary antibodies to eNOS (1:200), ET-1 (1:200) or GAPDH (1:1000) as an internal control. After washing, the blots were incubated with second antibody (horseradish peroxidase-labeled IgG) for 1 h at room temperature. Protein was then visualized with enhanced chemiluminescence solution and X-ray film. The immunoreactive bands were scanned and analyzed by Labwork's digital scanning densitometer.

Statistical analysis

All data were expressed as mean ± SEM. SPSS 17.0 was used for statistical analysis. Concentration-response data was fitted to a sigmoid plot using GraphPad Prism version 4.0. Relaxations (%) were expressed as the percentage of the contraction induced by 10^{-6} mol/L of phenylephrine. Statistical analyses were performed using one-way analysis of variance (ANOVA) with post hoc test for multiple comparisons. The criterion for significance was P<0.05.

RESULTS

Effect of 2,3,4',5-tetrahydroxystilbene-2-O-beta-D-glucoside (TSG) on the fasting serum insulin (FINS), fasting blood glucose (FBG), homeostasis model assessment-insulin resistance (HOMA-IR) and body weight (BW)

As indicated in Table 1, compared with the control group, FBG (14.6 ± 1.9 mmol/L), FINS (32.31 ± 3.32 mIU/L) and HOMA-IR (13.23 ± 2.61) were significantly increased in the diabetic group (P<0.01 for all). These results indicated that the diabetes model was successfully established. Compared with the diabetes group, FINS (P<0.01) and HOMA-IR (P<0.05) were significantly decreased in SV group. FBG, FINS and HOMA-IR were significantly decreased by treatment with TSG (60 or 120 mg/kg/day) compared with the diabetes group (P<0.05 for all). As shown in Figure 1, compared with the control group, the body weight (BW) of rats was significantly increased in the diabetic group from the third week (P<0.01 for all). BW of rats was significantly decreased by treatment with SV and TSG (60 or 120 mg/kg/day) compared with the diabetes group from the fourth week (P<0.05 for all).

Effect of TSG on the levels of serum malondialdehyde (MDA), super oxide dismutase (SOD), tumor necrosis factor-alpha (TNF-α) and free fatty acids (FFA)

Diabetic rats had higher MDA (P<0.01), TNF-α (P<0.01), FFA (P<0.01) levels and lower SOD (P<0.05) level compared to the control group (Table. 2). The level of MDA, FFA, TNF-α were significantly decreased and the levels of SOD were significantly increased in SV and TSG (60 and 120 mg/kg/day) group compared with the diabetes group (all P<0.05) (Table 2).

Effects of TSG on acetylcholine-induced endothelium-dependent relaxation

In diabetic rats, endothelium-dependent relaxation to acetylcholine in the aorta was markedly impaired compared with control rats (P<0.01). The maximal relaxation was decreased by 43% (P<0.01) in diabetic rats compared with controls. SV, TSG improved endothelium-dependent relaxation (P<0.05). Treatment with TSG (60 and 120 mg/kg/day) significantly increased the maximal relaxation by 18% (P<0.05) and 34% (P<0.01), respectively. However, SV and TSG-H showed no difference on endothelium-dependent relaxation compared with the diabetes group (P>0.05, Figure 2).

Effect of TSG on the expression of endothelial nitric oxide synthase (eNOS) and endothelin-1 (ET-1)

The mRNA and protein expressions OF eNOS and ET-1 were measured by RT-PCR and Western Blot. As indicated in Figures 3 and 5, the level of eNOS mRNA and protein expressions were significantly reduced by 38% (P<0.01) and 42% (P<0.01), respectively; while ET-1 mRNA and protein expressions were increased by 1.6-fold (P <0.01) and 1.4-fold, respectively (P <0.01) in the diabetic group compared to the control group (Figures 4 and 6). SV, TSG-L and TSG-H treatments significantly prevented the decrease in eNOS mRNA and protein expressions (P<0.05) (Figures 3 and 5), increase in ET-1 mRNA and protein expressions level (P<0.01) (Figures 4 and 6). However, there was no significant difference between SV and TSG-H group (P>0.05) (Figures 3 to 6).

DISCUSSION

The present study provided evidence that TSG improved

Table 2. Effect of 2,3,4',5-tetrahydroxystilbene-2-O-beta-D-glucoside (TSG) on the levels of serum malondialdehyde (MDA), super oxide dismutase (SOD), tumor necrosis factor-alfa (TNF-α) and free fatty acids (FFA) in each group.

	MDA (nmol/ml)	SOD (U/ml)	TNF-α (ng/ml)	FFA (mmol/L)
NC	5.41 ± 0.98	126.17 ± 13.01	0.30 ± 0.04	0.29 ± 0.03
DM	16.32 ± 2.81$^{\#\#}$	88.90 ± 11.81$^{\#}$	1.43 ± 0.06$^{\#\#}$	3.50 ± 0.44$^{\#\#}$
SV	9.51 ± 1.20*	117.65 ± 16.10*	0.76 ± 0.04*	1.92 ± 0.31*
TSG-L	13.51 ± 1.91*	109.24 ± 15.07*	1.00 ± 0.04*	2.98 ± 0.35*
TSG-H	9.61 ± 1.43*	116.24 ± 14.21*	0.78 ± 0.02*	1.95 ± 0.29*

NC: Control group; DM: diabetic group; SV: simvastatin group; TSG-L: low dose TSG group (60 mg/kg per day); TSG-H: high dose TSG group (120 mg/kg per day). Data are the mean ± SEM (n=10). $^{\#}P$<0.05, $^{\#\#}P$<0.01 vs. NC group; *P<0.05 vs. DM group.

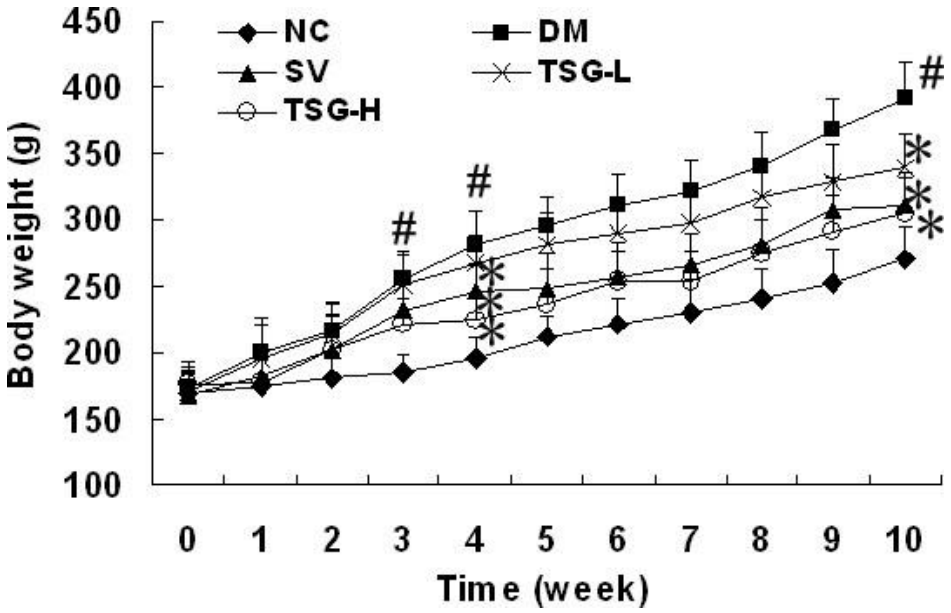

Figure 1. Effects of SV and TSG on the body weight (BW) of rats. Sprague-Dawley rats were treated by high-fat diet for 4 weeks and intraperitoneal injection of streptozotocin (40 mg/kg) to induce type 2 diabetes. TSG (60 or 120 mg/kg per day) was administered by oral gavage for 6 weeks. NC: Control group; DM: diabetic group; SV: simvastatin group; TSG-L: low dose TSG group (60 mg/kg per day); TSG-H: high dose TSG group (120 mg/kg per day). Data are the mean ± SEM (n = 10). $^{\#}P$<0.05 vs. NC group; *P<0.05 vs. DM group.

endothelium-dependent relaxation (EDR) of the aorta and IR, decreased the levels of serum MDA, TNF-α and FFA, and increased the level of serum SOD in diabetic rats. Moreover, our results also showed that TSG significantly up-regulated the eNOS mRNA and protein expressions, and significantly suppressed ET-1 mRNA and protein expressions in aorta of diabetic rats. At present, there are mainly 5 types of diabetes animal model, namely: spontaneous diabetic animal model, transgenic animal models of diabetes mellitus, diabetic animal model induced by streptozotocin or diet and diabetic animal model induced collectively by streptozotocin and diet. Diabetes is a multifactorial disease involving genetic and environmental factors. No one model can completely simulate human type 2 diabetes. Each model at least simulates some aspects of type 2 diabetes. The type 2 diabetes rats model induce by high-fat diet and intra-peritoneal injection of STZ is an ideal diabetes animal model for the researching vascular complications of type 2 diabetes. Hence in our study, the rats' model of type 2 diabetes was established by high-fat diet and STZ. Insulin resistance is mainly a pathophysiological base of type 2 diabetes. A large number of studies have shown that IR is closely related to vascular endothelial dysfunction and the degree of EDR injury is positively correlated with IR (Marchesi et al., 2009; Toblli et al., 2010). Hence, improving insulin resistance and protection endothelial function has become the key of treatment type 2 diabetes.

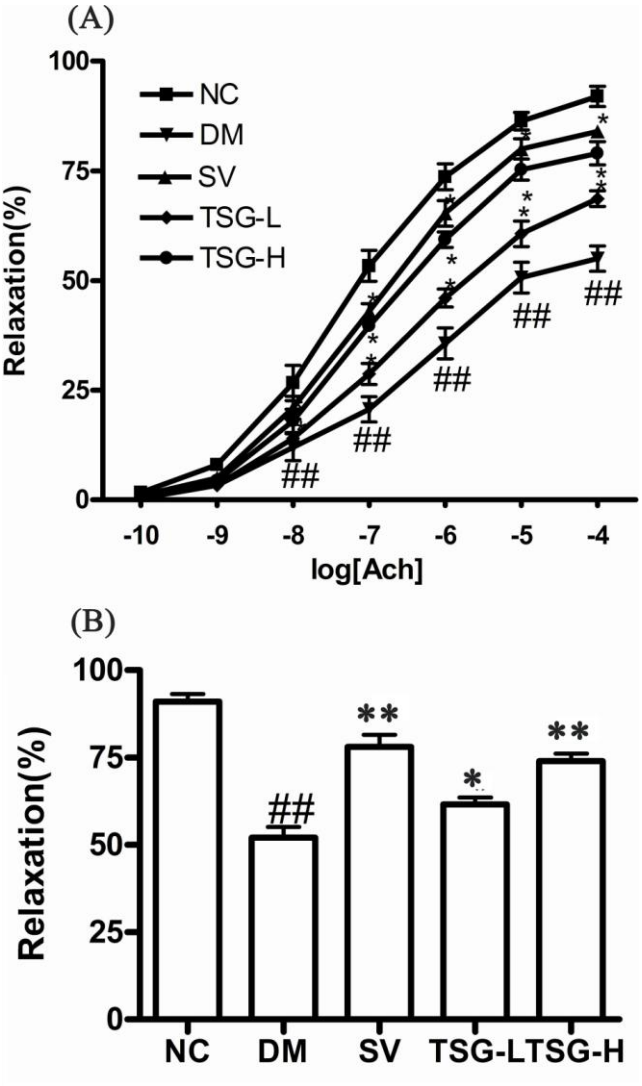

Figure 2. Effects of SV and TSG on acetylcholine-induced endothelium-dependent relaxation (A) and the maximal relaxation (B). NC: Control group; DM: diabetic group; SV: simvastatin group; TSG-L: low dose TSG group (60 mg/kg per day); TSG-H: high dose TSG group (120 mg/kg per day). Data are the mean ± SEM (n=10). $^{\#\#}P<0.01$ vs. NC group; $^{*}P<0.05$, $^{**}P<0.01$ vs. DM group.

HMG-CoA reductase inhibitors simvastatin is a lipid lowering drugs used to decrease cardiovascular events in type 2 diabetes. Some researchers have shown that simvastatin lowered blood lipid, possessed anti-inflammatory and anti-atherosclerosis activity, and protected endothelial diastolic function in the diabetes (Uysal et al., 2009; Heljić et al., 2009). Our results showed that simvastatin and TSG all improved insulin resistance and prevented endothelial dysfunction in the diabetic rats by high-fat diet and STZ. These suggested that TSG could be used to prevent and/or treat the vascular complications of type 2 diabetes just as simvastatin. Meanwhile, the mechanisms with which TSG improved insulin resistance and prevented endothelial dysfunction in the diabetic rats need the further study.

Insulin resistance is featured by pathway-specific impairment in phosphatidylinositol 3-kinase (PI3K)-dependent signaling, which may induce imbalance of secretion NO and endothelin-1 in endothelium (Kim et al., 2006). In mice with targeted deletion of the insulin receptor in vascular endothelium, expressions of both eNOS and ET-1 mRNA levels were reduced by approximately 30~60% in endothelial cells, aorta and heart (Vicent et al., 2003). Moreover, it was reported that *Prunella vulgaris* significantly reduced the expressions of ET-1, and remarkably increased the expression of eNOS in the aorta in db/db mice with type 2 diabetes (Hwang et al., 2012). High insulin level blunts PI3K-dependent effects of insulin such as induction of eNOS expression and production of NO, and insulin signaling through Ras/MAPK pathways is substantially enhanced under the condition of high insulin level, which ultimately induces the impairment of vasorelaxation in response to acetylcholine, an endothelium-dependent vasodilator. This was also confirmed by our study. The results showed that EDR effect was blunted in the aortic rings of diabetic rats compared with the aortic rings of normal rats and TSG improved relaxation response to acetylcholine of isolated aortic rings in dose-dependent manner in diabetic conditions. Furthermore, our results showed that TSG significantly up-regulated the eNOS mRNA and protein expressions and significantly down-regulated ET-1 mRNA and protein expressions of the aorta in diabetic rats. So we guessed that TSG improved EDR, which was associated with changes in eNOS and ET-1 in aorta induced by TSG.

MDA level is used as an index of oxidative status. MDA is a product derived from the lipoperoxidative processes that takes place as a consequence of the oxidative insult. On the other hand, SOD is an enzymatic scavenger that can convert superoxide anion to hydrogen peroxide. Thus, it exerts defensive effects against oxidative damage. Many experiments have shown that MDA level was increased and SOD level was decreased during oxidative stress in diabetic rats (Atawodi, 2005; Rajalakshmi et al., 2009; Meng and Cui, 2008; Likidlilid et al., 2010; Farzadi1 et al., 2011). Our results suggested that TSG could markedly decrease MDA level and increase SOD activity in dose-dependent manner in diabetic rats. Therefore, these results elucidated that TSG inhibited the oxidative stress and reduced free radicals in diabetic rats. We deduced that the protective effect of TSG was associated with scavenging oxygen free radical induced by TSG in the diabetic rats.

Previous studies have also showed that TSG possessed an anti-inflammatory effect (Zeng et al., 2011). Increased level of TNF-α is one of the textures of chronic inflammation. TNF-α activates the serine kinases, including IKKβ, which is a pathway as a target for insulin

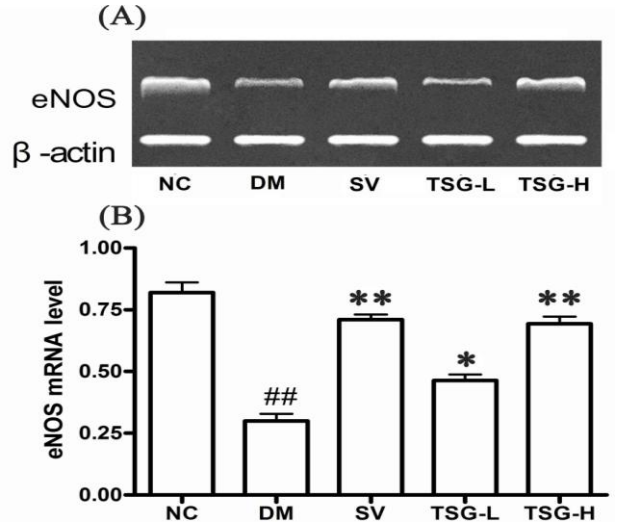

Figure 3. Effect of 2,3,4',5-tetrahydroxystilbene 2-O-β-D-glucoside (TSG) on the mRNA expression of endothelial nitric oxide synthase (eNOS). NC: Control group; DM: diabetic group; SV: simvastatin group; TSG-L: low dose TSG group (60 mg/kg per day); TSG-H: high dose TSG group (120 mg/kg per day). (A) RT-PCR for eNOS and β-actin in samples obtained from NC, DM, SV, TSG-L and TSG-H group. (B) Summarized data for the effect of TSG on eNOS mRNA level (normalized by β-actin). Data are the mean ± SEM (n=10). $^{\#\#}P$<0.01 vs. NC group; *P<0.05, **P<0.01 vs. DM group.

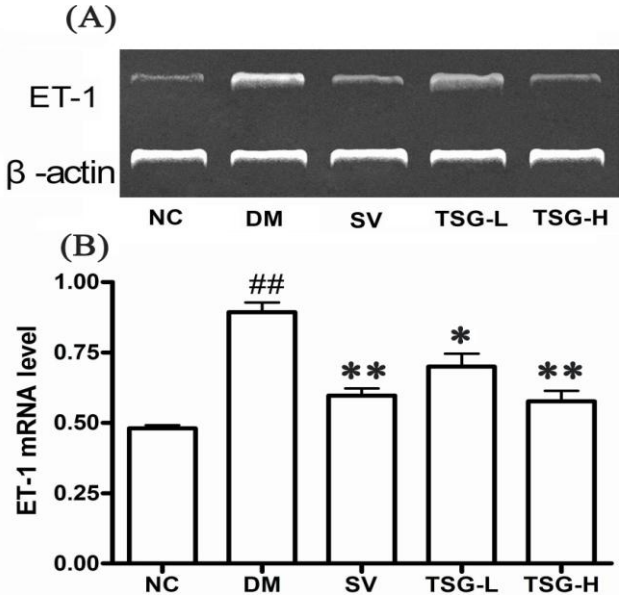

Figure 4. Effect of 2,3,4',5-tetrahydroxystilbene 2-O-β-D-glucoside (TSG) on the mRNA expression of endothelin-1 (ET-1). NC: Control group; DM: diabetic group; SV: simvastatin group; TSG-L: low dose TSG group (60 mg/kg per day); TSG-H: high dose TSG group (120 mg/kg per day). (A). RT-PCR for ET-1 and β-actin in samples obtained from NC, DM, SV, TSG-L and TSG-H group. (B) Summarized data for the effect of TSG on ET-1 mRNA level (normalized by β-actin). Data are the mean ± SEM (n=10). $^{\#\#}P$<0.01 vs. NC group; *P<0.05, **P<0.01 vs. DM group.

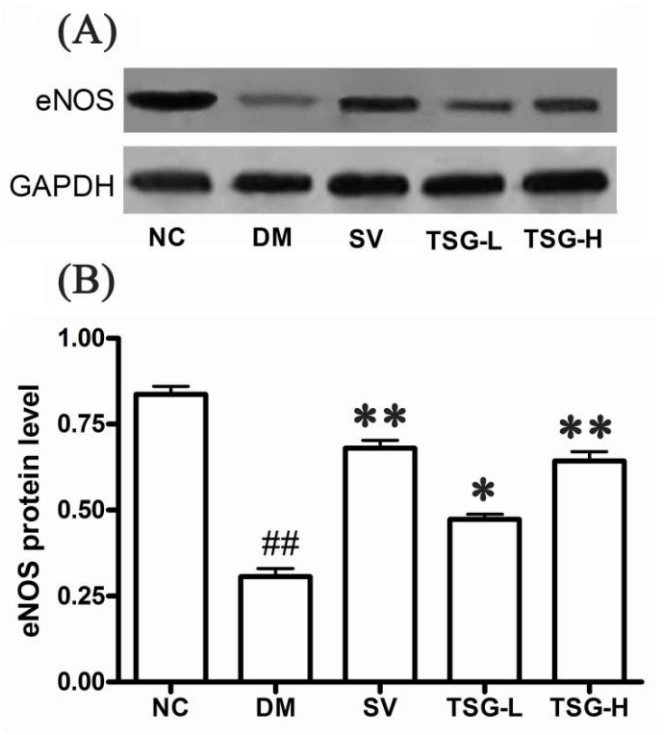

Figure 5. Effect of 2,3,4',5-tetrahydroxystilbene 2-O-β-D-glucoside (TSG) on the protein expression of endothelial nitric oxide synthase (eNOS). NC: Control group; DM: diabetic group; SV: simvastatin group; TSG-L: low dose TSG group (60 mg/kg per day); TSG-H: high dose TSG group (120 mg/kg per day). (A) Representative immunoblots for eNOS and GAPDH in samples obtained from NC, DM, SV, TSG-L and TSG-H group. (B) Summarized data for the effect of TSG on eNOS protein levels (normalized by GAPDH). Data are the mean ± SEM (n=10). $^{\#\#}P$<0.01 vs. NC group; *P<0.05, **P<0.01 vs. DM group.

sensitization (Yuan et al., 2001). TNF-α also increases mitochondrial reactive oxygen species (ROS), which in turn activates apoptosis signal-regulating, increases serine and tyrosine phosphorylation of insulin receptor substrate-1 (IRS-1) and inhibits the activity of PI3K in response to insulin stimulation (Nishikawa and Akawi, 2007). In the present study, our results have shown that TSG reduced serum levels of TNF-α in a dose-dependent manner. This suggested that the anti-inflammatory action of TSG might contribute to its effect of improving EDR and insulin resistance in the diabetic rats. Free fatty acids (FFAs), which are increased in metabolic syndrome, inhibits insulin signaling and induces insulin resistance (Brownlee, 2005; Wang et al., 2006). Moreover, FFAs increases ROS production in the vasculature. ROS in turn activates PKC-α, PKC-ß, and PKC-δ, decreases expression of eNOS, increases expression of ET-1, and activates the NF-κB which is the core of proinflammatory gene expression (Brownlee, 2005). Our data showed that TSG (60 or 120 mg/kg) inhibited the level of FFAs in a dose-dependent manner in diabetic rats. So, TSG inhibited lipotoxicity in the diabetic rats, which might be

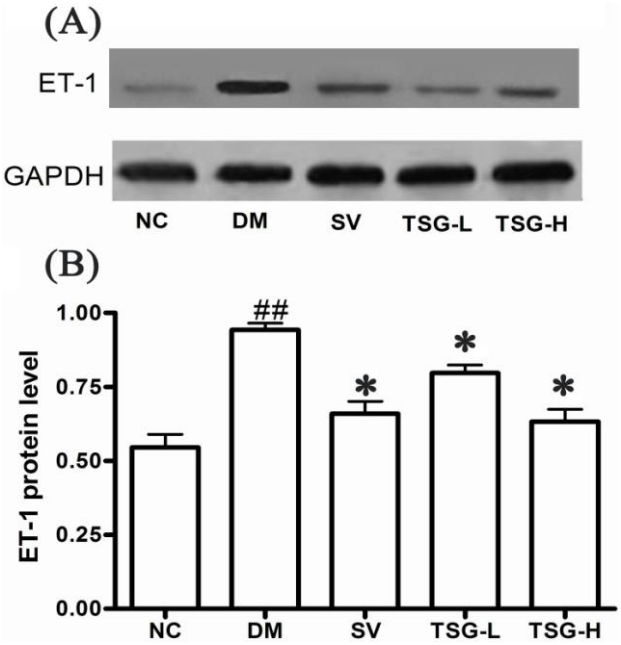

Figure 6. Effect of 2,3,4',5-tetrahydroxystilbene 2-O-β-D-glucoside (TSG) on the protein expression of endothelin-1 (ET-1). NC: Control group; DM: diabetic group; SV: simvastatin group; TSG-L: low dose TSG group (60 mg/kg per day); TSG-H: high dose TSG group (120 mg/kg per day). (A). Representative immunoblots for ET-1 and GAPDH in samples obtained from NC, DM, SV, TSG-L and TSG-H group. (B). Summarized data for the effect of TSG on ET-1 protein levels (normalized by GAPDH). Data are the mean ± SEM (n=10). $^{\#\#}P<0.01$ vs. NC group; $^{*}P<0.05$ vs. DM group.

involved in improving EDR and insulin resistance induced by TSG.

In conclusion, our results demonstrated that TSG prevents vascular endothelial dysfunction in the type 2 diabetes rats, and the mechanism may be related to its ability of anti-oxidation, anti-inflammation, anti-lipotoxicity, improving insulin resistance, up-regulation of eNOS expression and down-regulation of ET-1.

ACKNOWLEDGEMENTS

This work was supported by the Natural Science Foundation of Jiangsu Province (no. BK2009162) and a project funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions.

Abbreviations: TSG, 2,3,4',5-Tetrahydroxystilbene 2-O-β-D-glucoside; **STZ,** streptozotocin; **EDR,** endothelium-dependent relaxation; **eNOS,** endothelial nitric oxide synthase; **ET-1,** endothelin-1; **FINS,** fasting serum insulin; **FBG,** fasting blood glucose; **MDA,** malondialdehyde; **SOD,** superoxide dismutase; **TNF-α,** tumor necrosis factor-alpha; **FFA,** free fatty acids; **SV,** simvastatin; **HOMA-IR,** homeostasis model assessment-insulin resistance; **RT-PCR,** reverse transcriptase-polymerase chain reaction.

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