

Effects of grape seed proanthocyanidin extract on renal injury in type 2 diabetic rats

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Abstract. Grape seed proanthocyanidin extract (GSPE) is known to be an effective natural polyphenol capable of removing free radicals *in vivo*. It has been reported that GSPE has biological functions including antioxidant, anti-cancer, anti-hyperglycemic, anti-radiation, and prevention and treatment of cardiovascular diseases. This study aims to investigate the effects of GSPE on renal injury in type 2 diabetic rats induced with low-dose streptozotocin and a high-carbohydrate/high-fat diet. Rats (n=12 per group) were administered GSPE at either a low (125 mg/kg·bw), medium (250 mg/kg·bw) or high (500 mg/kg·bw) dose, while control rats and diabetes mellitus group rats received no specific treatment. After 16 weeks, GSPE slightly increased body weight and decreased food consumption, water intake and urine volume in rats. Diabetic rats treated with GSPE demonstrated decreased fasting blood glucose, serum insulin, HbA1c and systolic blood pressure ($P<0.05$). GSPE significantly improved renal function parameters, reduced the expression of tissue inhibitor of metalloproteinase-1 and also increased the activity of matrix metalloproteinase-9. Moreover, GSPE (particularly at a dose of 500 mg/kg·bw) increased the activity of antioxidant enzymes and reduced the levels of c-reactive proteins ($P<0.01$) in serum and the expression of tumor necrosis factor- α , monocyte chemoattractant protein-1 and intercellular adhesion molecule-1 ($P<0.05$) in the kidney.

These findings suggest that GSPE ameliorates renal injury in type 2 diabetic rats through its antioxidative activity and anti-inflammatory effects.

Introduction

The incidence of diabetes mellitus (DM) is increasing worldwide relentlessly and rapidly. Type 2 diabetes mellitus (T2DM) accounts for 90-95% of all cases. It was estimated that 92.4 million adults in China (20 years and older) suffered from diabetes in 2010 while a further 148 million people were in the pre-diabetic state (1). Diabetic nephropathy (DN) is the primary microvascular complication of DM, and is also a major cause of chronic renal failure. It is estimated that approximately 50% of DM patients will suffer complications with DN within ten years of diagnosis, and the proportion of cases of DN in end-stage renal failure (ESRF) has been increasing every year (2). In the United States, DN is the leading cause of ESRF (3). In addition, the speed of development to ESRF in patients with DN is approximately 14 times as fast as that of normal kidney diseases (4).

The pathogenesis of DN is complex and has not yet been fully elucidated. Hyperglycemia has been proven to be the major driving force of the progression of DN (5). A previous study has indicated that oxidative stress is one of the mechanisms by which hyperglycemia causes DN (6). Oxidative stress refers to the imbalance between the production of reactive oxygen species (ROS) and the ability of endogenous antioxidative systems to scavenge these ROS (7). It is a systemic process which may occur in any tissue. Under normal conditions, ROS produced in the cellular metabolism is efficiently scavenged by the intrinsic antioxidant defense mechanisms. However, in the hyperglycemic state of DM, excessive ROS are produced in renal tissue due to the damaged scavenging ability. Excessive ROS leads to renal histological changes and functional abnormalities of oxidizing lipids, proteins and nucleic acids. A previous study has revealed that oxidative stress plays a critical role in the development and progression of DN (8). Interventions could ameliorate DN through the reduction of oxidative stress and the increase of renal antioxidant enzyme activity (9).

Inflammation is a further significant risk factor of DN, and has been scrutinized in recent years. Currently, anti-inflammatory drugs are used in the management of DN. Studies have reported that kidney inflammation is crucial

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Abbreviations: FBG, fasting blood glucose; hs-CRP, high-sensitivity c-reactive protein; SCr, serum creatinine; CCr, endogenous creatinine clearance rate; BUN, blood urea nitrogen; SOD, superoxide dismutase; CAT, catalase; MDA, malondialdehyde; TNF- α , tumor necrosis factor- α ; TIMP-1, tissue inhibitor of metalloproteinase-1; ICAM-1, intercellular adhesion molecule-1; MCP-1, monocyte chemoattractant protein-1; MMP-9, matrix metalloproteinase-9

Key words: diabetic nephropathy, antioxidative activities, rats, inflammatory, grape seed proanthocyanidin extract

in promoting the development and progression of DN (10). Inflammation may be a key factor which is activated by the metabolic, biochemical and hemodynamic derangements known to exist in the diabetic kidney (11). Hyperglycemia, advanced glycoxidation end products (AGEs) and immune complexes in DM decrease the release of chemokines and the upregulation of cell adhesion molecules (12). These events promote the infiltration of renal monocytes and lymphocytes. One study on drugs which have significant effects on DN has reported that these drugs work through their anti-inflammatory effects (13). However, existing drugs only partially alleviate the inflammatory response, and do not deter the progress of DN. If efficacy and safety are taken into consideration, there are few anti-inflammation drugs that truly function in the treatment of DN patients. Therefore, it is of particular urgency to identify chemicals which are safe and effective to combat DN.

Grape seed proanthocyanidin extract (GSPE) is a potent antioxidant extracted from grape seeds and skins. GSPE is reported to be highly bioavailable and provides significantly greater protection against the damage of oxidative stress than vitamins C and E and β -carotene (14). GSPE may be rapidly absorbed by the gastrointestinal tract and reaches a peak level after 45 min. It has a half-life of 5 h. Fourteen percent of it is evacuated by the biliary system in 11 h while 70% is evacuated in the form of CO₂, urine and feces after 24 h. Another study demonstrated that GSPE mediated a protective effect against doxorubicin-induced cardiac injury through antioxidant, anti-inflammatory and antiapoptotic mechanisms (15). Therefore, the use of GSPE against oxidative stress and inflammation offers the possibility of a protective effect on renal injury induced by DM. Previous studies have demonstrated that GSPE protects against renal injury induced by T1DM (16). However, there is limited information on its effects on renal injury induced by T2DM. This study aims to identify its effects and possible mechanisms.

Materials and methods

Materials and reagents. GSPE (lot no. 1003007-24) was donated by JF-Natural Limited Company (Tianjin, China). The proanthocyanidin content was 95% when analyzed using high-performance liquid chromatography with gas chromatography-mass spectrometry detection. It contained 56% dimers, 12% trimers, 6.6% tetramer and small amounts of other polymeric forms. The high-carbohydrate/high-fat diet was purchased from Beijing Ke'ao Limited Company (Beijing, China), and included 66% basal diet, 15% lard, 10% sucrose, 6% casein and 3% egg yolk.

Commercial kits used for the determination of superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA) and protein in kidney were purchased from Jiancheng Institute of Biotechnology (Nanjing, China). The antibodies of tumor necrosis factor- α (TNF- α), monocyte chemoattractant protein-1 (MCP-1), intercellular adhesion molecule-1 (ICAM-1) and tissue inhibitor of metalloproteinase-1 (TIMP-1) were purchased from Zhongshanjinqiao Biotechnology Limited Company (Beijing, China). Matrix metalloproteinase-9 (MMP-9) was purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). Ethanol, acetaldehyde and isopropanol

were of analytical grade and purchased from Beijing Chemical Company (Beijing, China).

Animals and treatment. Seventy male Sprague-Dawley rats (6 weeks old, weighing 200-250 g) were obtained from the Animal Service of the Health Science Center, Peking University, China. The rats were kept in a filter-protected, air-conditioned room with controlled temperature (21-25°C), relative air humidity (40-50%) and a 12-h light/12-h dark cycle (lights on between 07.30 and 19.30). Animal treatment and maintenance were carried out strictly in accordance with the Principles of Laboratory Animal Care (NIH publication no. 85-23, 1985) and the guidelines of the Peking University Animal Research Committee.

Experimental design. Rats were randomly divided into two groups. Group 1 (n=12, normal control) was fed the basal diet. Group 2 (n=58) was fed a high-carbohydrate/high-fat diet for 4 weeks. Then after fasting for 15 h, rats in group 2 were intraperitoneally injected with streptozotocin (STZ; 0.01 mol/l, 30 mg/kg-bw). Another STZ injection was administered after 7 days. STZ was freshly dissolved in ice-cold citrate buffer (pH 4.5). Rats in group 1 were injected with the citrate buffer vehicle at the same time. Fourteen days after the STZ injection, the fasting serum glucose of rats in group 2 was tested. Diabetes was defined as fasting serum glucose >11.1 mmol/l in caudal vein blood and rats having the symptoms of polyuria, polyphagia and emaciation. A total of 48 rats in group 2 satisfied the above conditions and were used in the next experiments. The diabetic rats (n=48) were randomly divided into four groups (n=12 in each): one DM control group and three groups for low (125 mg/kg-bw), medium (250 mg/kg-bw) and high (500 mg/kg-bw) doses of GSPE. Distilled water was used as the solvent for GSPE. The rats in the GSPE intervention groups were given GSPE intragastrically while the others received an equal volume of distilled water during the experimental period. The study lasted for a total of 16 weeks. Food consumption, water intake and urine volume of rats were measured through metabolic cages. Systolic blood pressures of all rats were measured. Blood was collected from the femoral artery and the rats were then sacrificed by cervical dislocation after collecting 24 h volume urine. Serum was separated by centrifugation at 3,000 rpm for 15 min. Portions of the kidney were then obtained for biochemical assays.

Biochemical assays. Systolic blood pressure was measured by the BP-98A intelligent non-invasive blood pressure monitor (Softron Biotechnology Limited Company, Beijing, China). The levels of fasting blood glucose (FBG) and high-sensitivity c-reactive protein (hs-CRP) in serum were detected using an automatic biochemistry analyzer (Hitachi, Tokyo, Japan), as were serum creatinine (SCr) and blood urea nitrogen (BUN) in urine. SOD and CAT activity and the MDA level in renal tissues were determined by detection kits according to the manufacturer's instructions.

Western blot analysis. Total protein was extracted from the frozen renal tissues using RIPA lysis buffer (1% Triton X-100, 1% deoxycholate, 0.1% SDS) and 1 mM phenylmethanesulfonyl

Table I. Effect of grape seed proanthocyanidin extract on body weight, food intake, water intake and urine volume in rats after 16 weeks.

Parameters	Normal rats	DM rats	Low GSPE rats	Medium GSPE rats	High GSPE rats
Initial body weight (g)	425.70±5.87	442.23±10.15 ^a	452.43±12.78	440.22±8.17	454.50±9.42
Final body weight (g)	585.38±13.23	415.78±9.45 ^a	434.53±10.89	447.65±12.74 ^c	450.63±11.67 ^c
Initial food intake (g/d)	16.86±0.88	23.43±1.24 ^a	19.16±1.56	21.13±0.98	20.16±1.42
Final food intake (g/d)	15.50±0.56	24.67±1.55 ^b	27.50±1.01	20.75±1.32 ^c	18.14±1.42 ^c
Initial water intake (ml/d)	40.21±4.32	80.76±5.05 ^a	78.14±9.97	82.14±7.74	86.13±6.94
Final water intake (ml/d)	35.77±5.11	121.67±11.67 ^b	115.71±8.65	78.59±5.67 ^d	71.57±5.43 ^d
Initial urine volume (ml/d)	7.57±1.73	36.50±8.39 ^b	33.26±6.38	40.58±2.32	38.85±3.59
Final urine volume (ml/d)	6.71±1.95	100.80±9.96 ^b	90.13±10.55	70.83±10.50 ^c	55.75±5.12 ^d

DM, diabetes mellitus; GSPE, grape seed proanthocyanidin extract. Data are expressed as the mean ± standard deviation of 12 rats per group. ^aP<0.05, ^bP<0.01 versus normal control rats; ^cP<0.05, ^dP<0.01 versus DM control rats.

fluoride. Following ultrasonication for 5 min, extracts were centrifuged at 12,000 rpm for 15 min at 4°C, and the supernatants containing protein were retained. The concentrations of protein were measured with the bicinchoninic acid method (Beyotime[®], Institute of Biochemistry, China). Then 40-μg protein samples were resolved on 15% Tris-glycine polyacrylamide minigels for TNF-α, MCP-1 and TIMP-1 and 5% for ICAM-1 and MMP-9, and then transferred to polyvinylidene fluoride (PVDF) membranes. Membranes were blocked with 5% skimmed milk in TBST for 1 h. Primary antibodies were used overnight at 4°C and dilutions were as follows: TNF-α (1:200), MCP-1 (1:200), TIMP-1 (1:200), MMP-9 (1:200) and ICAM-1 (1:500), all from Santa Cruz. Secondary antibodies were used at the following dilutions: goat antibody to mouse IgG (1:5000) and goat anti-rabbit (1:5000), both from ZSGB-BIO, Beijing, China. Signals were visualized using the enhanced chemiluminescence system and analyzed densitometrically using Image Pro Plus version 6.0 software (Media Cybernetics, Inc., Silver Spring, MD, USA). PVDF membranes were reprobated with β-actin (at 1:1000, Cell Signaling Technology, Inc., Beverly, MA, USA) to verify equal protein loading on the gel.

Statistical analysis. The data are expressed as the mean ± standard deviations. The analyses were carried out with SPSS 13.0 statistical software (SPSS, Inc., Chicago, IL, USA). Data were first tested for homogeneity and then evaluated by means of a one-way ANOVA test followed by Tukey's post hoc least significant difference test, if variances were equal, or Tamhane's T3 test, if variances were not equal. P<0.05 was considered to indicate a statistically significant result.

Results

Body weight, food consumption, water intake and urine volume values. As shown in Table I, the body weight was lower in DM rats than in normal rats at the end of the study (P<0.05). Food consumption, water intake and urine volume were increased in DM rats compared with normal

rats throughout the study period (P<0.01 for each). However, GSPE treatment slightly increased body weight and decreased food consumption, water intake and urine volume in a dose-dependent manner.

Fasting blood glucose, serum insulin, HbA1c and systolic blood pressure values. As shown in Fig. 1, FBG, serum insulin, HbA1c and systolic blood pressure were markedly increased in DM control rats compared with normal control rats (P<0.05). Following administration of GSPE (particularly at a dose of 500 mg/kg-bw), the above parameters were decreased to a certain extent.

Renal function index values. BUN, SCr, creatinine clearance (CCr), 24-h urine protein and kidney index (kidney weight/body weight) were used to evaluate renal injury. From Table II it may be noted that some renal injury occurred in DM control rats including renal hypertrophy and renal dysfunction (P<0.01 or P<0.05 compared with normal control rats). However, GSPE treatment was able to improve these renal function parameters (P<0.01 or P<0.05).

MMP-9 and TIMP-1 expression in renal tissues. As shown in Fig. 2, low-dose streptozotocin and a high-carbohydrate/high-fat diet treatment significantly decreased the expression of MMP-9 and increased the expression of TIMP-1 compared with that observed in the normal control rats (P<0.05). The 500 mg/kg-bw GSPE treatment markedly increased the expression of MMP-9 and reduced the expression of TIMP-1 when compared with diabetic control rats (P<0.05).

SOD and CAT activity and MDA levels in renal tissues. Parameters of oxidative stress are shown in Table III. The activity of SOD and CAT in the kidneys of the DM control rats was significantly lower than that in normal control rats (P<0.01) while MDA levels were significantly higher (P<0.05). The SOD and CAT activity in high-dose GSPE rats was significantly higher than that in DM control rats (P<0.01 and P<0.05, respectively). The MDA levels in high-dose GSPE-treated

Table II. Effect of grape seed proanthocyanidin extract on 24-h urine protein, blood urea nitrogen, serum creatinine, creatinine clearance and renal mass in rats.

Parameters	Normal rats	DM rats	Low GSPE rats	Medium GSPE rats	High GSPE rats
24-h urine protein (mg)	77.96±3.83	524.76±8.75 ^b	539.50±6.30	445.39±4.59 ^c	231.04±4.07 ^d
BUN (mmol/l)	4.38±0.26	12.25±5.09 ^b	7.97±0.89	7.15±0.77	5.12±1.56 ^d
SCr (μ mol/l)	30.38±8.39	48.47±10.32 ^a	43.12±9.66	39.63±11.21	31.12±9.95 ^d
CCr (mmol/l)	2.03±0.23	6.79±1.04 ^b	6.03±0.53	5.07±0.60	3.35±1.01 ^d
Kidney weight/body weight (%)	0.46±0.02	0.76±0.02 ^b	0.82±0.02	0.74±0.04	0.54±0.02 ^d

DM, diabetes mellitus; GSPE, grape seed proanthocyanidin extract; BUN, blood urea nitrogen; SCr, serum creatinine; CCr, creatinine clearance. Data are expressed as the mean \pm standard deviation of 12 rats per group. ^aP<0.05, ^bP<0.01 versus normal control rats; ^cP<0.05, ^dP<0.01 versus DM control rats.

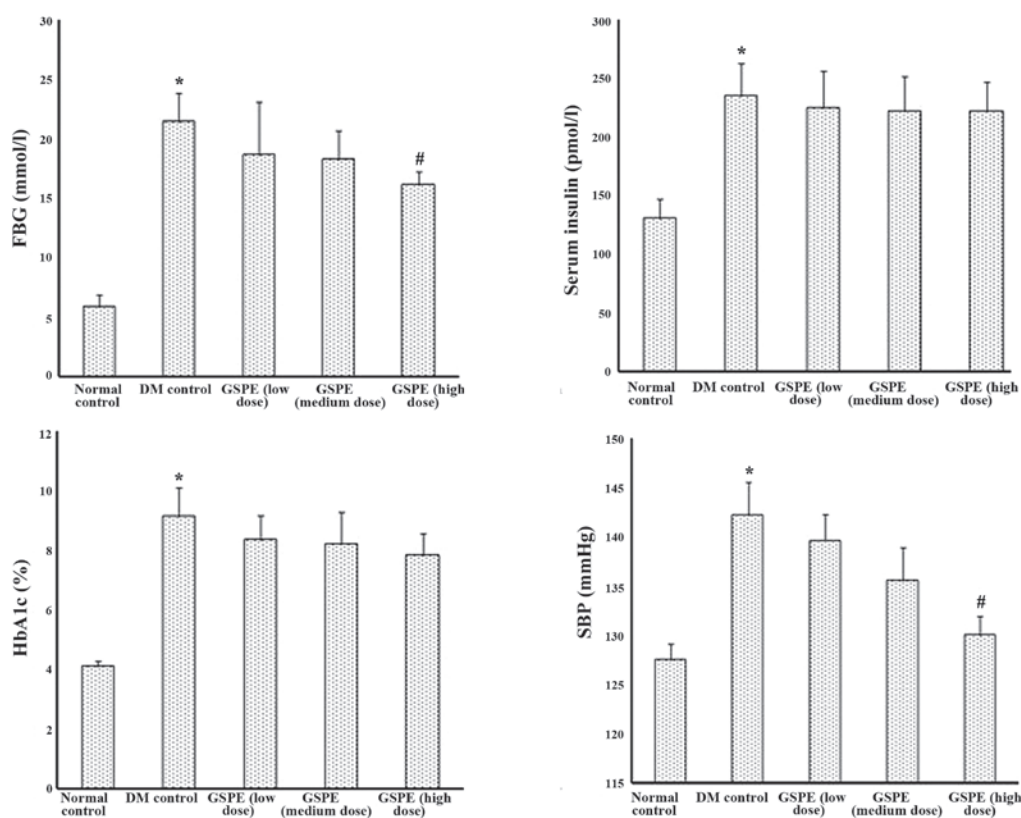


Figure 1. Effects of grape seed proanthocyanidin extract (GSPE) on fasting blood glucose (FBG), serum insulin, HbA1c and systolic blood pressure (SBP) in rats. DM, diabetes mellitus. Data are expressed as the mean \pm standard deviation of 12 rats per group. *P<0.05 versus normal control rats; #P<0.05 versus DM control rats.

rats were significantly lower than those in DM control rats (P<0.01).

Serum high sensitivity c-reactive protein levels and expression of TNF- α , MCP-1 and ICAM-1 in renal tissues. Parameters of inflammation were determined as shown in Figs. 3 and 4. Compared with normal control rats, serum hs-CRP in DM control rats was significantly increased (P<0.01), while in GSPE medium- and high-dose groups it was significantly lower than in DM control rats (P<0.01). Inflammatory cytokines, including TNF- α , MCP-1 and ICAM-1, were all significantly elevated in the kidneys of DM control rats when

compared with normal controls (P<0.05 for each). However, GSPE (particularly at a dose of 500 mg/kg·bw) decreased their expression when compared with the DM control group (P<0.05 for each).

Discussion

Due to its poor prognosis and the rapidly increasing number of cases, type 2 DN has raised great concern. In this study, we successfully built a T2DM model of rats using a high-carbohydrate/high-fat diet for 4 weeks and two low-dose streptozotocin injections. Following 16 weeks of treatment,

Table III. Effect of grape seed proanthocyanidin extract on superoxide dismutase and catalase activity and malondialdehyde levels in renal tissues of rats.

Parameters	Normal rats	DM rats	Low GSPE rats	Medium GSPE rats	High GSPE rats
SOD (U/mg·pro)	69.45±5.27	53.71±3.45 ^b	60.72±10.10	62.58±4.35	67.78±7.11 ^d
CAT (U/mg·pro)	38.46±2.75	28.01±2.64 ^b	30.70±2.76	32.38±5.14	35.65±2.32 ^c
MDA (nmol/mg·pro)	0.59±0.18	1.15±0.31 ^a	0.82±0.16	0.72±0.16	0.53±0.25 ^d

DM, diabetes mellitus; GSPE, grape seed proanthocyanidin extract; SOD, superoxide dismutase; CAT, catalase; MDA, malondialdehyde. Data are expressed as the mean ± standard deviation of 12 rats per group. ^aP<0.05, ^bP<0.01 versus normal control rats; ^cP<0.05, ^dP<0.01 versus DM control rats.

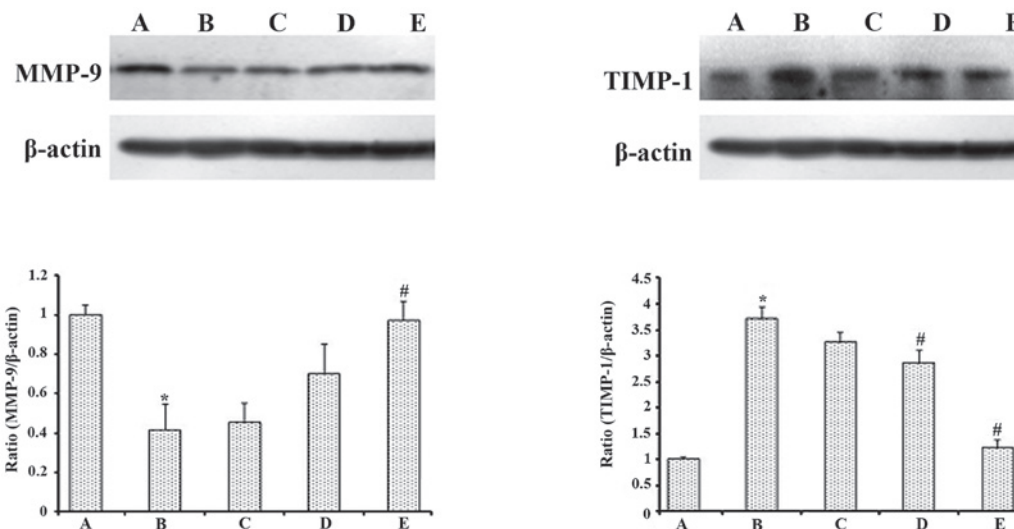


Figure 2. Effect of grape seed proanthocyanidin extract (GSPE) on matrix metalloproteinase-9 (MMP-9) and tissue inhibitor of metalloproteinase-1 (TIMP-1) in renal tissues of rats. β -actin protein levels were used as a control. Data are expressed as the mean ± standard deviation of four rats per group. *P<0.05 versus normal control rats; #P<0.05 versus diabetes mellitus (DM) control rats. (A) Normal control group; (B) DM control group; (C) low-dose GSPE group; (D) medium-dose GSPE group; (E) high-dose GSPE group.

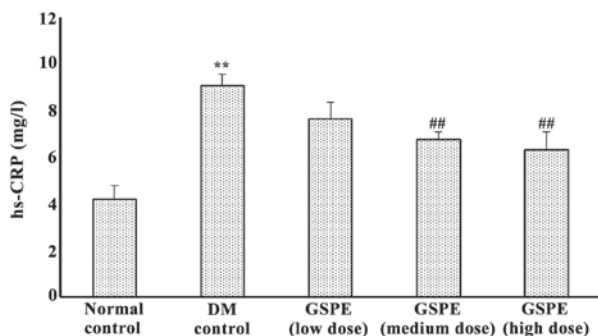


Figure 3. Effect of grape seed proanthocyanidin extract (GSPE) on serum high-sensitivity c-reactive protein (hs-CRP) levels in rats. Data are expressed as the mean ± standard deviation of 12 rats per group. **P<0.01 versus normal control rats; ##P<0.01 versus diabetes mellitus (DM) control rats.

GSPE was demonstrated to improve renal function, decrease the levels of FBG, serum insulin, HbA1c and systolic blood pressure, and suppress oxidative stress and inflammatory response.

At the end of this study, nephritic structural and functional damages were observed in diabetic control rats as demonstrated by the BUN, SCr, endogenous CCr, 24-h proteinuria,

kidney weight/body weight, and expression of MMP-9 and TIMP-1. Increased glomerular filtration rate and proteinuria are the main physical signs of DN. BUN, SCr, CCr and 24-h urine protein are the main indicators of renal function. In the present study, the BUN, SCr, CCr and 24-h urine protein of DM rats were significantly higher than those in normal rats, which implied glomerular hyperfiltration and renal dysfunction in the DM rats. However, GSPE improved these renal function parameters. This is similar to the results observed in a previous study by Li *et al* (17). These authors also indicated that GSPE was capable of decreasing proteinuria, attenuating the progression of nephropathy in diabetic rats.

Glomerular extracellular matrix (ECM) accumulation and base-membrane thickness are the main pathological changes in DN (18). MMP-9 is considered to be essential for ECM turnover in kidneys, and the downregulation of MMP-9 levels has been demonstrated in streptozotocin-induced diabetic rats (19,20). Previous studies have indicated that an imbalance between MMPs and their inhibitors (TIMPs) contributes to nephropathy and that they may be potential targets for therapeutic interventions (21,22). In the present study, decreased MMP-9 activity and increased TIMP-1 activity were observed in diabetic rats. GSPE promoted the upregulation of MMP-9

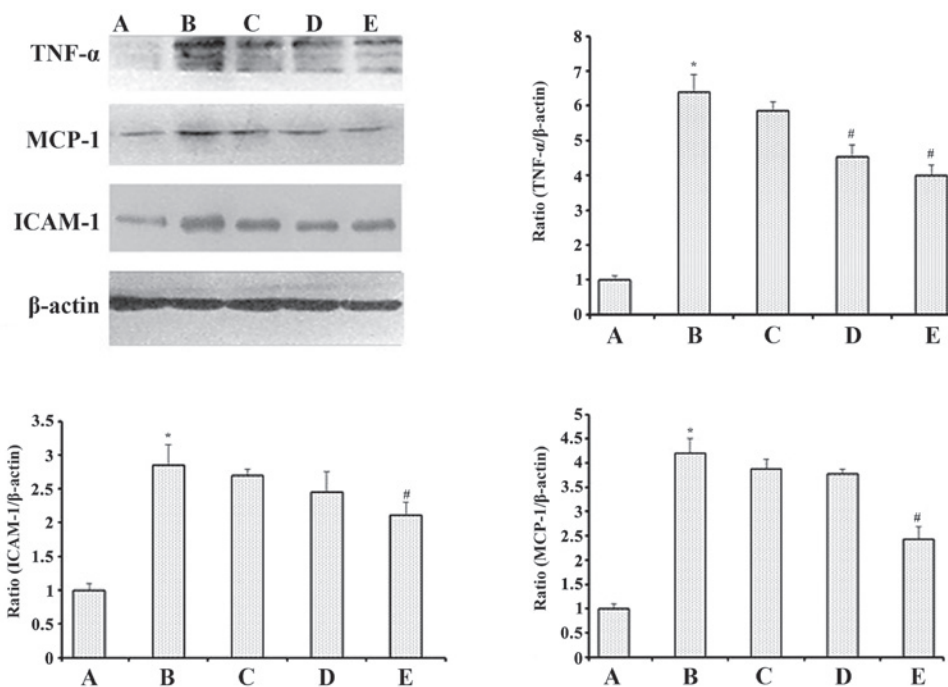


Figure 4. Effect of grape seed proanthocyanidin extract (GSPE) on tumor necrosis factor- α (TNF- α), monocyte chemoattractant protein-1 (MCP-1) and intercellular adhesion molecule-1 (ICAM-1) in renal tissues of rats. β -actin protein levels were used as a control. Data are expressed as the mean \pm standard deviation of four rats per group. * P <0.05 versus normal control rats; # P <0.05 versus diabetes mellitus (DM) control rats. (A) Normal control group; (B) DM control group; (C) low-dose GSPE group; (D) medium-dose GSPE group; (E) high-dose GSPE group.

and downregulation of TIMP-1, which may be a potential mechanism of the protective effect. This finding may be significant as there are few reports on MMPs and their inhibitors in studies involving GSPE in DN.

Hyperglycemia is a typical feature of DM, which is also one of the most common symptoms. Long-term hyperglycemia is the main cause of DN, thereby imposing a great risk to patients. One prospective diabetes study from the UK demonstrated that the risk of microalbuminuria and clinical DN in DM patients could be significantly reduced if intensive treatment was administered to control the levels of blood glucose (23). Pinent *et al* (24) observed that GSPE had a hypoglycemic effect on STZ-induced diabetic rats by delaying the absorption of glucose in intestines. In addition, El-Alfy *et al* (25) demonstrated that GSPE could lower hyperglycemia in mice induced by alloxan monohydrate in an antagonistic manner. GSPE, as an effective antioxidant, protects islet tissue from damage, and may also promote residual islet B cells to secrete insulin. A previous study revealed that 250 mg/kg-bw GSPE could not decrease FBG and HbA1c levels in diabetic rats (26). In the present study, we noted that 500 mg/kg-bw GSPE slightly decreased the levels of FBG.

Hypertension is always accompanied by hyperglycemia, and is another major cause of DN. Glomerular capillary pressure due to high blood pressure may cause increased capillary tension and thickening of the arterial wall, thus leading to ischemia and endothelial cell damage. Then mesangial cells and matrix proliferate and promote glomerular metabolism and compensatory hypertrophy, which eventually results in glomerulosclerosis. This damage could cause the blood pressure to increase in turn, creating a vicious cycle thereafter. Therefore, controlling the blood pressure to a normal level is

also essential in the treatment of DN. In our study, the systolic blood pressure in GSPE-treated rats was significantly lower than that in normal rats, which indicated that GSPE was able to reduce blood pressure. Cui *et al* (27) fed ouabain-induced hypertensive rats with GSPE and noted a lowered blood pressure, making GSPE a potential natural reagent in hypertension treatment. Li *et al* (17) also observed a similar result. GSPE may enhance the production and release of nitric oxide, reduce the expression of endothelin-1 in the vascular endothelium, and thus have an effect on hypertension (28).

Increased oxidative stress in DN induces a number of ROS, including peroxide hydrogen (H_2O_2), superoxide anion (O_2^-) and hydroxyl radical ($\cdot OH$). ROS activate nuclear transcription factor- κB and activator protein-1 by activating protein kinase C (PKC) and mitogen-activated protein, which results in large amounts of cytokines and growth factors in the kidney (29,30). Microvascular endothelial cells are damaged as a result, and a number of negative consequences induced by oxidative stress, such as mitochondrial DNA deletions, will accelerate the development of DN (31,32). Numerous studies have suggested that antioxidant therapy reduces renal injury in DN (33-35). SOD is the main antioxidant enzyme *in vivo*, while CAT is an enzyme scavenger decomposing hydrogen peroxide into hydrogen and water, and MDA is the most abundant by-product of lipid peroxidation. These three may be considered as indicators of oxidative stress. The present results reveal that DM rats possessed a higher MDA level and lower SOD and CAT activity levels in the kidney than those of normal control rats, which confirmed the involvement of oxidative stress in renal injury induced by DM. It was also noted that GSPE increased the activity of SOD and CAT, but decreased the levels of MDA. In other

words, GSPE reduced the levels of ROS and protected the kidneys from injury induced by oxidative stress, which may be one of the potential mechanisms by which it exerts its beneficial effects on DN.

Possibly the most significant results in this study were the notable anti-inflammatory effects of GSPE and its pivotal role in the treatment of DN. An increasing number of inflammatory signal pathways and cytokines are being investigated and deemed new molecular targets for treating DN. In the progress of DN, certain pro-inflammatory cytokines and ROS become activated, which induces mesangial cells to secrete type IV collagen, laminin and fibronectin, and leads to glomerulosclerosis (36,37). Moreover, inflammation is involved in the activation of certain signal transduction pathways *in vivo*, including the polyol, AGE, ROS and PKC pathways, which promotes the expression of the specific cytokines and induces the expression of the gene within the cell as well as protein dysfunction, eventually leading to the occurrence of microvascular complications (38). The present study revealed a severe inflammatory reaction in the serum and kidney. The levels of TNF- α , MCP-1 and ICAM-1 were all significantly higher than those of normal controls. Previous studies have reported that renal injury was attenuated when their levels were decreased (39-41). One of mechanisms of protecting the kidney may be that GSPE decreased the levels of inflammatory cytokines.

In addition, inflammation and oxidative stress are not assumed to take effect separately. They are interrelated and interactive with each other in a number of diseases, including hypertension and cardiovascular disease (42,43). However, information on the correlation between oxidative stress and inflammation in DN is limited. Further studies are necessary to identify whether either of these play a significant role in the treatment of DN.

In summary, this study indicated that oxidative stress and inflammatory reaction may, at least in part, cause renal injury in T2DM rats. GSPE may alleviate renal injury and exert its antioxidative effect *in vivo* by increasing the activity of SOD and CAT, decreasing the levels of MDA, and decreasing the levels of inflammatory cytokines including hs-CRP, TNF- α , MCP-1 and ICAM-1. However, there are several inflammatory pathways *in vitro*. Further studies are required to investigate the exact inflammatory pathways by which GSPE protects against renal injury, as well as its specific mechanism of action.

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