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Effects of Grape Seed Proanthocyanidin Extract on Oxidative Stress Induced by Diabetes in Rat Kidney

Esrafil Mansouri[1], Marzieh Panahi[*1], Mohammad Ali Ghaffari[2] and Ali Ghorbani[3]

[1]Dept. of Anatomical Sciences and [2]Dept. of Biochemistry, Cellular and Molecular Research Center, Faculty of Medical Sciences, Ahwaz Jondishapur University of Medical Sciences, Ahwaz; [3]Dept. of Nephrology, Golestan Hospital, Ahwaz Jondishapur University of Medical Sciences, Ahwaz, Iran

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ABSTRACT

Background: This study examined the effect of grape seed proanthocyanidin extract (GSPE) on lipid peroxidation content and activity of tissue antioxidant enzymes, including catalase, superoxide dismutase and glutathione peroxidase in diabetic rats. **Methods:** Thirty male rats were divided into three groups of 10 rats each: control, diabetic and diabetic groups that received 500 mg/kg GSPE for 6 weeks. Diabetes was induced by a single intraperitoneal injection of streptozotocin (50 mg/kg body weight). Rats with fasting blood glucose levels above 250 mg/dl were used as diabetic animals. The first 24-hour urinary albumin excretion (UAE) was measured two weeks after diabetes induction and then each week until the end of the experimental period in all groups. Lipid peroxidation content and activities of catalase, superoxide dismutase and glutathione peroxidase were measured in kidney homogenate supernatants. Statistical significance of differences was assessed with one-way ANOVA by SPSS followed by Tukey's t-test. $P<0.05$ was assumed statistically significant. **Results:** UAE in diabetic nephropathy rats were significantly higher than in control. In addition, an increase in lipid peroxidation content and decrease in catalase, superoxide dismutase and glutathione peroxidase activities in kidney of diabetic nephropathy rats were observed. The GSPE administration did not affect on body weight, but significantly decreased lipid peroxidation and augmented the activities of antioxidant enzymes studied in kidney of diabetic nephropathy rats as well as reduced UAE and decreased kidney weight. **Conclusion:** The results suggested that GSPE could ameliorate diabetic nephropathy rats through reduction of oxidative stress and increase in renal antioxidant enzyme activity. *Iran. Biomed. J. 15 (3): 100-106, 2011*

Keyword: Grape seed proanthocyanidin extract (GSPE), Diabetic nephropathy, Oxidative stress, Antioxidant enzymes

INTRODUCTION

Diabetes is the leading cause of end-stage renal diseases and 67% of all patients with diabetes have nephropathy [1]. The severity of diabetic nephropathy is one of the major factors determining the prognosis of diabetic patients. Several lines of evidence have indicated that hyperglycemia causes diabetic nephropathy through a variety of mechanisms, including the induction of oxidative stress [2] and the acceleration of glycation reaction [3]. Oxidative stress induces the production of highly reactive oxygen radicals that are toxic to cells [4].

The mechanism by which hyperglycemia causes free radical generation, thus causes oxidative stress is complex. Hyperglycemia promotes glycosylation of circulating and cellular protein and may initiate a series of auto-oxidative reactions that culminate in the formation and accumulation of advanced glycosylation end-products (AGE) in tissue proteins. The AGE has oxidizing potential and can promote tissue damage by free radicals [5]. The activation of hyperglycemia-induced secondary mediators, such as protein kinase C (PKC) and mitogen-activated protein kinase, and also cytokine production are responsible for oxidative stress-induced renal injury in the diabetic condition [6].

Nowadays, an intense search for novel type of antioxidants is being carried out from numerous plant materials. Many plant extracts and plant products have been shown to possess a significant antioxidant activity. Polyphenolic flavonoids, widely distributed in plants, have been recognized for their interesting clinical properties. Several of these flavonoids, including silymarin, catechin and quercetin have

*Corresponding Author; mobile: (+98-916) 6166551; Fax: (+98-611) 3332036; E-mail: marzpanah@yahoo.com or marpanahi@ajums.ac.ir

shown protective effects on experimental diabetes by enhancing the activity of antioxidant enzymes [7].

Natural grape seed proanthocyanidins are a combination of biologically active polyphenolic flavonoids including oligomeric proanthocyanidins [8]. These proanthocyanidins have demonstrated a marked spectrum of biological, pharmacological, therapeutic, and chemoprotective properties against oxygen free radicals and oxidative stress [9]. Bagchi *et al.* [10] have discovered that grape seed proanthocyanidins provide significantly greater protection against free radicals, free radical-induced lipid peroxidation and DNA damage than vitamin C, E and β-carotene. Such a remarkable spectrum of biochemical and cellular functions holds promise for the prevention and treatment of a variety of human disorders caused by oxidative stress.

The present study was undertaken to assess the protective effect of grape seed proanthocyanidin extract (GSPE) on diabetic nephropathy. In addition, we evaluated parameters of stress oxidative in rat kidneys in the course of streptozotocin-induced diabetic nephropathy as well as the effect of GSPE treatment on renal function, lipid peroxidation and activities of anti-oxidants in kidney of streptozotocin (STZ)-induced diabetic nephropathy rats. The results of the study could serve as a step toward the development of a mechanism-based therapeutic approach for the management of diabetic nephropathy; hence provide the basis for the usefulness of the potent antioxidants, GSPE.

MATERIALS AND METHODS

Male Sprague-Dawley rats (n = 30, 140-180 g) were prepared from Animal House Central of Ahwaz Jondishapur University of Medical Science (Ahwaz, Iran). All animals were housed in cages with 12/12 h light/dark cycle at 21 ± 2°C. All experimental animals were carried out in accordance with the guidelines of the Ethical Committee of Ahwaz Jondishapur University of Medical Sciences (Ahwaz, Iran).

Chemicals. GSPE (95% purity) were purchased from Hangzhou Joymore Technology Co. Ltd. (China). The other chemicals and reagents were obtained from Sigma-Aldrich Chemical Co. (St. Louis, USA).

Induction of diabetes. The animals were fasted for 24 h prior to the induction of diabetes. Diabetes was induced by a single intraperitoneal injection of STZ (50 mg/kg body weight), freshly dissolved in citrate buffer (0.1 M, pH 4.5), while control rats were injected with vehicle buffer only. Blood samples were obtained from the tail vein of the animals at 72 h after STZ injection and fasting blood glucose levels were determined with a glucose strip test in a glucometer (EasyGluco Blood Glucose Monitoring system, Infopia, Korea). Rats with fasting blood glucose levels above 250 mg/dl were used as diabetic animals.

Experimental design. A total of 30 rats were used and divided into three groups of 10 rats each. The groups were divided as follows: group 1, control; group 2, untreated diabetic and group 3, treated diabetic (GSPE, 500 mg/kg body weight). The GSPE was given in normal saline solution by oral gavages for 6 weeks (8-10 AM everyday) from the beginning of fifth week after the induction of diabetes, since we found a significant increase in UAE rate in diabetic rats in comparison with control rats at the end of fourth week. Rats were kept individually in metabolic cage with access to drinking water for measurement of 24-h UAE after two weeks the induction of diabetes then each week until the end of the experimental period. UAE was measured by quantitative reaction with bromocresol green [11] using bovine serum albumin as standard. A volume of 10 µl of sample and standard were mixed separately with 1 ml of BCG then the absorbance was read at 625 nm. At the end of the experiment, six rats were selected randomly from every investigated group, and then were sacrificed under ether anesthesia.

Preparation of renal homogenate. Immediately after the rats were killed, the left kidney was dissected and rinsed in isotonic saline and then blotted dry and weighed. After weighing, kidney tissue was minced and a homogenate was prepared with 5% (w/v) potassium phosphate buffer (0.1 M, pH 7.4) using a homogenizer (Heidolph Silent Crusher M, Germany). Homogenate was then centrifuged at 16,000 × g for 20 min to remove nuclei and cell debris. This supernatant was used for lipid peroxidation and antioxidant reactivity measurement.

Estimation of lipid peroxidation. Degree of lipid peroxidation in kidney tissue homogenate of all the experimental animals was determined in terms of thiobarbituric acid reactive substances (TBARS) formation [12]. A volume of 500 µl of supernatant was mixed with 1.5 ml trichloroacetic acid (10%) and after centrifugation (4,000× g for 10 min), 1.5 ml of supernatant was added to 2 ml TBA (0.67%) and heated at 100°C for 30 min. After cooling, the sample was extracted with 2 ml n-butanol and after centrifugation at 4,000× g for 15 min, the organic phase was collected. The absorbance was read spectrophotometrically at 535 nm using a blank containing all the reagents except the tissue homogenate. Values were expressed as nmol/mg

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protein. As 99% of the TBARS was malondialdehyde (MDA), TBARS concentrations of the samples were calculated from a standard curve using 1,1,3,3-tetramethoxypropane.

Catalase activity assay. Catalase activities were assayed using the method described by Claiborne [13]. The reaction mixture (1 ml) consisted of 50 mM potassium phosphate (pH 7.0), 19 mM H_2O_2, and a 20-50 ml sample. The reaction was initiated by the addition of H_2O_2, and absorbance changes were measured at 240 nm (25°C) for 30 s. The molar extinction coefficient for H_2O_2 is 43.6 M^{-1} cm^{-1}. One unit is defined as one µmol of hydrogen peroxide consumed per minute, and the specific activity is reported as units per milligram of protein.

Superoxide dismutase (SOD) activity assay. The assay for SOD activity was made according to the method of Suttle [14] using Ransod kit (Randox Labs., Crumlin, UK). This method is based on the formation of red formazan from the reaction of 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride and superoxide radical (produced in the incubation medium from xanthine oxidase reaction), which is assayed in a spectrophotometer at 505 nm. The inhibition of the produced chromogen is proportional to the activity of the SOD present in the sample. A 50% inhibition is defined as 1 unit of SOD, and specific activity is expressed as units per milligram of protein.

Glutathione peroxidase (GPx) activity assay. GPx activity was determined using the Ransel kit (Randox Labs., Crumlin, UK) according to Paglia and Valentine [15]. GPx catalyzes the oxidation of glutathione by cumene hydroperoxide. In the presence of glutathione reductase and NADPH, the oxidized Glutathione (GSSG) was immediately converted to the reduced form with a concomitant oxidation of NADPH to NADP+. The decrease in absorbance was monitored with a spectrophotometer at 340 nm. One GPx unit is defined as 1 µmol of NADPH consumed per minute, and specific activity is reported as units per milligram of protein.

Protein determination. Protein was measured by the method of Bradford [16] using bovine serum albumin as standard. A volume of 20 µl of sample or standard was mixed with 1 ml Bradford reagent, and then the absorbance was read by a spectrophotometer at 595 nm after 5 min.

Statistical analysis. All data are expressed as the mean ± SE. Statistical significance of differences was assessed with one-way ANOVA by SPSS for Windows (version 18) followed by Tukey's *t*-test. $P<0.05$ was assumed as statistically significant.

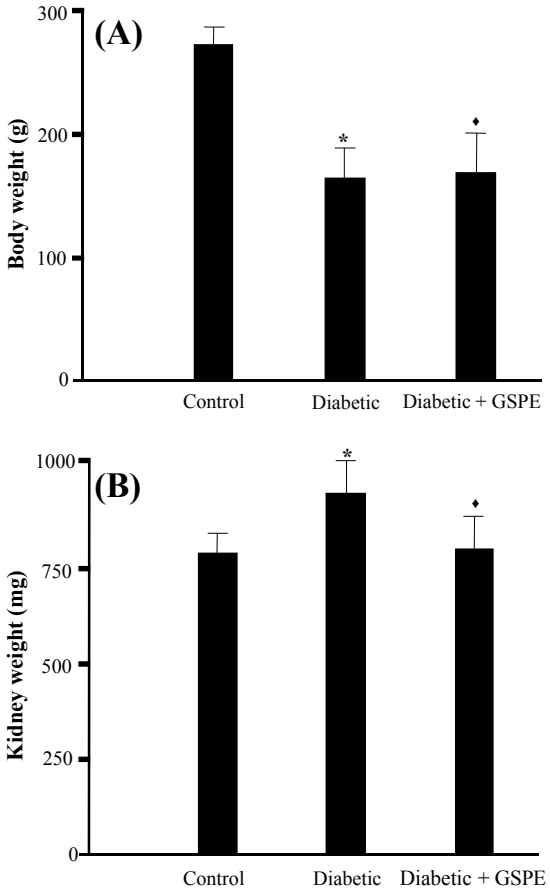

Fig. 1. Body weight **(A)** and kidney weight **(B)** changes among different groups: control, diabetic, diabetic treated with GSPE ($^{*}P<0.05$; and $^{\blacklozenge}P<0.05$ as compared with control and diabetic, respectively).

RESULTS

Body and kidney weight. In untreated diabetic group, body weight was decreased significantly compared to the control group. The body weight of diabetic rats, which were administered GSPE, did not differ from that of diabetic rats not taking GSPE (Fig. 1A). In untreated diabetic group, kidney weight was increased significantly compared to the control group. After administration of GSPE, a significant decrease of kidney weight was found in this group of rats in comparison to diabetic group of rats not receiving GSPE (Fig. 1B).

Twenty-four-hour albumin excretion in urine. A significant increase of 24-hour albumin excretion in urine of diabetic group was observed in comparison to the control groups. In the diabetic group which received GSPE, a decrease of 24-hour albumin excretion in urine was observed in comparison to diabetic group of rats not receiving GSPE; these differences were significant (Fig. 2).

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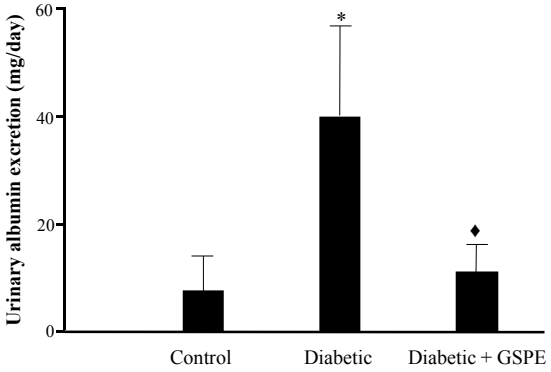

Fig. 2. Changes in 24-hour albumin excretion during the experimental period among different groups: control, diabetic, diabetic treated with GSPE ($^{*}P<0.05$; and $^{\blacklozenge}P<0.05$ as compared with control and diabetic, respectively).

Renal lipid peroxidation content. Renal lipid peroxidation content in diabetic rats was significantly higher in comparison to the control group. GSPE administration significantly reversed this increase (Fig. 3).

Superoxide dismutase activity in rat kidneys. SOD activity in diabetic rats significantly decreased compared to the control group of rats and GSPE treatment significantly inhibited decrease of SOD activity (Fig. 4A).

Glutathione peroxidase activity in rat kidneys. GPx activity in diabetic rats was significantly lower than the control group. A significantly higher renal GPx activity was found in diabetic rats receiving GSPE comparing to diabetic rats not receiving GSPE (Fig. 4B).

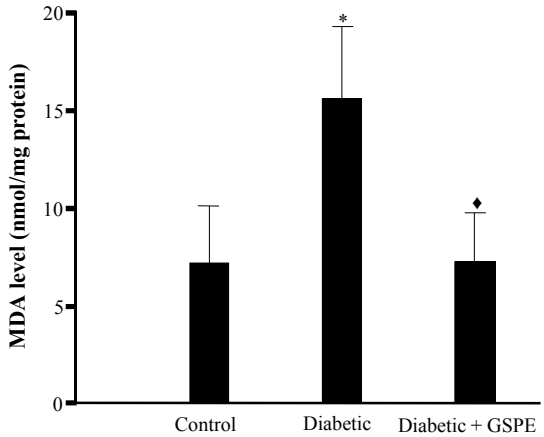

Fig. 3. Renal level of malondialdehyde among different groups: control, diabetic, diabetic treated with GSPE ($^{*}P<0.05$ and $^{\blacklozenge}P<0.05$ as compared with control and diabetic, respectively).

Catalase activity in rat kidneys. Catalase activity in diabetic rats was significantly decreased compared to the rats control group. Higher renal catalase activity in group receiving GSPE was found statistically significant when compared to the group not receiving GSPE (Fig. 4C).

DISCUSSION

Oxidative stress, induced by hyperglycemia in diabetes, is a major cause for development and progression of diabetic microvascular complications such as nephropathy [17]. Diabetic nephropathy is a state in which oxidative stress increases and anti-oxidant status reduces, as documented previously [18].

In this study, we observed a significant decrease in the body weight of diabetic group of rats in comparison to control group of rats. STZ-induced diabetes in animals caused a weight loss due to abnormal carbohydrate and lipid metabolism and increased catabolism of protein. For this reason, weight reduction is being used as a marker of diabetes mellitus induced by STZ [19]. We observed that the administration of GSPE did not influence on the body weight of diabetic group. Our data are in agreement with the studies of other papers [20, 21].

In the diabetic rats, a significant increase in the kidney weight was observed. It might be due to development of renal hypertrophy in rats with stereptozotocin-induced diabetes that was observed by Ku *et al.* [22]. The GSPE administration successfully prevented the enlargement of the kidney. This result is in agreement with the study of Liu *et al.* [23].

In this study, we found an increased 24-hour albumin excretion in diabetic group of rats' urine. Increased albumin excretion in urine is caused by renal glomeruli filtration barrier damage, but the mechanism of this phenomenon has not been thoroughly recognized. Disturbed basement membrane metabolism evoked among others by the severity of pro-oxidative stress in diabetes may be one of the possible mechanisms. It has been stated that AGE interaction with AGE receptor and oxidative stress may affect increased permeability of vessels in diabetes. Increased lipid peroxidation of cell membranes evoked by oxygen-reactive forms may be the reason for these disturbances [24].

Results of Our study are in agreement with other study [25]. In this study, the level of albumin excretion in GSPE- treated diabetic rats is significantly lower than the untreated diabetic rats, but still statistically higher than the control group. This result is similar to that reported in previous study [26].

We also observed an increase in the level of lipid peroxidation in diabetic rats. Based on the results of Ha

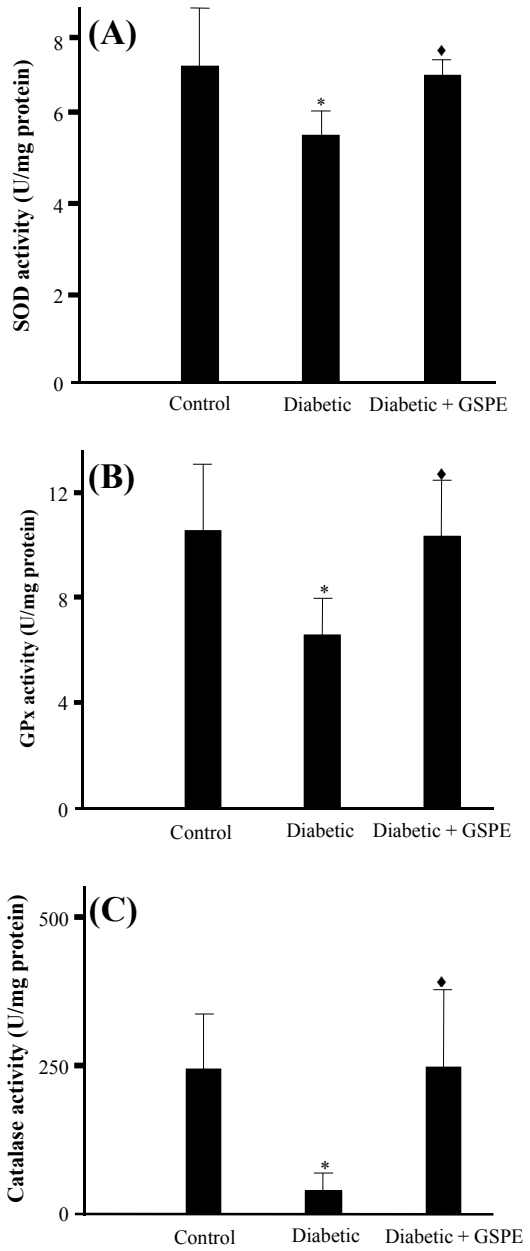

Fig. 4. Renal SOD **(A)**, GPx **(B)** and CAT **(C)** activities among different groups: control, diabetic, diabetic treated with GSPE ($^{*}P<0.05$; and $^{\blacklozenge}P<0.05$ as compared with control and diabetic, respectively).

and Kim studies [24], raised glucose concentration triggers the system of PKC, which in consequence leads to intensified lipid peroxidation in diabetes. It is suggested that PKC activation initiates oxidative stress in renal glomeruli in diabetes, whereas other mechanisms such as glucose auto-oxidation, polyol pathway activation and glycation play a significant role only in later periods of peroxidative damage of renal glomeruli [24]. Also, increase of lipid peroxidation might be due to the increased generation of different radical species. These radicals have been documented to stimulate degradation of DNA, lipids, and carbohydrates leading to hyperglycemia and related glucose auto-oxidation [27]. These results are in agreement with the previous finding [28]. In the present study, GSPE significantly decreased lipid peroxidation in the diabetic rats that this result was also observed by other authors [29-31].

In the present study, we observed a significant decrease in catalase, SOD and GPx in diabetic rats. Hyperglycemia reduces the synthesis and activities of a number of antioxidant enzymes including SOD and GPx, presumably by glycation. It is well established that diabetic patients have a lower antioxidant defense, enzymatic (SOD, catalase and GPx) and non-enzymatic (vitamin C, E or A, free radical scavengers) [32]. The role of oxidative stress in the pathogenesis of diabetic nephropathy is not only through overproduction of ROS but also through the reduction of antioxidant enzyme activities, auto-oxidation of glucose, impaired glutathione metabolism, formation of lipid peroxides and non-enzymatic protein glycosylation [33]. Our observations are similar to the previous study [28]. In the present study, GSPE significantly increased antioxidant enzyme activity in diabetic rats. These results are in agreement with the studies of other authors [29-31]. GSPE showed a protective effect on experimental diabetic nephropathy that it might be due to inhibition of AGE formation. The reason that GSPE inhibit AGE formation is correlated with the antioxidative activities of GSPE [31] and the excellent free radical scavenging activity of GSPE could be a possible reason for this reversal effect of lipid peroxidation level and antioxidant enzymes activities [10].

In some reports, SOD and GPx activities were increased [34, 35]. The differences between our observations and the results presented by Rauscher *et al.* [34, 35] may be due to different doses of STZ.

The present study showed that the beneficial effect of GSPE on diabetic was still active even if GSPE treatment was started at the time when albumin excretion became apparently increase in diabetic animals. On the other hand, GSPE can improve the activity of antioxidant enzymes (SOD, GPx and catalase) in kidney of rats with diabetic and decrease the content of MDA. It indicates that the antioxidant function of GSPE maybe work by increasing the activity of body's antioxidant enzymes. Considering that pharmacological treatment usually starts after some interval from the onset of diabetes in the clinical field, we believe that the present experimental design can be modeled to actual clinical application.

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