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Effects of Grape Seed Proanthocyanidin Extract in Attenuating Diabetic Complications in Streptozotocin-Induced Diabetic Rats

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Abstract

Oxidative stress is a major cause for development and progression of diabetic mediated peripheral and central complications. The aim of this work is to evaluate the role of Grape Seed Proanthocyanidin (GSPE) extract in attenuating diabetic complications. Forty-five adult male Wistar rats were divided into three groups. Control, non-treated diabetic and diabetic rats treated with GSPE. Diabetes was induced by intraperitoneal injection of streptozotocin. After eight weeks; urine, blood and brain, heart, kidneys, liver tissue homogenate parameters were evaluated. The results showed a reduction in both renal and hepatic function in non-treated group as well as an elevated serum inflammatory markers (TNF-α, hs-CRP and IL-6). In addition, there were an elevation in both serum and different tissue homogenate oxidative stress parameters (SOD, GPx, Catalase and MDA). Treatments with GSPE improve the oxidative stress status in different tissue and improve renal and kidney functions. These finding suggested that GSPE is effective as adjuvant therapy for protection from diabetic complications as well as its potent antioxidant and anti-inflammatory activity.

Keywords: Proanthocyanidins; Oxidative stress; Inflammatory markers; Glutathione peroxidase; Catalase; Malondialdehyde; Superoxide dismutase; TNF-α; hs-CRP; IL-6

Introduction

Diabetes Mellitus (DM) is an important metabolic disorder with a global prevalence estimated to be 9% among adults [1] and 15.4% among adult Egyptian population [2]. In 2012, an estimated 1.5 million deaths were directly caused by diabetes. More than 80% of diabetes deaths occur in low- and middle-income countries [3]. Diabetes is the eleventh most important cause of premature mortality in Egypt, and is responsible for 2.4% of all years of life lost. Similarly, diabetes is the sixth most important cause of disability burden in Egypt [4].

Diabetic macrovascular complication is subjected to a high incidence of vascular diseases such as stroke, myocardial infarction, and peripheral vascular diseases [5]. Pathological changes in major blood vessels leading to functional and structural abnormalities in diabetic vessels include endothelial dysfunction, reduced vascular compliance, and atherosclerosis. Almost half of diabetic patients die of cardiovascular disease [6].

DM is conjoined with the presence of microvascular complications. This complications lead to nephropathy, retinopathy, and peripheral neuropathy [7]. Diabetic peripheral neuropathy is the major reason for loss of protective limb mechanical sensations, traumatic ulcerations and therefore amputations [8]. Diabetic retinopathy is an important cause of blindness, and occurs because of long-term accumulated damage to the small blood vessels in the retina. 1% of global blindness can be attributed to diabetes [9]. DM is the major cause of end-stage kidney diseases. About 67% of diabetic patients have nephropathy [10].

Oxidative stress is a widely accepted participant in the development of DM and progression of its complications [11]. Excessively high levels of free radicals cause damage to cellular proteins, membrane lipids and nucleic acids, and eventually cell death [12]. Moreover, DM is accompanied by elevated lipid peroxidation, reduced antioxidant enzymes activities, as well as, altered glutathione redox situation [13]. During diabetes, constant hyperglycemia stimulates glycosylation of circulating and cellular protein. It may start a chain of auto-oxidative reactions which in turn lead to formation and aggregation of advanced glycosylation end products (AGEs) in tissue proteins. AGEs can promote tissue damage by free radicals [14].

Grape Seed Proanthocyanidin Extract (GSPE) is a natural product containing a mixture of biologically active polyphenolic flavonoids [15]. GSPE has marked properties against free radicals and oxidative stress [16]. This antioxidant activity is higher than that of vitamin E, C and β-carotene [17]. GSPE have been reported to possess a variety of potent properties, including anti-nonenzymatic glycation, anti-inflammation, anti-atherosclerosis, anti-tumor, and so on immune function modulator, antithrombotic agent, and LDL oxidation inhibitor [18]. It has a protective effect on various forms of cardiac disorders, reduce hypoxic-ischemic brain injury, prevent diabetic nephropathy from progressing, and protect gastric mucosa [13].

The aim of this study was to investigate the biochemical changes and the protective effects of GSPE against macrovascular and microvascular complications of DM induced in rats by STZ.

Methods

Chemicals

GSPE was obtained as Gervital® capsules, each containing 150 mg GSPE, (MEPACO, Egypt). STZ was obtained from Sigma-Aldrich Chemical Co. (St. Louis, USA).

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Animals

This study was carried out on healthy adult male albino Wistar rats weighing 200–250 g. Animals were purchased from national research Institute, Giza, Egypt. Rats were maintained in the animal house, faculty of pharmacy, Damanhour University, Egypt. Rats were housed in polypropylene cages, they maintained under standardized condition (12 h light/dark cycle, 25°C, 35–60% humidity) and allowed free access to conventional pellet food and purified drinking water *ad libitium*. Procedures involving animals and their care were in conformity with the institutional guidelines and in compliance with national and international laws and the guide for the care and use of laboratory animals.

Induction of diabetes

In overnight fasted rats, DM was induced by a single intraperitoneal (i.p.) injection of 65 mg/kg STZ (freshly dissolved in citrate buffer, 0.1 M, pH 4.5). Hyperglycemia was confirmed by elevated blood glucose levels at 72 h and then on day 7 after injection. Fasting blood glucose was estimated after 72 h. and after 7 days. Rats with level higher than 250 mg/dL were considered diabetic and were used for studies.

Experimental design

This study was carried out on 45 adult albinos Wister rats classified into 3 groups each consisting 15 animals:

Group 1: Normal healthy control rats (distilled water, orally).

Group 2: Diabetic non-treated rats (distilled water, orally).

Group 3: Diabetic rats treated with GSPE (200 mg/kg/day, orally).

All the treatments were started 1 week after injection of STZ. All the treatments were given daily for 8 weeks.

Δ Body weight %	Control	Diabetic	GSPE
	118%	74%#	83%#@
#Significant from normal healthy control group @Significant from diabetic non-treated group			

Table 1: Change in body weight (%) in different studied groups.

Parameters	Control	Diabetic	GSPE
	M ± SEM	M ± SEM	M ± SEM
Urine volume ml/day	9.62 ± 0.51	65.1# ± 5.41	24.6#@ ± 1.92
Urinary albumin mg/day	9.4 ± 1.67	35.3# ± 4.18	18.3#@ ± 1.34
Urinary protein mg/day	14.2 ± 2.31	40.2# ± 3.66	20.7#@ ± 1.32
Plasma glucose (mg/dL)	85.12 ± 6.27	315.42# ± 25.1	165.56#@ ± 12.45
Serum TC mg/dL	83.52 ± 6.42	188.0# ± 13.5	123.8#@ ± 9.85
Serum HDL-C mg/dL	41.81 ± 3.20	15.6# ± 0.95	31.2#@ ± 2.63
Serum TAG mg/dL	72.00 ± 5.30	135.0# ± 12.3	93.1#@ ± 8.10
Serum Total protein g/dL	8.10 ± 0.60	3.90# ± 0.21	6.50#@ ± 0.46
Serum Albumin g/dL	4.70 ± 0.39	2.10# ± 0.09	3.90#@ ± 0.21
Serum ALT (IU/L)	30.12 ± 2.50	73.3# ± 5.70	38.14#@ ± 3.10
Serum AST (IU/L)	80.43 ± 6.60	156.2# ± 12.1	92.08@ ± 8.20
Serum ALP (U/L)	215.6 ± 17.3	320.4# ± 28.1	250.1@ ± 22.1
Serum Urea mg/dL	17.82 ± 1.10	53.22# ± 4.65	22.10#@ ± 1.63
Serum Creatinine mg/dL	0.42 ± 0.03	1.66# ± 0.09	0.53#@ ± 0.04
Serum MDA mmol/mL	23.1 ± 2.17	35.3# ± 2.93	27.9#@ ± 2.14
Serum SOD U/mL	17.8 ± 1.26	9.75# ± 0.31	14.2#@ ± 0.42
Serum GPx U/mL	152.3 ± 14.6	61.3# ± 4.52	98.4#@ ± 7.12
Serum Catalase U/mL	208.7 ± 13.2	145.3# ± 12.2	192.3@ ± 15.2
#Significant from normal healthy control group @Significant from diabetic non-treated group			

Table 2: M ± SEM of different measured parameters among different studied groups.

Sampling

After 8 weeks, each rat was kept in metabolic cages for 24 h for urine collection. Urine samples were centrifuged at 1400 rpm for 5 min after proper dilution. The supernatant was collected for the determination of urinary albumin and protein levels using standard kits.

Then, the rats were fasted overnight then all rats were weighed. Under light ether anesthesia, blood samples were collected from the retro orbital plexus using glass capillaries with and without fluoride. Blood was allowed to clot for 20 min, and it was then centrifuged at 5000 rpm/15 min. Serum and plasma were separated into aliquots and stored at -20°C.

Glucose was assessed in plasma. Creatinine, urea, total protein, albumin, alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate transaminase (AST), total cholesterol (TC), triacylglycerol (TAG), high density lipoprotein (HDL-C), catalase, thiobarbituric acid reactive substances (malondialdehyde, MDA), glutathione peroxidase (GPx), superoxide dismutase (SOD), tumor necrosis factor alpha (TNF-α), high sensitivity C-reactive protein (hs-CRP) and interleukin 6 (IL-6) were estimated in serum using standard kits.

At the end of the experiment, the animals were sacrificed. Livers, kidneys, hearts and brains were immediately rinsed in isotonic saline and blotted dry. Tissues were minced and a homogenate was prepared. Homogenate was then centrifuged at 16,000 × g/20 min. Supernatant was used for the measurement of MDA, SOD, catalase and GPx levels using standard kits.

Statistical analysis

Data analysis was achieved using GraphPad prism version 6.00 (GraphPad software, SanDiego, CA). Results were expressed as means ± standard error of mean (M ± SEM). Statistical significant difference was determined by one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test. Probability values (*P*) less than 0.05 considered statistically significant.

Results

Body weight

A significant decrease in the body weight was noticed in diabetic group as described in Table 1. However, treatment with GSPE suppressed this weight loss. Δ body weight % of each group were calculated in comparison to its starting Δ body weight %.

Fasting blood glucose concentrations

Plasma glucose concentration in diabetic rats was significantly elevated in relative to normal control group. Treatment with GSPE was significantly ameliorated plasma glucose levels when compared to diabetic non-treated rats.

Changes in 24 h urine volume, urinary protein and albumin

Regarding control group, 24 h urine volume and urinary total protein and albumin were higher in diabetic group as mentioned in Table 2. On the other hand, administration of GSPE was significantly reduced urine volume, urinary protein and albumin excretion as compared to diabetic non-treated group.

Lipid parameters

In additions, Table 2 shows that, the concentrations of serum TC and TAG were significantly increased in the diabetic animals. Whereas the concentration of serum HDL-C was significantly decreased. The supplementation of GSPE was significantly decreased TC and TAG, and increased the HDL-C concentration.

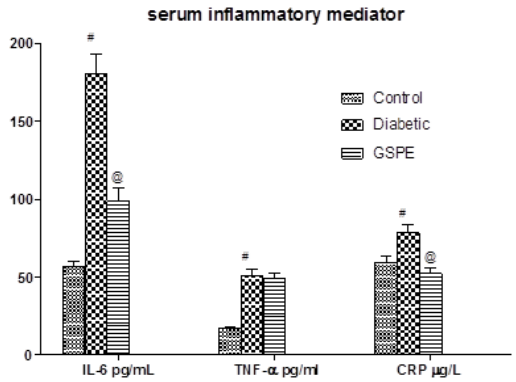

Figure 1: M ± SEM of serum inflammatory mediators among different studied groups.

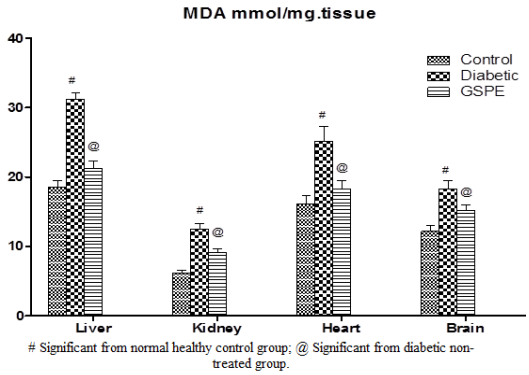

Figure 2: M ± SEM of MDA in different tissues mmol/mg, tissue among different studied groups.

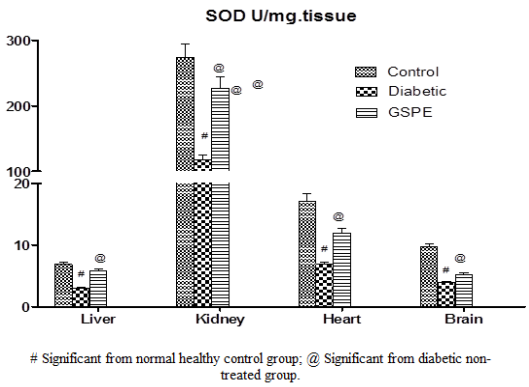

Figure 3: M ± SEM of SOD in different tissues U/mg tissue, among different studied groups.

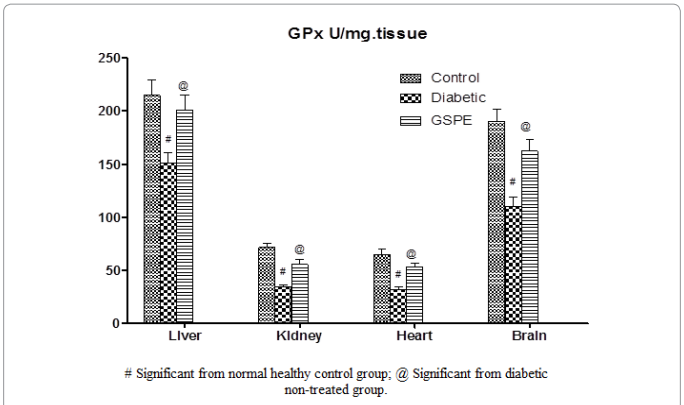

Figure 4: M ± SEM of GPx in in different tissues U/mg, tissue among different groups.

Liver and kidney functions

Also, Data in Table 2 illustrates the changes happen in liver and kidney functions. There was a significant increase in serum level of AST, ALT, ALP, creatinine and blood urea in diabetic rats in comparison with normal healthy control one. Treatment with GSPE was significantly ameliorated these parameters when related to diabetic non-treated group. On the other hand, serum level of total protein and albumin were significantly reduced in diabetic non-treated rats. Treatment with GSPE was significantly improving both parameters when related to diabetic non-treated group.

Inflammatory markers

Figure 1 demonstrates the inflammatory markers of different studied groups. IL-6, TNF-α, and hs-CRP were significantly higher in diabetic rats. Treatment with GSPE was significantly ameliorated serum level of IL-6 and hs-CRP in different studied groups when related to diabetic non-treated group. GSPE treatment did not affect serum level of TNF-α significantly when related to diabetic non-treated group.

Oxidative stress markers

Regarding to oxidative stress markers in different tissues. Figure 2 illustrate a significant increase in MDA level in liver, kidney, heart and brain tissues of diabetic non-treated group. Treatment with GSPE was significantly ameliorated tissue level of MDA when related to diabetic non-treated rats except heart tissue.

In respect to antioxidant enzymes in different tissues, Figures 3-5 demonstrate a significant reduction in SOD, GPx and catalase activity in different tissues of diabetic non-treated group. Treatments with GSPE were significantly improving these parameters when related to diabetic non-treated group.

In addition, Table 2 demonstrates serum level of MDA, SOD, GPx and catalase which revealed a significant increase in serum level of MDA in diabetic non-treated rats. Treatments with GSPE was significantly ameliorated MDA level compared to diabetic non-treated group. On the other hands, there were a significant reduction in serum antioxidant activity in diabetic animals which is a result of a decrease in the level of serum GPx, SOD and catalase. Treatments with GSPE was significantly improved the antioxidant enzymes activity when related to diabetic non-treated group.

Discussion

DM is associated with peripheral and central complications.

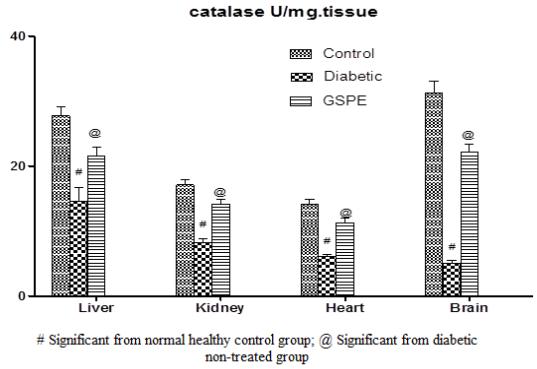

Figure 5: M ± SEM of catalase in different tissues U/mg, tissue among different studied groups.

Hyperglycemia induced oxidative stress is responsible for development and progression of diabetic mediated complications, possibly via the formation of free radicals [19,20]. Antioxidants have the capability to delay or halt the oxidation of cellular substrates. The antioxidants function to eliminate superoxide, and the activation of detoxifying proteins [21].

In this study, we aimed to evaluate the role of GSPE in decreasing oxidative stress as well as attenuating brain, heart, kidney and liver injury in STZ-induced diabetic rats.

The results of this study showed a significant reduction in the body weight of diabetic rats. Weight reduction is used as a marker of STZ-induced diabetes. It leads to abnormal metabolism of carbohydrate and lipid as well as elevated catabolism of structural proteins and increased muscle atrophy [22]. Supplementation with GSPE for 8 weeks significantly controls the body weight loss during diabetes. Weight gain may be is a result of anti-hyperglycemic properties [23]. These results were in agreement with previous reports of Taskinen et al. [24]. In addition, diabetic rats developed severe polyuria because of osmotic diuresis. However, there were significant decreases in urinary volume in treated group. This improvement is related to the normalization of plasma glucose level [25].

Our results showed a significant increased glucose level in diabetic group which significantly declined in treated groups. This elevation in glucose level is a result of decreased insulin levels in diabetic rats which is a result of β-cells destruction [26]. The hypoglycemic effect of GSPE is attributed to increasing circulating levels of insulin [25]. This finding was supported by previous study [27].

In addition, there were an increased level of 24 h urinary total proteins and albumin excretion in diabetic rat. These increases are significant criteria of early glomerular filtration barrier damage, disturbed basement membrane metabolism, and alterations in renal histology and hemodynamics [28]. One of probable mechanisms is the severity of oxidative stress [29]. The treated groups showed a significant decrease in urinary total proteins and albumin excretion levels. Results of our study were in agreement with other studies [30,31].

In addition, serum levels of TAG, TC and HDL-C were significantly affected in diabetic group (elevation in TC and TAG and reduction in HDL-C). Changes in the metabolism of plasma lipoprotein is an ordinary feature of diabetes, known as diabetic dyslipidemia [32,33]. Hyperlipidemia is a feature of overproduction and/or defective removal of one or more lipoproteins [34]. This results are in correspondence with other results on animals and humans [35,36]. Treatment with GSPE significantly ameliorated serum lipid profile of diabetic rats. These improvements are in harmony with the previous studies [25,37].

Moreover, a significant decrease in serum levels of total protein and albumin and increases of serum levels of ALT, AST, and ALP indicate the damage happen to liver cells in diabetic group [38]. It was reported that abnormalities in liver functions is more common in diabetic patients regarding non-diabetic one [39]. Also, STZ-induced diabetes causes liver tissue injury [40]. Treatment with GSPE significantly improves hepatic status. This may due to cellular regeneration and stability of cell membrane which in turn prohibit the permeation of intracellular enzymes [41].

In addition, increased protein metabolism, albuminuria and microproteinuria may attribute to the reduction in serum levels of total protein and albumin [42]. Also, this work showed elevation in the level of serum creatinine and urea in the diabetic rats. Increased urea and creatinine indicates progressive renal damage and diabetic nephropathy [43]. Also, high production of urea may be a result of increased proteins catabolism in liver and plasma [44]. The treatment with GSPE showed a significant reduction in creatinine and urea serum levels. these results were in correspondence with previous works [27,45].

Regarding to oxidative stress biomarkers in the serum and in liver, kidney, heart and brain homogenate, these results showed a significant decrease of the activity of antioxidant enzymes (GPx, SOD and catalase) and a significant MDA level elevation in diabetic rats. A marked reduction in MDA level with a significant increase in GPx, SOD and catalase activities were shown after GSPE administration. Wadsworth et al. [46] and Halliwell [47] stated that increased brain oxidative stress is linked to neurodegenerative diseases development. These results are supported by previous studies [15,16,48].

Several works stated that oxidative stress does a major function in the pathogenic pathway of diabetic injuries. Free radicals such as superoxide and lipid peroxidation can induce cell and tissue damage [45]. Oxidative stress in tissue is characterized by lipid peroxidation. O_2 reacts with PUFA to create lipid products as MDA, and a high production of free radicals. This series of reactions will lead to cell membrane injury, cell necrosis as well as inflammation [49]. SOD, GPx and catalase are scavenging antioxidants that protect the cells and tissue against injuries mediated by oxidative stress [50]. The insufficient antioxidants activities account for increased H_2O_2 and O_2^-, which in turn produce HO^-. SOD dismutases O_2^- to H_2O_2, then to H_2O by GPx or catalase [51]. The actions of SOD, GPx and catalase are integral. So, alteration in their activity will lead to lipid peroxides accumulation which will account for oxidative stress [52].

Due to glycation, hyperglycemia will decrease antioxidant enzymes activities and production [53]. Lower antioxidant defense is a known feature in diabetic patients [54]. In addition to its role in progression of diabetic complications via decreasing antioxidant activities, Oxidative stress is attributed to ROS overproduction, glucose autoxidation, dysfunction of glutathione metabolism, lipid peroxides formation and glycosylation of non-enzymatic protein [55].

In diabetes, β-oxidation of fatty acids is stimulated by fatty acyl CoA oxidase enzyme, which stimulated by low level of circulating insulin [56]. Increase of MDA formation is due to production of free radical species. These radicals attributed to a stimulated destruction of DNA, carbohydrates and lipids. This will lead to hyperglycemia and glucose autoxidation [17]. GSPE may exert there effect via improving oxidative stress status [45,57].

TNF-α inhibit the activity of kinase enzyme in insulin-signaling pathway. So, it affects this pathway in different insulin-responsive

tissues such as endothelial cells, skeletal muscle and fat [58]. TNF-α can elevate plasma TAG level [59], and stimulate fat cells lipolysis [60]. Also, states in insulin resistant contribute to the elevated level of IL-6 [20,61]. In the same manner, the vascular inflammation is characterized by increased hs-CRP [62].

Thus, TNF-α, IL-6 and hs-CRP play a significant function in insulin resistance and vascular inflammation process [62,63]. Oxidative stress stimulates overexpression of signaling genes, which in turn stimulate secretion of proinflammatory cytokines [64,65]. The results of this study demonstrated a significant elevation in serum levels of TNF-α, IL-6 and hs-CRP of diabetic rats. These results is supported by previous work [66,67]. The treatment with GSPE showed a significant reduction in IL6 and hs-CRP.

The result of this work emphasizes that the use of GSPE in diabetes has a great ameliorating effect against macrovascular and microvascular diabetic complications. It decreases lipid profile protecting from hypercholesterolemia, hypertriglyceridemia, atherosclerosis as well as cardiovascular disorders. In addition, GSPE can protect from nephropathy and affected liver functions as well as its protection from neurodegenerative diseases.

Conclusion

The present study showed the effectiveness of GSPE as adjuvant therapy for protection from diabetic complications in liver, kidneys, heart and brain. These effects are attributed to their potent antioxidant and anti-inflammatory activity.

Declarations

Conflict of interests

The authors declared that they have no conflict of interest.

Authors contributions

All authors read and approved the final manuscript.

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