

Protective effect of red grape seeds proanthocyanidins against induction of diabetes by alloxan in rats

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Abstract

It has been documented that impaired homeostasis in diabetes mellitus is associated with increased production of reactive oxygen species and depletion of the antioxidant defense systems. Natural grape seed proanthocyanidins (GSP) are potent free radical scavengers and hence provide significant protection against oxidative stress. Accordingly, the present study focused on investigating the possible protective role of GSP against free radical-mediated damage in pancreatic tissues of alloxan-induced diabetes in rats. The results revealed that oral administration of 50 and 100 mg kg⁻¹ (body weight) of GSP for 72 h significantly increased pancreatic glutathione (GSH) levels and inhibited the increase in lipid peroxidation caused by alloxan ($p < 0.001$). On the other hand, a significant reduction in pancreatic total nitrate/nitrite content ($p < 0.001$) was observed. Furthermore, GSP caused significant decline in the hyperglycemia induced by alloxan ($p < 0.001$). Such antihyperglycemic effect of GSP was accompanied by a significant increase in serum insulin levels in diabetic rats following 72 h of administration ($p < 0.001$). In conclusion, the study suggests that GSP are effective in ameliorating the damage to pancreatic tissue in experimental diabetes mellitus. Such effect may be related to their potent antioxidant properties as evidenced by the increase in pancreatic GSH and reduction of lipid peroxidation as well as total nitrate/nitrite levels.

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1. Introduction

Diabetes is a chronic metabolic disorder that continues to present a major worldwide health problem. It is characterized by absolute or relative deficiencies in insulin secretion and/or insulin action associated with chronic hyperglycemia and disturbances of carbohydrate, lipid, and protein metabolism. As a consequence of the metabolic derangements in diabetes, various complications develop including both macro- and microvascular dysfunctions [1]. New biochemical and molecular advances have contributed to a more profound understanding of the pathogenesis of diabetes and its complications [2]. Recently, increased oxidative stress has been proven to play a pivotal role in the etiology and pathogenesis of diabetes mellitus and its complications [3].

The role of oxidative stress in both type I and type II diabetes mellitus is currently under intensive scientific investigation [4–6]. It is believed that insulin-dependent diabetes mellitus (IDDM) results from the destruction of insulin-producing pancreatic β -cells by multiple factors, including viruses, chemical toxins, and autoimmune responses [7–9]. The exact cellular mechanism of β -cell destruction remains unclear. However, it has been established that locally produced reactive oxygen species (ROS) and nitric oxide (NO) induced after cytokine stimulation are involved [10,11]. Recent studies by Kaneto et al. [12] and Matsuoko et al. [13] have proven that ROS lead to damage of β -cells through the induction of apoptosis and suppression of insulin biosynthesis. Similarly, the development of type II diabetes has been associated with pancreatic β -cell dysfunction, and once hyperglycemia becomes apparent, β -cell function progressively deteriorates [14].

Previous studies have shown that sustained hyperglycemia, a characteristic of diabetes, increases intracellular

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ROS in pancreatic β -cells hence leading to cellular dysfunction [15,16]. Pancreatic β -cells are particularly susceptible to the deleterious effects of ROS because of their low expression of the antioxidant enzymes genes as compared to other tissues [17,18]. Hence, the cellular antioxidant status is an important determinant of its susceptibility to oxidative damage.

Reduced glutathione (GSH) is an endogenous antioxidant that acts as a first line defense system against prooxidant status [19]. Anathan et al. [20] showed a significant reduction in plasma GSH levels in experimental diabetic rats. Similarly, depleted GSH levels have been repeatedly reported in several tissues of experimental diabetic animals, including eye, aorta, kidney as well as small intestine [21–23]. Furthermore, significant decreases in plasma as well as erythrocyte GSH levels have been documented in diabetic patients [24,25].

Lipid peroxidation is a key marker of oxidative stress. It is the result of a chain reaction evoked by ROS and eventually leads to extensive membrane damage and dysfunction [19]. Significant increases in lipid peroxidation products, measured as thiobarbituric acid reactive substances (TBARS), have been reported in diabetes [26,27].

Nitric oxide (NO) has also been proposed to play a significant role in diabetes mellitus. Studies have revealed that NO is an important destructor and/or mediator for the insulinitis during diabetic development [28,29]. Mechanistic studies showed that the NO attack results in mitochondrial dysfunction and chromosomal DNA damage in pancreatic islet cells eventually damaging the cells and hence leading to failure in insulin secretion [30,31]. The increased oxidative stress experienced by tissues in diabetic patients as a result of hyperglycemia leads to loss of control over the NO production pathway. The overproduction of NO in such patients induces various complications and damage in many organs including the eye, kidney, and cardiovascular systems in both type I and type II diabetes [32].

Alloxan is a classical diabetogen that specifically damages pancreatic β -cells. It has been suggested that alloxan destroys β -cell function by inhibiting the enzyme glucokinase (GK) through oxidation of two thiol groups in the glucose-binding site of the enzyme [33]. In addition, there is substantial evidence that ROS participate in this destructive pathway [34]. It has been documented that alloxan and its reduced derivative dialluric acid are potent generators of superoxide anions and hydrogen peroxides. Redox cycling then proceeds in presence of traces of catalyzing metal ions leading to the generation of hydroxyl radicals from hydrogen peroxide [35]. The role of ROS in alloxan-induced destruction of β -cells has been substantiated by the finding that transgenic mice overexpressing antioxidants are protected against alloxan-induced diabetes [36]. Such data support the use of alloxan-induced diabetes as a model for the oxidative stress status experienced by diabetic patients.

As a result of the plethora of scientific evidence advocating the involvement of oxidative stress in the pathogenesis

of diabetes and its complications, interest has grown in the usage of natural antioxidants as a new strategy for alleviating the oxidative damage in diabetes. A recent study by Yilmaz et al. [37] revealed that the combination of alpha lipoic acid, ascorbic acid-6-palmitate, and fish oil reduced the oxidative stress in streptozotocin-induced diabetic rats by elevating the levels of reduced glutathione (GSH) and raising the level of unsaturated fatty acids. Similarly, β -carotene therapy for 14 days increased GSH levels in diabetic rats and exacerbated the increased glutathione peroxidase activity thus reducing oxidative stress [38]. Additionally, supplementation of antioxidant vitamin C has been shown to lower glycosylated hemoglobin in diabetic patients [39]. 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 significant antioxidant activity. Sabu and Kattan [40] reported that ellagic and gallic acid derivatives possess potent free radical scavenging properties that correlated well with their anti-diabetic effects. Polyphenolic flavonoids, widely distributed in plants, have been recognized for their interesting clinical properties. Several of these flavonoids, including silymarin, catechin, and quercetin, have shown protective effects in experimental diabetes by enhancing the activity of antioxidant enzymes [20,41,42].

Natural grape seed proanthocyanidins (GSP) are a combination of biologically active polyphenolic flavonoids including oligomeric proanthocyanidins [43]. These proanthocyanidins have demonstrated a marked spectrum of biological, pharmacological, therapeutic, and chemoprotective properties against oxygen free radicals and oxidative stress [44–46]. Bagchi et al. [47] have discovered that GSP provide significantly greater protection against free radicals and free radical-induced lipid peroxidation and DNA damage than vitamins C, E and β -carotene. Such 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 thus undertaken to assess the protective effect of GSP on oxidative damage induced by alloxan in rat pancreatic tissue and their possible role in ameliorating the development of diabetes. The results of the study could serve as a step toward the development of a mechanism-based therapeutic approach for the management of diabetes and hence provide the basis for the usefulness of the potent antioxidants, GSP.

2. Materials and methods

2.1. Animals

Adult male Wistar rats (200–220 g) were procured from the animal house facility at King Saud University. All animals were housed in cages with 12/12 h light/dark cycle at $21 \pm 2^\circ\text{C}$. The animals were given Purina rat chow and water

ad libitum. The animals were kept under observation for one week prior to the start of treatment. All animal experiments were carried out in accordance with King Saud University Ethical Committee Acts.

2.2. Chemicals

Grape seed proanthocyanidins (GSP) (95% purity) were purchased from Alpha-medicine (Egypt). All other chemicals and reagents were of analytical grade and were obtained from Sigma–Aldrich Chemical Co. (St. Louis, USA).

2.3. Induction of diabetes

The animals were fasted for 24 h prior to the induction of diabetes. Alloxan monohydrate, freshly prepared in normal saline, was immediately injected intravenously (150 mg kg^{-1} BW) through tail vein to induce diabetes. This dose of alloxan was previously tested and proven to increase blood glucose level above 200 mg dl^{-1} .

2.4. Experimental design

A total of 80 rats were used and were divided into eight groups of 10 rats each. The groups were divided as follows: group 1, control rats (vehicle only); group 2, untreated diabetic (alloxan induced then vehicle only); groups 3–8 were given GSP (50 mg kg^{-1} BW for groups 3–5 or 100 mg kg^{-1} BW for groups 6–8) 1 h prior to alloxan injection. The GSP was given in normal saline solution by oral gavage. GSP treatment was continued for the respective groups for either 24, 48, or 72 h once daily. At the end of the experimental period (after 24, 48, or 72 h of alloxan injection), the animals were fasted overnight (18 h) and then sacrificed by decapitation, blood was collected, and the pancreas was dissected out. The blood was centrifuged at 3000 rpm for 20 min and the clear serum separated. Dissected tissues and sera were kept at -80°C until further analysis.

2.5. Biochemical analyses

2.5.1. Estimation of serum glucose and insulin

Serum glucose was estimated using a commercially available kit (Adamco Ltd., SA) according to the method described by Trinder [48]. Serum insulin level was determined with an enzyme-linked immunosorbant assay (ELISA) kit (Biosource, Europe) [49].

2.5.2. Measurement of pancreatic lipid peroxidation

The level of thiobarbituric acid reactive substances (TBARS), a commonly used marker for lipid peroxidation and malondialdehyde (MDA) production, was measured spectrophotometrically by the method of Uchiyama and Mihara [50]. The results were expressed as nmol MDA per gram wet weight.

2.5.3. Determination of pancreatic total nitrate/nitrite level

Total nitrate/nitrite content, an indicator of NO production, was estimated in pancreatic tissue homogenate based on Griess reaction [51] according to the procedures of a commercially available kit (R&D Systems, UK).

2.5.4. Estimation of total pancreatic glutathione

Total glutathione was measured in pancreatic tissue homogenate by the reaction of the sulfhydryl groups (SH) in the non-protein fractions of pancreatic tissues with 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB or Ellman's reagent). The product was measured spectrophotometrically at 412 nm [52].

2.6. Statistical analysis

The results were analyzed by one-way ANOVA test followed by Tukey's multiple-comparison post hoc test. Graph-Pad Prizm 3.0 software was used. The results were expressed as mean \pm S.E.M., $n = 10$. p -Values < 0.05 were considered to be statistically significant.

3. Results

3.1. Effect of GSP on serum glucose and insulin

As shown in Fig. 1, alloxan treatment produced significant increase in serum glucose level with respect to the control group. The hyperglycemia was more pronounced after 48 h. The administration of GSP significantly reduced the increase in serum glucose concentration induced by alloxan. Such effect was more obvious with the high dose of GSP (100 mg kg^{-1} BW) and seemed to be time dependent. The protective effect of GSP on β -cell function, as evidenced by the elevated levels of serum insulin, was prominent only following 72 h of alloxan treatment, whereas the high dose of

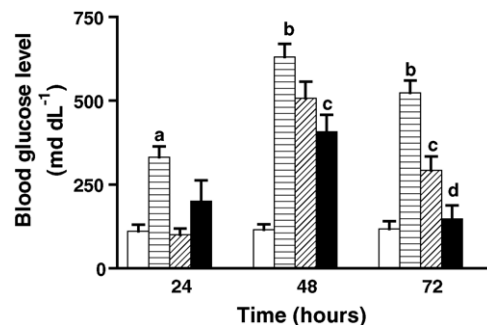


Fig. 1. Effect of red grape seeds proanthocyanidins (GSP) on fasting serum glucose level in alloxan-induced diabetic rats: (□) control; (▨) untreated diabetic; (▧) diabetes + 50 mg GSP; (■) diabetes + 100 mg GSP. Values are represented as means \pm S.E. ($n = 10$). Untreated diabetic animals were compared to control group, ^a $p < 0.05$, ^b $p < 0.001$. GSP-treated groups were compared with their respective untreated diabetic group, ^c $p < 0.05$, ^d $p < 0.001$.

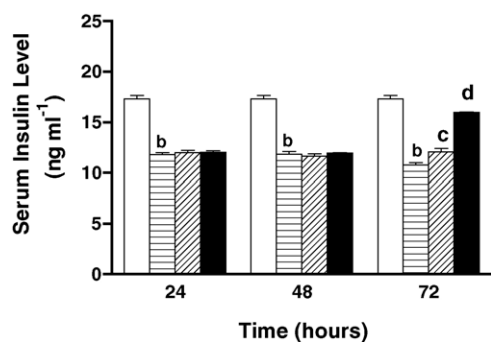


Fig. 2. Effect of red grape seeds proanthocyanidins (GSP) on serum insulin level in alloxan-induced diabetic rats: (□) control; (▨) untreated diabetic; (▧) diabetes + 50 mg GSP; (■) diabetes + 100 mg GSP. Values are represented as means \pm S.E. ($n = 10$). Untreated diabetic animals were compared to control group, ^b $p < 0.001$. GSP-treated groups were compared with their respective untreated diabetic group, ^c $p < 0.05$, ^d $p < 0.001$.

GSP antagonized the severe hypoinsulinemia induced by alloxan and nearly normalized the serum insulin level (Fig. 2).

3.2. Effect of GSP on pancreatic lipid peroxidation

Alloxan produced a significant increase in pancreatic malondialdehyde (MDA) level following 72 h of diabetes induction ($p < 0.05$). The administration of GSP ameliorated the alloxan-induced elevation in lipid peroxidation. Moreover, the high dose of GSP normalized the value of MDA production as compared to control rats. The results are shown in Fig. 3.

3.3. Effect of GSP on total pancreatic nitrate/nitrite content

Fig. 4 demonstrates that alloxan caused a significant increase in total pancreatic nitrate/nitrite content especially after 48 and 72 h compared to the control group (73% and 201%, respectively). Diabetic animals treated with GSP showed a significant reduction in the pancreatic nitrate/nitrite

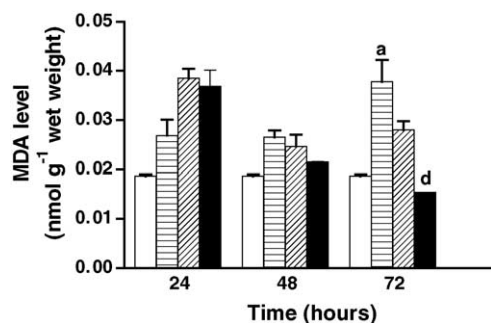


Fig. 3. Effect of red grape seeds proanthocyanidins (GSP) on pancreatic malondialdehyde (MDA) level in alloxan-induced diabetic rats: (□) control; (▨) untreated diabetic; (▧) diabetes + 50 mg GSP; (■) diabetes + 100 mg GSP. Values are represented as means \pm S.E. ($n = 10$). Untreated diabetic animals were compared to control group, ^a $p < 0.05$. GSP-treated groups were compared with their respective untreated diabetic group, ^d $p < 0.001$.

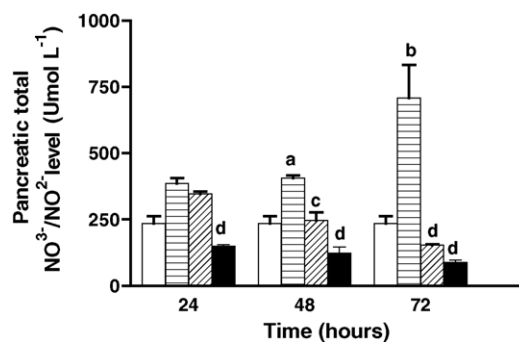


Fig. 4. Effect of red grape seeds proanthocyanidins (GSP) on pancreatic nitrate/nitrite level in alloxan-induced diabetic rats: (□) control; (▨) untreated diabetic; (▧) diabetes + 50 mg GSP; (■) diabetes + 100 mg GSP. Values are represented as means \pm S.E. ($n = 10$). Untreated diabetic animals were compared to control group, ^a $p < 0.05$, ^b $p < 0.001$. GSP-treated groups were compared with their respective untreated diabetic group, ^c $p < 0.05$, ^d $p < 0.001$.

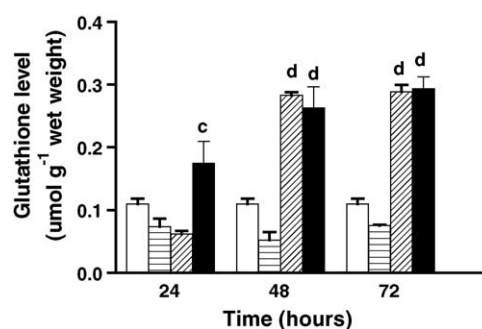


Fig. 5. Effect of red grape seeds proanthocyanidins (GSP) on pancreatic glutathione level in alloxan-induced diabetic rats: (□) control; (▨) untreated diabetic; (▧) diabetes + 50 mg GSP; (■) diabetes + 100 mg GSP. Values are represented as means \pm S.E. ($n = 10$). GSP-treated groups were compared with their respective untreated diabetic group, ^c $p < 0.05$, ^d $p < 0.001$.

level. Such effect was obvious at both doses used following 48 as well as 72 h.

3.4. Effect of GSP on pancreatic glutathione (GSH) level

Data represented in Fig. 5 illustrate that alloxan treatment consistently reduced pancreatic GSH content by approximately 38% as compared to the control animals. Although this effect was non-significant, treatment with GSP significantly elevated the pancreatic GSH level starting 24 h post treatment ($p < 0.05$). The GSH content reached a maximum level (approximately 250% increase) at 48 h and this effect was sustained until 72 h following treatment ($p < 0.001$).

4. Discussion

Alloxan-induced diabetes is a well-documented model of experimental diabetes. This compound causes severe necrosis of pancreatic β -cells [53]. This effect was previously explained on the basis of alloxan's ability to produce hydrogen

peroxide and other free radicals, including O_2^\bullet and $\bullet OH$ that damage β -cells hence leading to their death [54]. The sensitivity of β -cells to oxidative stress has been attributed to their low levels of antioxidants compared with other tissues [17]. Beta cell dysfunction eventually culminates in reduction in insulin release leading to hyperglycemia. The alloxan-induced sustained hyperglycemia aggravates the oxidative stress status by autooxidation of glucose and its primary and secondary adducts [55]. Furthermore, evidence suggests that oxidative stress induced by hyperglycemia may constitute the key and common events in the pathogenesis of different diabetic complications [56]. Accordingly, maintenance of β -cell oxidant status and their protection against oxidative damage might delay the onset of diabetes as well as the progression of its complications.

The current study revealed that alloxan significantly induced hyperglycemia accompanied by hypoinsulinemia. Such effect might be explained by the possible pancreatic damage caused by the observed significant rise in lipid peroxidation as well as total nitrate/nitrite content. Interestingly, GSP restored the oxidant status of pancreatic tissue and prevented the hyperglycemia and hypoinsulinemia induced by alloxan. Such results suggest a protective effect of GSP against alloxan action.

The observed increase in the level of lipid peroxides in alloxan-treated rats 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 autooxidation [57]. These results are in agreement with previous findings whereby alloxan-treated rats showed marked increase in pancreatic cells lipid peroxidation [58,59]. The marked protective effect of GSP against pancreatic tissue lipid peroxidation observed in this study is consistent with previously published reports. Whereby, GSP offered significant decreases in lipid peroxidative damage in brain, liver, as well as gastrointestinal mucosa subjected to different stress models [44,60].

The data presented suggest a notable contribution of NO to the overall free radical pool of the diabetic pancreas, which could result in secondary reactions leading to an elevated steady-state free radical concentration in this organ. Whereby, alloxan-treated rats showed significant elevation of total pancreatic nitrate/nitrite levels. Such findings coincide with previously published studies that proved that production of NO by β -cells, in presence of alloxan, has been implicated in the development of diabetes [61,62]. Nitric oxide reacts with superoxide radical to form the noxious peroxynitrite that contribute in the pathogenesis of diabetic complications [63]. Recently, Stadler et al. [64] reported an increased level of NO in kidneys of streptozotocin-induced diabetic rats, thus supporting the role of NO, ROS, and peroxynitrite-derived species in the development of early diabetic tissue injury.

The data presented revealed a marked protective effect of GSP against alloxan-induced elevation of total nitrate/nitrite level in pancreatic tissue. Whereby, concurrent treatment with

GSP normalized the pancreatic NO levels. The effect of GSP on NO was recently studied by Johnson-Varghese et al. [65]. Their study demonstrated that GSP offered protection against hyperoxic and NO-mediated injury to fetal rat type II pneumocytes. Furthermore, pretreatment with grape seed proanthocyanidins extract exerted cytoprotective effect in rat glial cultures against nitrosative oxidative stress [66].

In this study, alloxan treatment led to depletion of pancreatic GSH content, although such effect was non-significant. Depletion of reduced glutathione (GSH) could significantly affect the overall redox potential of the cell. Previous studies have documented that GSH level was reduced in diabetic patients and in experimental models [2,22,23].

In the current study, the decrease in pancreatic GSH effect was reversed by the administration of GSP. In addition, GSP elevated the level of GSH significantly compared to the control group. A possible explanation for this effect is that these compounds function as free radical scavengers and therefore increase the available free GSH which detoxify the reactive intermediary oxygen products of lipid peroxidation induced by alloxan [45]. Another plausible mechanism is that GSP induced the production of GSH by pancreatic cells. A study by Zou et al. [67] has shown that GSP effectively delayed oxidative insult to human erythrocytes induced by 2,2'-azobios-(amidinopropane) dihydrochloride. Such effect was strongly associated with significant protection against erythrocyte GSH loss.

In summary, the results of the present study indicate that GSP possess potent protective effect on the induction of diabetes by alloxan. The data provided suggest that the mechanism underlying such protection is mediated via prevention and restoration of pancreatic antioxidant defense systems. Restoration of pancreatic antioxidant status led to normalization of the release of insulin, hence maintaining glucose serum levels within the normal range. Moreover, the role of GSP in stimulating insulin release by pancreatic β -cells cannot be ignored. An additional mechanism of the antihyperglycemic action of GSP may be through stimulating the surviving pancreatic cells to release more insulin. In this context, other plant materials have been shown to exhibit insulin-release stimulatory effect [68]. A recent study by Pinet et al. [69] has also shown that grape-seed procyanidins possess an insulinomimetic activity on insulin sensitive cell lines.

5. Conclusion

Based on the oxidative stress hypothesis of alloxan action, it was considered as an adequate model for investigating the role of free radicals in the pathology of diabetes mellitus. The present study demonstrates that GSP, a potent antioxidant, can exert anti-diabetic effects by preserving pancreatic β -cell function. The data presented provide additional benefits of GSP administration and may offer a promising natural and safe new trend for the prevention or delay of diabetic complications.

Acknowledgements

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