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ORIGINAL ARTICLE

Grape seed extract attenuates hyperglycaemia-induced in rats by streptozotocin

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Abstract The current study aimed to investigate the possible beneficial effects of grape seed extract (GSE) on some metabolic and biochemical changes associated with streptozotocin (STZ; 50 mg/kg; i.p.)-induced hyperglycaemia in male rats. Blood samples were used to determine serum levels of glucose, insulin, total cholesterol (TC) and triglycerides (TG). Some biochemical markers for oxidative stress viz., serum lipid peroxides level (measured as malondialdehyde; MDA) and total anti-oxidant capacity as well as serum nitric oxide (NO) level were assessed. Hyperglycaemic animals received GSE (100 and 300 mg/kg/day) orally on daily basis for 28 consecutive days and their effects were determined 24 h after the administration of the last dose. Results of the present study revealed that STZ-induced hyperglycaemia is associated with decreased serum insulin level with increased levels of TC and TG. Hyperglycaemia was also associated with increased level of serum MDA together with decreased total antioxidant capacity and level of serum NO. GSE succeeded to improve the serum glucose level in STZ-treated rats in a dose dependent manner. It also showed a restoration of the increased serum level of TC, TG and MDA and of the suppressed insulin and total antioxidant capacity as well as the decreased plasma level of NO. From our results it can be concluded that GSE has beneficial effects against the biochemical changes associated with STZ-induced hyperglycaemia. These beneficial effects might be related to the ability of GSE to improve hyperglycaemia in addition to its anti-oxidant property.

1. Introduction

Diabetes mellitus (DM) is one of the most common chronic diseases and is associated with some morbidities such as obesity and hypertension.[1,2] It is a progressive disease constituting a complex syndrome involving insulin dysfunction and is initially characterized by gross abnormalities in glucose homoeostasis.[3] DM is characterized by chronic hyperglycaemia resulting from defects in insulin secretion and/or insulin action. The chronic hyperglycaemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially eyes, kidneys, nerves, heart, and blood vessels.[4]

DM is amongst the silent killers, since many people are not aware that they have the disease until they develop one of its life-threatening complications.[5] It well documented that DM

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not only contributes to the hyperglycaemia, but may play a role in other metabolic abnormalities. These include high levels of plasma triglycerides (TG), low levels of high density lipoproteins, hypertension, abnormal fibrinolysis, and coronary heart disease. This constellation of abnormalities is often referred to as the metabolic syndrome.[6,7]

There is considerable evidence that oxidative stress resulting from increased production and/or inadequate removal of free radicals including reactive oxygen species (ROS) plays a key role in the pathogenesis of late diabetic complications.[8] It has been reported that in uncontrolled diabetes, the levels of endogenous anti-oxidants such as superoxide dismutase, vitamin E, and lipoic acid are markedly reduced. Enhanced oxidative stress status arising from excess release of ROS has been documented in diabetic animals and cells cultured under high glucose conditions,[9,10] and associated with the pathogenesis of diabetic endothelial dysfunction. ROS may be generated as a result of prolonged exposure of cells to hyperglycaemia that results in non-enzymatic glycation of plasma proteins[11] which then undergo further spontaneous reactions to produce free radicals such as superoxide anion, the foundation molecule of other ROS.[12]

Superoxide anion is also formed by enzymatic activities of cyclooxygenase, xanthine oxidase, uncoupled endothelial nitric oxide synthase (eNOS) and NADPH oxidase.[13] Amongst these enzymes NADPH oxidase enzyme system has attracted much of the attention. It has been characterized as the main source of ROS in coronary microvascular endothelial cells[14] and has later been coupled to oxidative stress-mediated endothelial dysfunction in the central retinas of diabetic rats.[15]

Grape Seed Extract (GSE) are industrial derivatives from whole grape seeds that have a great concentration of vitamin E, flavonoids, linoleic acid and phenolic oligo proanthocyanidins (also known as procyanidins), polymers of catechin.[16] Proanthocyanidins were shown to have cardioprotection against cardiac ischemia[17] and have a role in lowering cholesterol levels.[18] However, many other benefits of GSE proanthocyanidins have also been uncovered including potent antioxidant activity and cytotoxicity to cancer cells.[17]

The present study was devoted to investigate the influence of grape seed extract (GSE) on some metabolic and biochemical changes associated with hyperglycaemia in streptozotocin (STZ)-treated rats. Gliclazide was also used as a reference drug. Hyperglycaemia was induced by a single intraperitoneal (i.p.) injection of streptozotocin. The extract was administered orally once per day for 28 consecutive days and its effect was evaluated 24 h after the administration of the last dose. For the assessment of the metabolic changes, the serum levels of glucose, insulin, total cholesterol (TC) and TG were determined. For the assessment of the biochemical changes, the blood levels of some relevant biomarkers for oxidative stress and NO were determined. Plasma lipid peroxides level (measured as malondialdehyde; MDA) and total antioxidant capacity activity were taken as *in vivo* reliable indices for the contribution of free radical generation and in turn, oxidative stress in STZ-induced hyperglycaemia. Plasma nitrate/nitrite level was used as a convenient marker for NO formation. The effects produced by these treatments are compared with the standard drug gliclazide.

2. Materials and Methods

2.1. Drugs and chemicals

Grape seed extract (Mepaco, Egypt) and gliclazide (Servier, Egypt) were used in the present investigation. The drugs were freshly prepared in distilled water and given orally. The concentration of either drug was adjusted so that each 100 g animal body weight received 0.5 ml, containing the required dose. Streptozotocin (STZ), N-(1-Naphthyl) ethylene-diamine dihydrochloride (NEDD) were purchased from Sigma–Aldrich, USA. Thiobarbituric acid (TBA) and Sulphanilamide were purchased from Fluka (Italy) and Merck (Germany), respectively. All other chemicals were of the highest commercially available grade.

2.2. Animals

Adult male albino rats, weighing 180–200 g, were used in all experiments of this study. They were obtained from the Animal House Colony of the National Research Center (Dokki, Giza, Egypt), and were housed under conventional laboratory conditions throughout the period of experimentation. The animals were fed standard rat pellet diet and allowed free access to water. This study was conducted in accordance with ethical procedures and policies approved by the Animal Care and Use Committee of National Research Center.

2.3. Induction of hyperglycaemia

Hyperglycaemia was induced by a single i.p. injection of STZ (50 mg/kg).[19] Rats were weighed and injected with STZ dissolved in a citrate buffer (0.1 M, pH 4.5). After 48 h blood samples were withdrawn from the retro-orbital venous plexus under light ether anaesthesia and the serum was separated by centrifugation for the determination of glucose level. Only rats with serum glucose levels more than 250 mg/dl were selected and considered as hyperglycaemic animals that have been subjected to further experimentation.

2.4. Experimental design

Hyperglycaemic rats were weighed and randomly allocated into four groups (8–10 rats each). One group served as a hyperglycaemic control, another group was treated with gliclazide 5 mg/kg as a reference drug while the other two groups were treated orally with GSE (100 mg/kg/day) and GSE (300 mg/kg/day)[20] for 28 consecutive days, respectively. Drug treatment was started 48 h after STZ injection (time at which hyperglycaemia was confirmed). In addition, a universal normal group which received only the citrate buffer (8–10 rats) was used. Twenty-four hours after the last dose of either drug treatment, serum was collected for the determination of serum levels of insulin, TC, TG, MDA and NO levels as well as total antioxidant capacity. Serum glucose level was measured thrice, the first 48 h after STZ injection, the second after 14 day treatment period and the third at the end of the experiment.

2.4.1. Assessment of metabolic and biochemical parameters

From each 18 h food-deprived rat, blood sample was withdrawn from the retro-orbital venous plexus using heparinized capillary tubes. The blood samples were centrifuged at 3000 rpm for 10 min and the sera were obtained. An aliquot of the separated serum was then used for the determination of glucose level, TC and TG while the rest was stored at −70 °C for the subsequent determination of insulin, MDA, NO levels and the total antioxidant capacity.

2.4.2. Determination of serum glucose level

Glucose level was determined as quinine amine using a test reagent kit (Stanbio, USA) according to the method of Tinder (1969).[21] The absorbance was measured at 510 nm and the results were expressed as mg/dl.

2.4.3. Determination of serum insulin level

Serum insulin concentration was determined by enzyme-linked immunosorbent assay by using a diagnostic kit (Crystalchem, USA) according to the method of Judzewitsch et al. (1982).[22] The absorbance was measured at 450 nm using ELISA reader and the results were expressed as μIU/ml.

2.4.4. Determination of serum triglyceride level

Triglycerides were estimated by enzymatic methods by using diagnostic kit (Biodiagnostic, Egypt) according to the method of Fossati and Prencipe (1982).[23] The absorbance was measured at 510 nm and the results were expressed as mg/dl.

2.4.5. Determination of serum total cholesterol level

Total cholesterol was estimated by enzymatic methods by using diagnostic kit (Biodiagnostic, Egypt) according to the method of Allain et al. (1974).[24] The absorbance was measured at 500 nm and the results were expressed as mg/dl.

2.4.6. Determination of serum lipid peroxide level

Lipid peroxide level was determined as thiobarbituric acid (TBA)-reactive substances according to the method of Mihara and Uchiyama (1978).[25] The absorbance was measured at 534 nm and the results were expressed as nmol/ml.

2.4.7. Determination of serum nitric oxide level

The total amount of NO was indirectly estimated in terms of its main metabolites, nitrate and nitrite by the Griess reaction using NEDD and sulphanilamide as described by Miranda et al. (2001).[26] The absorbance was measured at 540 nm and the results were expressed as nmol/ml.

2.4.8. Determination of total antioxidant capacity

Total antioxidant capacity was determined by ability to eliminate a certain amount of the provided H_2O_2 using a test reagent kit (Biodiagnostic, Egypt) according to the method of Koracevic et al. (2001).[27] The absorbance was measured at 510 nm and the results were expressed as mM/l.

3. Statistical analysis

Data are expressed as means ± SEM. Statistical significance was taken as $P < 0.05$ for all experiments, using one way analysis of variance (ANOVA) followed by Tukey's multiple comparison test to judge the difference between various groups for the parametric parameters.

4. Results

4.1. Metabolic parameters

4.1.1. Effect of GSE on serum glucose level

A single i.p. injection of STZ (50 mg/kg) produced an elevation of serum glucose level which was evidenced 48 h after administration. The elevation was found to be persistent during the period of investigation and reached the average value of 409% of the normal ones. Oral treatment of hyperglycaemic rats with GSE (100 mg/kg/day) and (300 mg/kg/day) for 2 weeks decreased the elevated serum glucose level reaching about 194% and 174% of the normal values, respectively. Similar treatment with gliclazide decreased the elevated serum glucose level reaching about 190% of the normal value. Oral treatment of hyperglycaemic rats with GSE 100 mg/kg/day

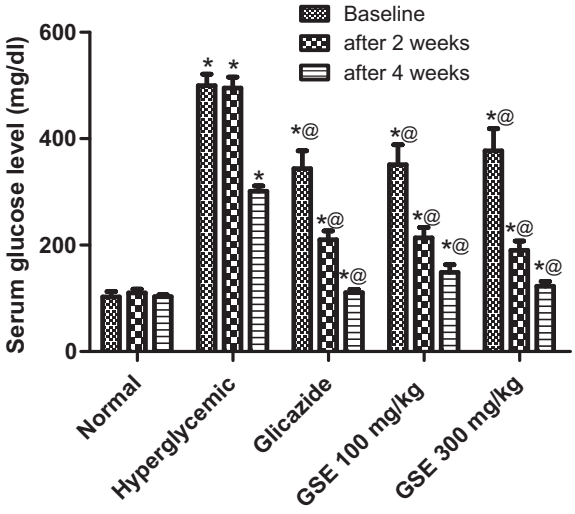

Figure 1 Effect of grape seed extract on serum glucose level in streptozotocin-treated rats. Rats were rendered hyperglycaemic by a single i.p. injection of streptozotocin (STZ; 50 mg/kg). Grape seed extract (GSE; 100 mg/kg and 100 mg/kg; p.o.) was administered for 28 consecutive days. Treatment with either drug was started 48 h after STZ injection. Blood samples from 18 h food-deprived animals were withdrawn using heparinized capillary tubes and serum was used for glucose determination 48 h after STZ injection, after 14 consecutive days of treatment and twenty-four hours after the last dose. Results are expressed as means ± SEM (n = 6–10). *Significant difference from normal rats $P < 0.05$. @ Significant difference from hyperglycaemic rats $P < 0.05$.

and 300 mg/kg/day for 4 weeks succeeded to cause a decrease in the elevated serum glucose level reaching about 144% and 119% of the normal values, respectively. Similar treatment with gliclazide succeeded to cause a decrease in the elevated serum glucose level reaching nearly the normal value (Fig. 1).

4.1.2. Effect of GSE on serum insulin level

Induction of hyperglycaemia with a single i.p. injection of STZ (50 mg/kg) was associated with a decrease in the serum insulin level. The decreased level reached about 6% of the normal value. Oral treatment of hyperglycaemic rats with GSE (100 mg/kg/day) and (300 mg/kg/day) for 4 weeks caused an increase in the serum insulin level reaching about 12% and 15% of the normal values, respectively. Similar treatment with gliclazide caused a decrease in the serum insulin level reaching about 40% of the normal value (Fig. 2).

4.1.3. Effect of GSE on serum TC level

Induction of hyperglycaemia with a single i.p. injection of STZ (50 mg/kg) was associated with an increase in the serum TC level. The increased level reached about 170% of the normal value. Oral treatment of hyperglycaemic rats with GSE (100 mg/kg/day) did not show any change in the serum TC level, however treatment with GSE (300 mg/kg/day) for 4 weeks showed a decrease in the serum TC level reaching about 114% of the normal value. Similar treatment with gliclazide succeeded to cause a decrease in the elevated serum TC level reaching nearly the normal value (Table 1).

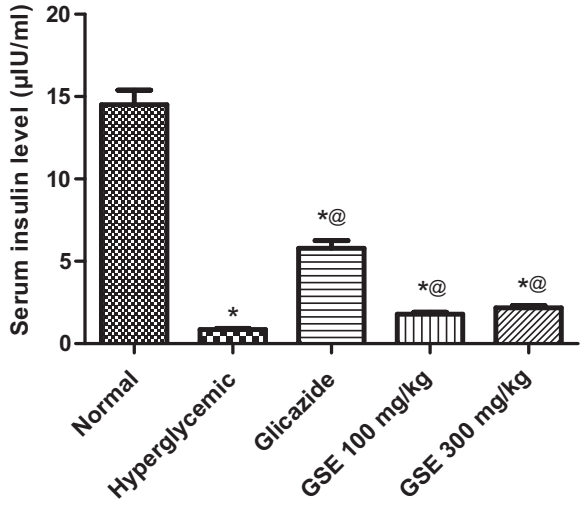

Figure 2 Effect of grape seed extract on serum insulin level in STZ-treated rats. Rats were rendered hyperglycaemic by a single i.p. injection of streptozotocin (STZ; 50 mg/kg). Grape seed extract (GSE; 100 mg/kg and 100 mg/kg; p.o.) were administered for 28 consecutive days weeks. Treatment with either drug was started 48 h after STZ injection. Twenty-four hours after the last dose, blood samples from 18 h food-deprived animals were withdrawn using heparinized capillary tubes and serum was used for insulin determination. Results are expressed as means ± SEM (n = 6–10). *Significant difference from normal rats $P < 0.05$. @ Significant difference from hyperglycaemic rats $P < 0.05$.

4.1.4. Effect of GSE on serum TG level

Induction of hyperglycaemia with a single i.p. injection of STZ (50 mg/kg) was associated with an increase in the serum TG level. The increased level reached about 260% of the normal value. Oral treatment of hyperglycaemic rats with GSE (100 mg/kg/day) did not show any change in the serum TG level, however treatment with GSE (300 mg/kg/day) for 4 weeks showed a decrease in the serum TG level reaching about 215% of the normal value. Similar treatment with gliclazide succeeded to cause a decrease in the elevated serum TC level reaching nearly the normal value (Table 1).

4.1.5. Effect of GSE on serum MDA level

Induction of hyperglycaemia with a single i.p. injection of STZ (50 mg/kg) was encountered with an elevated plasma MDA level. This elevation reached about 3-fold rise of the normal value. Oral treatment of hyperglycaemic rats with GSE 100 mg/kg/day and 300 mg/kg/day for 4 weeks showed a decrease in the serum MDA level reaching about 252% and 216% of the normal values, respectively. Similar treatment with gliclazide succeeded to cause a decrease in the elevated serum MDA level reaching nearly the normal value (Table 1).

4.1.6. Effect of GSE on serum NO level

Induction of hyperglycaemia with a single i.p. injection of STZ (50 mg/kg) was accompanied with a decreased plasma NO level. This decrease reached about 44% of the normal value. Oral treatment of hyperglycaemic rats with GSE 100 mg/kg/day and 300 mg/kg/day for 4 weeks showed an increase in the serum NO level reaching about 77% and 87% of the normal values, respectively. Similar treatment with gliclazide succeeded to cause a decrease in the elevated serum NO level reaching about 81% of the normal value (Table 1).

4.1.7. Effect of GSE on serum total antioxidant capacity

Induction of hyperglycaemia with a single i.p. injection of STZ (50 mg/kg) was accompanied with a decreased plasma NO level. This decrease reached about 39% of the normal value. Oral treatment of hyperglycaemic rats with GSE 100 mg/kg/day and 300 mg/kg/day for 4 weeks showed an increase in the serum NO level reaching about 86% and 89% of the normal values, respectively. Similar treatment with gliclazide succeeded to cause a decrease in the elevated serum NO level reaching about 88% of the normal value (Table 1).

5. Discussion

Results of the present study revealed that STZ-induced hyperglycaemia is associated with some metabolic and biochemical changes. Streptozotocin-induced hyperglycaemia in animals is considered to be a potent model for the preliminary screening of compounds active against diabetes and is widely used.[28] STZ, N-{methylnitrocarbamoyl}-d-glucosamine, is a potent DNA methylating agent and acts as a NO donor in pancreatic cells.[29] It selectively destroys the islets of Langerhans by oxidant production and producing inappropriate NO response.[30] It has been shown that toxic action of STZ is due to the generation of free radicals[31] which irreversibly destruct the β-islet cells of the pancreas, causing degranulation or reduction of insulin secretion. Low doses of STZ (50 mg/kg) only destroy

Table 1 Effect of grape seed extract on serum levels of triglycerides, total cholesterol, nitric oxide, malondialdehyde and total antioxidant capacity in STZ-treated rats.

	Normal	Diabetic	Glicazide	GSE 100 mg/kg	GSE 300 mg/kg
TC	72.67 ± 3.57	123.06 ± 9.33*	67.21 ± 2.10@	123.06 ± 9.33*	82.95 ± 7.95@
TG	51.16 ± 2.40	133.12 ± 11.29*	49.92 ± 1.85@	120.00 ± 11.04*	110.34 ± 6.57*@
NO	89.33 ± 4.90	38.94 ± 2.11*	72.23 ± 2.35*@	68.61 ± 3.37*@	77.87 ± 7.13*@
MDA	74.03 ± 3.26	216.98 ± 1.19*	86.64 ± 2.40@	186.69 ± 17.82*@	159.93 ± 9.59*@
Total antioxidant capacity	0.27 ± 0.019	0.16 ± 0.013*	0.36 ± 0.033*@	0.34 ± 0.027*@	0.35 ± 0.024@

Rats were rendered hyperglycaemic by a single i.p. injection of streptozotocin (STZ; 50 mg/kg). Grape seed extract (GSE; 100 mg/kg and 100 mg/kg; p.o.) was administered for 28 consecutive days weeks. Treatment with either drug was started 48 h after STZ injection. Twenty-four hours after the last dose, blood samples from 18 h food-deprived animals were withdrawn using heparinized capillary tubes and serum was used for determination of TG, TC, NO, MDA and total antioxidant capacity. Results are expressed as means ± SEM (n = 6–10).

* Significant difference from normal rats $P < 0.05$.

@ Significant difference from hyperglycaemic rats $P < 0.05$.

some population of pancreatic β-cells giving rise to type 2 DM.[29]

Persistent hyperglycaemia, the common characteristic of DM, is the cause of the most diabetic complications which can be normalized by the action of insulin.[32] Blood glucose level is strictly controlled by insulin secretion from pancreatic cells and insulin action on liver, muscle and other target tissues.[33]

In the present study, oral treatment of STZ-treated rats with GSE succeeded to lower the serum glucose levels probably partly due to its effect on insulin secretion and partly due to its insulin sensitizer effect. This was in accordance with Suwannaphet and his colleges who showed that GSE prevents body weight gain, hyperglycaemia and hyperinsulinaemia due to its ability to attenuate the impairment of insulin-stimulated glucose disposal in rats with insulin resistance.[34] Studies showed that GSE strongly inhibited both α-amylase and α-glucosidase activity, two key glucosidases required for starch digestion in humans. It has been suggested that procyanidins in GSE strongly inhibit α-amylase activity.[35] Similar treatment with gliclazide, a sulphonylurea antidiabetic drug succeeded to cause a decrease in the elevated serum glucose level reaching nearly the normal value is known to stimulate insulin secretion from the pancreas.[36]

Hyperlipidaemia is a major characteristic of DM.[37] DM induced hyperlipidaemia is attributable to excess mobilization of fat from the adipose tissue due to the under utilization of the glucose.[38] Moreover, studies suggested that hyperlipidemia is one of the most common features in STZ-induced hyperglycaemia in experimental rats.[39]

In this study, an increase in the levels of TC and TG has been observed in STZ-induced hyperglycaemic rats. Oral treatment of hyperglycaemic rats with GSE (300 mg/kg) significantly decreased the serum levels of TC and TG. Hence, we could say that GSE had beneficial effects on carbohydrate metabolism in hyperglycaemic rats. This antihyperlipidemic effect of GSE can be attributed to its ability to enhance the activity of enzymes involved in bile acid synthesis and its excretion leading to a decrease in the serum TC and TG levels.[40] This is in accordance with other studies that showed that GSE prevents body-weight gain.[34] Procyanidin extract supplementation prevents high-fat diet-induced obesity in hamsters by improving adipokine imbalance.[41]

Oxidative stress and overexpression of nitric oxide synthase (NOS) have been discussed as interrelated contributing factors in pancreatic β-cell dysfunction and destruction associated with diabetic conditions that lead to micro- and macrovascular complications. Hyperglycaemia is considered one of the factors responsible for the development of oxidative stress that results from enhanced formation of ROS by glucose autooxidation.[42] Hyperglycaemia may also play a role in the decreased NO production in type 2 DM, because high glucose per se inhibited endothelial NOS activity in the glomeruli, through a protein kinase C associated mechanism.[43] The decrease in production of NO during diabetic complications is supposed to be the consequence of reduced production of NO by NOS and inactivation of NO by ROS produced either by glycosylated proteins or directly from vascular endothelium as high level of HbA1c was observed in DM.[44] Moreover, NO is a key regulatory molecule with extensive metabolic, vascular, and cellular effects.[45,46] The regulation of NO metabolism is particularly important in type 2 DM.[47,48]

It was observed that the increase in the content of MDA was significant in animal models of DM and clinic diabetic sufferer.[49] The level of free radicals and lipid peroxidation *in vivo* was associated with elevated blood sugar levels in patients. When the free radicals increased, because of their special role in cell toxicity, they may damage the body to produce a series of roles, in which the pancreatic β cell damage is one of the important factors that triggers DM and directly affects further development of the disease.[50]

The present study provided a further evidence that STZ-induced hyperglycaemia in rats is accompanied by increased levels of MDA together with depleted levels of NO in the serum concomitantly with suppression in the total antioxidant capacity. It has been proven that induction of experimental hyperglycaemia with STZ results in enhanced lipid peroxidation. Oxidative stress in DM coexists with a reduction in the antioxidant capacity, which can increase the deleterious effects of the free radicals.[51,52]

Oral treatment of hyperglycaemic rats with GSE for 28 consecutive days succeeded to attenuate the lipid peroxidation as it depleted the MDA levels, thus, decreasing the oxidative stress. GSE helps to protect anti-oxidant enzymes. GSE significantly elevated the depleted NO levels by STZ. Many studies have been reported that the monomers catechin and epicatechin (monomeric flavanols) are the major phenolic compounds in GSE that show its anti-oxidant activity. Catechin shows antioxidant activity by inhibiting the oxidation of plasma lipids. Moreover, epicatechin is able to scavenge hydroxyl

radicals, peroxyl radicals, superoxide anion radicals.[53] Procyanidins are reported to have potent antioxidant activity both *in vitro* and *in vivo*.[54]

Moreover, previous studies attributed to the protective effect of GSE against cardiovascular events and atherosclerosis and also to its ability to enhance eNOS expression and NO production in hydrogen peroxide-treated human umbilical vein endothelial cells in a dose-dependent manner.[55]

In conclusion, GSE supplementation has beneficial and protective effects against some metabolic and biochemical changes associated with STZ-induced hyperglycaemia. These effects might be related to the ability of GSE to improve hyperglycaemia in addition to its anti-oxidant property.

6. Conflict of interest

None.

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