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# Antioxidant effects of a grape seed extract in a rat model of diabetes mellitus

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## Abstract

In the present study we investigated the anti-hyperglycaemic and antioxidant effect of grape seed extract, a polyphenolic flavonoid, in normal and streptozotocin-induced diabetic Wistar rats. Adult male Wistar rats were divided into three groups: Group I: non-diabetic control; Group II: diabetic control; Group III: diabetic rats treated with grape seed extract, administered via an intragastric tube (0.6 ml/rat), at a dose of 100 mg/kg for 20 consecutive days after the induction of diabetes mellitus. Diabetes was induced by an i.p. injection with streptozotocin for groups II and III. The TBARS, carbonylated proteins, were measured in the plasma and in the supernatant of liver homogenisates, and superoxide dismutase and catalase were measured in the haemolysates of RBCs and supernatant of liver homogenisates. The results showed that oral administration of grape seed extract (100 mg/kg/day) reduced the levels of lipid peroxides and carbonylated proteins and improved the antioxidant activity in plasma and hepatic tissue in rats treated with grape seed natural extract as compared with the diabetic control rats. These results suggested that the grape seed extract enhanced the antioxidant defence against reactive oxygen species produced under hyperglycaemic conditions, hence protecting the liver cells.

## Key words

diabetes mellitus, Reactive Oxygen Species, lipid peroxides, carbonylated proteins, CAT, SOD, polyphenols, streptozotocin

## Introduction

In diabetes mellitus, chronic hyperglycaemia produces multiple biochemical sequelae, and diabetes-induced oxidative stress could play a role in the symptoms and progression of the disease.<sup>1</sup> Oxidative stress may result in overproduction of oxygen free-radical precursors and/or decreased efficiency of the antioxidant system.<sup>2</sup> The oxygen free-radical generation is associated with auto-oxidation of glucose, impaired glutathione metabolism, alterations in the antioxidant enzymes and formation of lipid peroxides.<sup>3–5</sup> There are various endogenous defence mechanisms against free radicals, such as the enzymes GSH, SOD, GPx and CAT, whose activities eliminate superoxide, hydrogen peroxide and hydroxyl radicals.<sup>6</sup>

Oxidative stress is increased in experimental models of streptozotocin-induced diabetes mellitus.<sup>7</sup>

Fruits and vegetables contain a vast array of antioxidant components, mainly polyphenols and flavonoids.<sup>8,9</sup> Flavonoids possess several physiological properties: antioxidant, antibacterial, antiviral, antiinflammatory, antimutagenic and antitumoral activity, as well as the activation or inactivation of certain enzymes.<sup>10</sup> In plants, flavonoids generally

exist as glycosylated and sulphated derivatives.<sup>11</sup> Flavonoid glycosides are much more rapidly absorbed by humans than the aglycones.<sup>12</sup>

The purpose of the present study was to evaluate the role of grape seed extract on lipid peroxidation, protein oxidation and antioxidant status in the plasma and liver of diabetic mellitus induced animals.

## Materials and methods

### Preparation of grape seed extract

Red grapes (*Vitis vinifera* variety Burgund mare) seeds were obtained from Recas vineyard, Romania. The 1:1 BM

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was obtained as previously described.<sup>13</sup> The polyphenol content was determined by the Folin-Ciocalteu method and samples were standardized to 3 mEq GA/ml.<sup>13</sup>

### Animals

Ninety-day-old Wistar rats, weighing 225±25 g, were given a normocaloric standard diet (Hindustan Lever, Kolkata, India) and water *ad libitum*, while being maintained in a controlled environment (12 h light and dark cycle, 21–23°C). The animals were acclimatized to the laboratory conditions for one week before the start of the experiment. All experiments were conducted in accordance with the NIH<sup>14</sup> prescriptions and the protocol was approved by the Institutional Committee on Ethics of Animal Experimentation.

### Drugs and chemicals

All of the drugs and biochemicals were purchased from Sigma Chemical Company Inc., St Louis, MO, USA. The chemicals were of analytical grade.

### Induction of experimental diabetes

Streptozotocin was freshly dissolved in citrate buffer (0.01 M, pH 4.5) and maintained on ice prior to use. Diabetes was induced with a single i.p. injection of STZ (50 mg/kg) to overnight fasted rats.<sup>15</sup> Control rats were injected with citrate buffer. Diabetic status was confirmed in the STZ-treated rats by measuring the fasting plasma glucose after 72 h. Blood (0.2 ml) was collected into heparinized tubes by puncturing the retro-orbital plexus. The plasma was centrifuged at 2000 G, for 10 min. After removing the buffer coat, the packed RBCs were washed twice with cold isotonic physiological saline solution. Then a known volume of RBCs was lysed in cold phosphate buffer (at pH=7.4). The haemolysate was separated by centrifuging at 3000 G, for 10 min, at 2°C. Both plasma and haemolysates were used for biochemical analysis. Glucose level was estimated using a commercial glucose kit (Qualigens Diagnostics – Accu-Chek- ROCHE). Rats with plasma glucose levels above 13.89 mmol/L were considered diabetic<sup>16</sup> and used in experiments. Treatments with BM extract were started on day 3 after STZ-injection.

### Experimental design

Thirty rats divided into three groups of 10 animals each, were used to investigate the antioxidant effect of BM extract. Group I, non-diabetic control; Group II, diabetic control; Group III, diabetic + BM extract (100 mg/kg). The BM extract was suspended in CMC (0.01 g/ml) and orally administered via an intragastric tube (0.6 ml/rat) on a daily basis for 20 days. Non-diabetic control and diabetic control rats received CMC alone. After the last treatment (day 20), rats were fasted overnight and sacrificed by cervical decapitation. For each group, glucose plasmatic level was determined at the beginning of the experiment, 96 hours

after STZ administration (only for group II and group III) and at the end of the experiment.

### Sampling and preparation of biological materials

**Tissues** Liver tissues, stored in ice-cold containers, were homogenized using a Potter-Elvehjem homogenizer with physio-logical serum, centrifuged at 3,000 rpm and then the supernatants were collected. Biochemical parameters were measured in the homogenates on the day of sacrifice.

**Biochemical analysis** The plasmatic and hepatic levels of oxidative stress were estimated, by assessing lipid peroxides and carbonylated proteins concentrations.

Lipid peroxides were measured by the TBARS method.<sup>17</sup> The results were expressed in nmol MDA per mL of plasma and nmol MDA per g of tissue.

Carbonylated proteins as products of the reaction between the reactive oxygen species (ROS) and proteins were determined in the plasma and hepatic tissue homogenates, using the hydrochloric guanidine method.<sup>18</sup> The results were expressed in nmol per mg protein.

The activity of SOD was assayed as described by Kakkar *et al.*<sup>19</sup> A unit of the enzyme activity was defined as the enzyme reaction giving 50% inhibition of NBT reduction in 1 min under the assay conditions and expressed as specific activity in units/mg Hb, respectively units/mg protein.

The CAT activity was assayed according to Sinha<sup>20</sup> and expressed in  $\mu\text{mol H}_2\text{O}_2$  consumed/min/mg Hb, respectively units/mg protein.

**Statistical analysis** All analysis were expressed as mean values  $\pm$  SEM and analysed by Student's *t* test. Differences were considered significant at  $p < 0.05$ .

## Results

### Effect of BM extract on fasting plasma glucose levels

Fasting plasma glucose levels were increased in diabetic control rats. When treated with grape seed extract diabetic rats displayed significantly ( $p < 0.005$ ) decreased plasma glucose levels, close to the normal levels (Table 1).

### Effect of BM extract on TBARS and CP

The concentration of TBARS and CP in the plasma and liver of normal and diabetic rats is described in Table 1. In diabetic rats, TBARS and CP were significantly increased in the plasma and liver tissue ( $p < 0.05$ ). Treatment of diabetic rats with grape seed extract significantly decreased the concentration of both TBARS and CP in the plasma and liver tissue ( $p < 0.005$ ).

**Table 1.** Effect of grape seed extract on fasting plasma glucose levels, the levels of lipid peroxides and carbonylated proteins and antioxidant enzyme activities in plasma and hepatic tissues in normal and diabetic rats

	Normal control rats	Diabetic control rats	Diabetic rats treated with grape seed extract
Plasma glucose (mg/dL)	4.72±0.16	15.23±0.69*** p<0.001	5.28±0.26*++ p<0.005
Plasma TBARS (nmol/mL)	2.01±0.51	2.83±0.25*	2.06±0.48*++
Plasma CP (nmol/ mg protein)	0.61±0.06	2.52±0.52***	0.96±0.20*++
RBC SOD (U/mg Hb)	3.17±0.14	1.62±0.16*	3.005±0.09*++
RBC CAT(μmol H <sub>2</sub> O <sub>2</sub> utilized/min/mg Hb)	2.81±0.09	1.73±0.13*	2.63±0.07*++
Liver TBARS (nmol/g of tissue)	0.85±0.03	1.77±0.08*	0.96±0.07*++
Liver CP (nmol/mg prot)	1.005±0.73	3.50±0.69***	2.20±1.09*++
Liver SOD (Units/mg prot)	10.72±0.82	5.72±0.44*	9.90±0.75*++
Liver CAT (Units/mg prot)	75.2±1.46	44.9±1.38*	65.9±3.10*++

TBARS= thiobarbituric acid reactive substances, CP= carbonylated proteins, RBC= red blood cell lysate, SOD= superoxide dismutase, CAT= catalase  
 \*, \*\*, and \*\*\*= significant at P<0.05, P=0.005, and P<0.001, respectively, compared to normal control rats  
 +, ++, and +++= significant at P<0.05, P=0.005, and P<0.001, respectively, compared to diabetic control rats

### Effect of BM extract on enzymatic antioxidants

In diabetic rats, the activities of SOD and CAT were significantly decreased in the plasma and liver (Table 1) (p<0.034). The diabetic rats treated with BM extract exhibited a significant increase in the activities of SOD and CAT in the plasma and liver (p<0.005).

### Discussion

STZ diabetic rats develop most of the typical diabetic complications.<sup>21</sup> Various proteins, including haemoglobin, albumin, collagen, LDL or crystalline proteins undergo non-enzymatic glycation<sup>22</sup>. Glycation itself may induce the formation of oxygen-derived free radicals under diabetic condition.<sup>23</sup> In the present study the administration of BM extract decreased plasma glucose levels in diabetic rats and prevented STZ-induced oxidative stress. This suggests that treatment with the grape seed extract could ameliorate the oxidative stress caused by hyperglycaemia.

Lipid peroxidation is a characteristic of diabetes mellitus. Lipid peroxidation is a free-radical-induced process leading to oxidative deterioration of PUFA. Under physiological conditions, the concentrations of lipid peroxides in the tissues are low. Karpen *et al.*<sup>24</sup> reported elevated levels of lipid peroxides in the plasma of diabetic rats. Lipid peroxide-mediated tissue damage resulted in the development of both type I and II diabetes.

Low levels of lipid peroxides stimulate the secretion of insulin, but when the concentration of endogenous peroxides increases, it may initiate uncontrolled lipid peroxidation, thus leading to cellular infiltration and islet cell damage in type I diabetes<sup>25</sup>. The most commonly used indicators of lipid peroxidation are TBARS products.<sup>26</sup> The increased lipid peroxidation in the tissues of diabetic animals may be due to the observed increase in the concentration of TBARS

in the liver and kidney of diabetic rats.<sup>27</sup> Our results showed that in diabetic animals the levels of TBARS were high in the plasma and liver tissue, and were restored to normal values after the treatment with BM extract.

Carbonylation of proteins is a feature of irreversible oxidative damage, often leading to a loss of protein function, which is considered a widespread indicator of severe oxidative damage and disease-derived protein dysfunction. Whether moderately carbonylated proteins are degraded by the proteasomal system, heavily carbonylated proteins tend to form high-molecular-weight aggregates which are resistant to degradation and accumulate as damaged or unfolded proteins. STZ-induced oxidative damage in proteins was revealed by the increased content of carbonylated proteins in the plasma and liver tissue.<sup>28</sup> The treatment of STZ-injected animals with grape seed extract lowered the proteins' oxidant damage in rats' plasma and liver tissues.

Oxidative stress in diabetes coexists with a reduction in the antioxidant capacity, which can increase the deleterious effects of the free radicals. SOD protects tissues against oxygen free radicals by catalysing the removal of superoxide radical, converting it into H<sub>2</sub>O<sub>2</sub> and molecular oxygen, which both damage the cell membrane and other biological structures.<sup>29</sup> Catalase is a haem-protein, which is responsible for the detoxification of significant amounts of H<sub>2</sub>O<sub>2</sub>.<sup>30</sup> Reduced activities of SOD and catalase in the liver and pancreas during diabetes were reported, resulting in a number of deleterious effects due to the accumulation of superoxide radicals and hydrogen peroxide.<sup>31</sup> The grape seed extract treated rats showed reduced lipid peroxidation and protein oxidation which was associated with an increased activity of SOD and CAT.

The antioxidant activity of phenols is due to their redox properties that allow them to act as reducing agents by donating hydrogen, quenching singlet oxygen or acting as

**Abbreviations and acronyms**

BM extract	hydroethanolic extract
CAT	catalase
CMC	carboxymethyl cellulose
CP	carbonylated proteins
GA	gallic acid
GPx	glutathione peroxidase
GSH	reduced glutathione
Hb	haemoglobin
i.p.	intraperitoneal
LDL	low-density lipoprotein
MDA	malondialdehyde
NBT	tetrazolium tetrazolium
PUFA	polyunsaturated fatty acids
RBC	red blood cell
SOD	superoxide dismutase
TBARS	thioarbituric acid-reactive substances.

metal chelators. Red wines, as rich sources of biologically active polyphenols (catechins, epicatechins and gallic acid), have antioxidant and antitumoral properties. Red wines that contain > 200 different phenolic compounds might be important dietary sources of polyphenols.<sup>13, 32, 33</sup>

In conclusion, our results suggest that long-term daily administration of grape seed extract offers enhanced antioxidant potential and protection against tissue lipid peroxidation and protein oxidation, and could therefore be associated with reduced glucose levels in diabetic rats.

**References**

- Giugliano, D, Ceriello, A, Paolisso, G (1996) Oxidative stress and diabetic vascular complications. *Diabetes Care* 19: 257–67.
- Baynes, JW (1991) Role of oxidative stress in development of complications in diabetes. *Diabetes* 40: 405–12.
- McLennan, SV, Heffernan, S, Wright, L, et al. (1991) Changes in hepatic glutathione metabolism in diabetes. *Diabetes* 40: 344–8.
- Mullarkey, CJ, Edelstein, D, Brownlee, M (1990) Free radical generation by early glycation products: a mechanism for accelerated atherogenesis in diabetes. *Biochem Biophys Res Commun* 173: 932–8.
- Strain, JJ (1991) Disturbances of micronutrient and antioxidant status in diabetes. *Proc Nutr Soc* 50: 591–604.
- Soto, C, Recoba, R, Barron, H, Alvarez, C, Favari, L (2003) Silymarin increases antioxidant enzymes in alloxan-induced diabetes in rat pancreas. *Comp Biochem Physiol C Toxicol Pharmacol* 136: 205–12.
- Szkudelski, T (2001) The mechanism of alloxan and streptozotocin action in beta-cells of the rat pancreas. *Physiol Res* 50: 536–46.
- Potter, JD (1997) Cancer prevention: epidemiology and experiment. *Cancer Lett* 114: 7–9.
- Harborne, JB Plant phenolics. In: Bell, EA and Charlwood, BV (eds). *Encyclopedia of Plant Physiology, Vol. 8. Secondary Plant Products*. (pp.329-95). Berlin: Springer, 1986.
- Rice-Evans, C, Packer, L *Flavonoids in Health and Diseases*. New York: Marcel Decker, 1998.
- Middleton, E, Kandaswami, C, Theoharides, TC (2000) The effects of plant flavonoids on mammalian cells: Implications for inflammation, heart disease and cancer. *Pharmacol Rev* 52: 673–751.
- Hollman, PC, Katan, MB Absorption, metabolism and bioavailability of flavonoids. In: Rice-Evans, C and Packer, L (eds). *Flavonoids in Health and Disease* (pp483–522). New York: Marcel Decker, 1998.
- Postescu, ID, Tatomir, C, Chereches, G, Bric, I, Damian, G, Petrisor, D, et al. (2007) Spectroscopic characterization of some grape extracts with potential role in tumor growth inhibition. *J Optoelectronics Adv Mater* 3: 564–7.
- National Institute of Health: Guide for the Care and Use of Laboratory Animals, DHEW Publication (NIH), 2<sup>nd</sup> revised edn, Office of Science and Health Reports. Bethesda, MD: DRR/NIH, 1985.
- Kamalakkannan, N, Stanely, Mainzen, Prince, P (2006) The antihyperglycaemic and antioxidant effect of rutin, a polyphenolic flavonoid, in streptozotocin-induced diabetic Wistar rats. *Basic Clin Pharmacol Toxicol* 98: 97–103.
- Nagasawa, T, Tabata, N, Ito, Y, Aiba, Y, Nishizawa, N, Kitts, DD (2003) Dietary G- rutin suppresses glycation in tissue proteins of streptozotocin-induced diabetic rats. *Mol Cell Biochem* 252: 141–147.
- Satoh, K (1978) Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. *Clinica Chimica Acta* 90: 37–43.
- Reznik, AZ and Packer, L (1994) Oxidative damage to proteins: spectrophotometric method for carbonyl assay. *Meth Enzymol* 233: 357–63.
- Kakkar, P, Das, B, Viswanthan, PN (1984) A modified spectrophotometric assay of superoxide dismutase (SOD). *Indian J Biochem Biophys* 21: 130–2.
- Sinha, KA (1972) Colorimetric assay of catalase. *Anal Biochem* 47: 389–94.
- Ozturk, Y, Altan, VM, Yildizoglu, A (1996) Effect of experimental diabetes and insulin on smooth muscle functions. *Pharmacol Rev* 48: 69–112.
- Klein, R (1995) Hyperglycemia and microvascular and macrovascular disease in diabetes. *Diabetes Care* 18: 258–68.
- Gupta, BL, Nehal, M, Baquer, NZ (1997) Effect of experimental diabetes on the activities of hexokinase, glucose-6-phosphate dehydrogenase and catecholamines in rat erythrocytes of different ages. *Indian J Exp Biol* 35: 792–5.
- Karpen, CW, Pritchard, KA, Merola, AJ, Panganamala, RV (1982) Alterations of the prostacyclin–thromboxane ratio in streptozotocin induced diabetic rats. *Prostaglandins Leukot Med* 8: 93–103.
- Metz, SA (1984) Oxygenation products of arachidonic acid: third messengers for insulin release. *Prostaglandins* 27: 147–151.
- Lyons, TJ (1991) Oxidized low-density lipoproteins, a role in the pathogenesis of atherosclerosis in diabetes. *Diabet Med* 8: 411–9.
- Stanely, P, Prince, M, Menon, VP (2001) Antioxidant action of *Tinospora cordifolia* root extract in alloxan diabetic rats. *Phytother Res* 15: 213–8.

28. Cumaoglu, A, Cevik, C, Rackova, L, Ari, N, Karasu, C (2007) Effects of antioxidant stobadine on protein carbonylation, advanced oxidation protein products and reductive capacity of liver in streptozotocin-diabetic rats: role of oxidative/nitrosative stress. *Biofactors* 30: 171–8.
29. Arivazhagan, P, Thilagavathy, T, Pannerselvam, C (2000) Antioxidant lipoate and tissue antioxidants in aged rats. *J Nutr Biochem* 11: 122–7.
30. Cheng, L, Kellogg, EW, and Packer, L (1981) Photoactivation of catalase. *Photochem Photobiol* 34: 125–9.
31. Searle, AJ, Wilson, RL (1981) Glutathione peroxidase: effect of superoxide, hydroxyl and bromine free radicals on enzyme activity. *Int J Rad Biol* 34: 125–9.
32. Briviba, K, Pan, L, Rechkemmer, G (2002) Red wine polyphenols inhibit the growth of colon carcinoma cells and modulate the activation pattern of mitogen-activated protein kinases. *J Nutr* 132: 2814–8.
33. German, JB, Walzem, RL (2000) The health benefits of wine. *Rev Nutr* 20: 561–93.