

Amelioration of Alloxan Induced Diabetes mellitus and Oxidative Stress in Rats by Oil of *Eruca sativa* Seeds

M.A. El-Missiry^a A.M. El Gindy^b

Zoology Departments, Faculties of Science, ^aMansura University, Mansura, and ^bAl Azhar University, Cairo, Egypt

Key Words

Eruca sativa seed oil · Alloxan · Insulin · Glucose · Lipids · Free radicals · Glutathione · Superoxide dismutase · Lipid peroxidation

Abstract

Clinical research has confirmed the efficacy of several plant extracts in the modulation of oxidative stress associated with diabetes mellitus (DM). Oil of *Eruca sativa* seeds (ESS) is tried for prevention and treatment of DM induced experimentally by alloxan injection. A single dose of alloxan (100 mg/kg) produced a decrease in insulin level, hyperglycemia, elevated total lipids, triglycerides and cholesterol, decreased high-density lipoprotein and hepatic glycogen contents and elevated hepatic glucose-6-phosphatase activity. Concurrent with these changes, there was an increase in the concentration of malondialdehyde and 4-hydroxynonenal in the liver. This oxidative stress was related to a decreased glutathione (GSH) content and superoxide dismutase activity in the liver of alloxan-diabetic rats. ESS oil (0.06 ml/kg) on its own increased significantly hepatic GSH. Daily oral administration of ESS oil 2 weeks before or after diabetes induction ameliorated hyperglycemia, improved lipid profile, blunted the increase in malondialdehyde and 4-hydroxynonenal and stimulated the GSH produc-

tion in the liver of alloxan-treated rats. We suggested that ESS oil could be used as antidiabetic complement in case of DM. This may be related to its antioxidative properties and to the increase in hepatic GSH.

Copyright © 2000 S. Karger AG, Basel

Introduction

Oxidant free radicals have been implicated in the pathogenesis of type I diabetes mellitus (DM) [1, 2]. In addition, diabetic patients have significant defects of antioxidant protection [3, 4], and it is believed that the metabolic disorders in type I DM may be due to increased cellular oxidative stress and reduced antioxidant protection [5].

Chemoprevention involves the use of natural substances to reduce the risk of developing DM and its sequels. A dietary component capable of mediating chemopreventive activity is sulforaphane, an aliphatic isothiocyanate, that is found in cruciferous vegetables such as *Eruca sativa* seeds (ESS). Sulforaphane, the principal component of ESS oil, was found to be a potent inducer of phase II detoxicating enzymes due to its antioxidant functions [6]. Glucoraphanin, that possesses potential antioxidant properties, was obtained from glucoerucin isolated from ripe seeds of *Eruca sativa* ESS [7].

Table 1. Serum glucose, insulin, total lipids, triglycerides, total cholesterol and HDL cholesterol in various animal groups

	Nondiabetic groups			Diabetic groups		
	control	acetate control	ESS oil control	alloxan	ESS oil + alloxan	alloxan + ESS oil
Glucose, mg/100 ml	92.26 ± 2.68	89.42 ± 3.59	89.03 ± 5.19	272.65 ± 33.61 ^c	152.14 ± 14.84 ^{c,d}	188.95 ± 10.83 ^{c,d}
Insulin, pg/ml	33.6 ± 2.99	32.2 ± 2.56	40.5 ± 7.7	12.4 ± 1.21 ^c	22.0 ± 2.95 ^{c,d}	24.8 ± 1.36 ^{c,d}
Total lipids, g/l	6.39 ± 0.12	6.32 ± 0.06	6.38 ± 0.13	7.63 ± 0.07 ^c	6.68 ± 0.21	7.21 ± 0.16 ^c
Triglycerides, mg/100 ml	86.67 ± 3.82	85.00 ± 2.83	87.52 ± 10.95	155.83 ± 15.57 ^c	87.01 ± 4.75 ^d	97.16 ± 4.00 ^d
Total cholesterol, mg/100 ml	90.65 ± 3.49	94.12 ± 2.36	84.60 ± 7.25	116.47 ± 2.88 ^c	95.74 ± 2.97 ^d	100.00 ± 3.22 ^d
HDL cholesterol, mg/100 ml	31.72 ± 1.8	30.48 ± 1.7	34.39 ± 3.2	25.26 ± 1.3 ^c	27.70 ± 2.2	28.52 ± 1.1

All values are mean ± SE of 6–8 animals.

^c Significant ($p < 0.05$) when compared with the normal control group.

^d Significant ($p < 0.05$) when compared with the diabetic group.

Alloxan-induced DNA fragmentation in pancreatic islets and cell damage have been attributed to the production of toxic free radicals [8]. Thus, the alloxan system was considered adequate for the study of a pathology in which free radicals might have central role, such as DM. Since diet forms the mainstay in the management of DM, there is a suggestion for exploiting the antioxidative potency of seeds and their oils to the maximum extent. Therefore, the aim of the present work was to investigate the influence of ESS oil supplementation on biochemical and antioxidant parameters in alloxan-treated rats.

Materials and Methods

Alloxan was purchased from Sigma Chemical Co. ESS oil was freshly obtained from a local source. Male Wister rats (150–180 g) were used. The animals were divided into six groups, each consisting of 6–8 animals. The 1st group was the control group without any treatment. The 2nd group received acetate buffer used for alloxan. The 3rd group received a daily dose of ESS oil (0.06 ml/kg) intragastrically for 14 days. The 4th group received a single intraperitoneal injection of 100 mg alloxan/kg. The 5th group received alloxan followed by ESS oil at the same dose as 3rd and 4th groups. The 6th group received ESS oil first for 14 days, then alloxan.

At the end of the experimental period, the serum insulin level was estimated using kits from Bio-Mérieux. The concentrations of glucose, total lipids, triglycerides, cholesterol and high-density lipoprotein (HDL) cholesterol in serum were estimated by kits obtained from Stanbio. The glycogen content in the liver was determined by the anthrone method [9]. The activity of glucose-6-phosphatase (G-6-Pase) in the liver was assayed as previously described [10]. Lipid peroxidation in the liver was estimated by colorimetric assay of malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE) using a kit obtained from Wak-Chem Medical. The total superoxide dismutase (SOD) activity and the Glutathione (GSH) content in the liver were determined as described [11, 12]. Statistical analysis of the results was performed using ANOVA.

Results

ESS oil supplementation for 2 weeks on its own did not change the biochemical parameters in serum (table 1). Alloxan treatment produced a significant elevation in plasma glucose concentration. The supplementation of ESS oil 2 weeks before alloxan treatment or concurrent supplementation of ESS oil after alloxan treatment for 2 weeks showed a significant decrease in glucose level as compared with alloxan-diabetic rats, in spite of the significant increase of serum glucose as compared with the control group. In addition, alloxan produced a significant decrease in the serum insulin level. Supplementation of ESS oil 2 weeks before or 2 weeks after alloxan injection ameliorated the decline in insulin level and showed higher values as compared with diabetic rats but was still significantly lower than the normal control value. Moreover, alloxan-injected rats showed significant increases in the serum levels of total lipids, triglycerides and cholesterol with a significant decrease in serum HDL level (table 1). Supplementation of diabetic rats with ESS oil for 2 weeks before and 2 weeks after alloxan treatment greatly improved the levels of serum lipid parameters as compared with the diabetic group, but still these were significantly different from control values.

As shown in table 2, alloxan treatment significantly decreased the hepatic glycogen content and increased the activity of G-6-Pase. Oral administration of ESS oil for 2 weeks before and 2 weeks after alloxan treatment significantly ameliorated these effects as compared with the diabetic group; however, the glycogen content was still significantly lower than control levels. ESS oil administration alone did not change hepatic glycogen and the activities of G-6-Pase.

Table 2. Liver glycogen, G-6-Pase, GSH, lipid peroxidation product (MDA+4-HNE) and SOD activity per gram fresh tissue in various animal groups

	Nondiabetic groups			Diabetic groups		
	control	acetate control	ESS oil control	alloxan	ESS oil + alloxan	alloxan + ESS oil
Glycogen, mg/g	669.4±51.00	629.6±43.6	784.8±76.1	187.7±9.0 ^c	416.3±21.00 ^{c,d}	687.2±19.2 ^d
G-6-Pase, μmol/pi/min/g	1.17±0.07	1.22±0.11	1.29±0.14	1.47±0.10 ^c	1.29±0.05	1.19±0.24
MDA+4-HNE, μmol/g	5.05±0.33	5.06±0.27	4.62±0.41	9.67±0.99 ^c	5.50±0.4 ^d	4.47±0.26 ^d
GSH, μmol/g	1.72±0.16	1.89±0.19	3.38±0.25 ^c	1.24±0.16 ^c	2.01±0.11 ^d	1.76±0.12 ^d
SOD, U/g	45.80±4.02	44.18±3.38	47.60±10.07	31.81±3.49	39.88±3.84	33.62±1.75

Footnotes as in table 1.

Alloxan injection produced significant oxidative stress in the liver of diabetic rats 2 weeks after DM induction which was manifested by increased lipid peroxidation products, MDA and 4-HNE, with decreased GSH content and SOD activity (table 2). Administration of ESS oil for 2 weeks before and 2 weeks after alloxan treatment significantly decreased lipid peroxidation, with similar value as in control rats. The hepatic GSH content was increased significantly in ESS oil treated groups over the diabetic group. ESS oil alone induced a significant increase in hepatic GSH as compared with the control group. When ESS oil was supplemented 2 weeks before alloxan treatment, the SOD activity was restored and showed a significant increase as compared with diabetic rats, but was within the control level. On the other hand, oral supplementation of ESS oil for 2 weeks after alloxan injection did not modify the decreased activity of SOD in the liver.

Discussion

The present results demonstrated that ESS oil significantly ameliorated the adverse influence of alloxan. To the best of our knowledge, this is the first report utilizing ESS oil as antidiabetic adjuvant and antioxidative agent. Oral supplementation of ESS oil before or after alloxan treatment resulted in lower serum glucose levels, higher serum insulin values and an improved lipid profile as well as hepatic glycogen content and its regulating enzyme as compared with rats treated with alloxan alone. These changes were accompanied by a significant decrease in lipid peroxidation in the liver. These results are in accordance with those of other investigations using different seed oils [13, 14]. It is suggested that the active principles from plant sources might act by several mechanisms such

as stimulating insulin secretion, increasing repair/proliferation of β -cells, enhancing the effect of insulin and adrenaline and increasing the antioxidative capability [14, 15].

Several studies showed that alloxan produces a decrease in the activity of the antioxidant enzymes during the development of alloxan-induced type I DM in liver, pancreas and testis [16, 17]. In our experimental model of DM, it was observed that alloxan administration produced a significant decrease in hepatic GSH content and SOD activity accompanied by a significant increase in aldehydic products of lipid peroxidation, indicating an increased hepatic oxidative stress which may also occur in other tissues in alloxan-treated rats. The aldehydic products of lipid peroxidation such as MDA and 4-HNE are more cytotoxic and stable than reactive oxygen species and react quickly with cellular constituents [18]. Besides these negative effects MDA and 4-HNE are modulators of signal transduction pathways that disturb cellular activities [19]. This in turn may contribute to the disruption of intracellular and membrane redox state of many cells including liver and β -pancreatic cells, hence disturbing glucose regulation.

The improvement recorded before and after ESS oil treatment of alloxan-injected rats might suggest a protective and prophylactic influence of ESS oil against alloxan action that might be mediated through neutralization of oxygen free radicals produced by alloxan. A stimulating effect of the synthesis of GSH by ESS oil was observed in the present study. The GSH reacts with free radicals and is a crucial substrate for glutathione peroxidase and glutathione-S-transferase which take part in the cellular defense mechanisms against intermediate oxygen products [16, 17]. The ameliorative effect of ESS oil on hepatic lipid peroxidation produced by alloxan may be related to the significant rise in hepatic GSH induced by the active

components in oil of this plant seed. It is reported that sulforaphane, the principle medical component of ESS oil, increased the GSH level about twofold in cultured Hepa 1c1c7 cells, induced a 5- to 17-fold increase in the catalase activity by interaction with antioxidant-responsive element and induced mouse mammary glutathione-S-transferase activity after feeding of 3 mg sulforaphane/mouse intragastrically for 4 days without elevation of hepatic enzyme activities [20]. It may be relevant that the ratio of GSH/GSSG plays a critical role in glucose homeostasis of diabetes because thiol groups are important in intracellular and membrane redox state [16]. Oil of ESS induced an increase in hepatic GSH content which might enhance the GSH/GSSG ratio and decrease hepatic lipid

peroxidation, hence aldehydic concentration, and, therefore, improve serum glucose regulation.

Parallel to these events, hepatic SOD activity was increased in rats supplemented with ESS oil as compared with diabetic rats. SOD is responsible for removal of superoxide radicals [11]; thus, it may contribute to the modulation of redox state of liver cells as well as other important secretory cells such as β -pancreatic cells. The interplay of these events may contribute to the favorable effects of ESS oil on liver damage produced by the generation of free radicals. This is the case in the experimental DM induced by alloxan and probably the case in human DM type I.

References

- Oberley LW: Free radicals and diabetes. *Free Radic Biol Med* 1988;5:113-124.
- Baynes JW: Role of oxidative stress in development of complications in diabetes. *Diabetes* 1991;40:405-412.
- Giugliano D, Ceriello A, Paolisso G: Oxidative stress and diabetic vascular complications. *Diabetes Care* 1996;19:257-267.
- Maxwell SR, Thomason H, Sandler D, Leguen C, Baxter MA, Thorpe GH, Jones AF, Barnett AH: Antioxidant status in patients with uncomplicated insulin-dependent and non-insulin-dependent diabetes mellitus. *Eur J Clin Invest* 1997;27:484-490.
- Stefano AS, Marra G, Giardina B, Cotroneo P, Mordent A, Martorana GE, Manto A, Ghirlan-da G: Defective plasma antioxidant defenses and enhanced susceptibility to lipid peroxidation in uncomplicated IDDM. *Diabetes* 1997;46:1853-1858.
- Fahey JW, Talalay P: Antioxidant functions of sulforaphane: A potent inducer of phase II detoxicating enzymes. *Food Chem Toxicol* 1999;37:973-979.
- Iori R, Bernardi R, Gueyrard D, Rollin P, Palmieri S: Formation of glucoraphanin by chemoselective oxidation of natural glucorucin: A chemoenzymatic route to sulforaphane. *Bioorg Med Chem Lett* 1999;9:1047-1048.
- Takasu N, Komiya I, Asawa T, Nagasawa Y, Yamada T: Streptozocin- and alloxan-induced H_2O_2 generation and DNA fragmentation in pancreatic islets: H_2O_2 as mediator for DNA fragmentation. *Diabetes* 1991;40:1141-1145.
- Nicholas VC, Robert WL, Joseph HR: The determination of glycogen in liver and muscle by use of anthrone reagent. *J Biol Chem* 1956;220:583-593.
- Swanson MA: Glucose-6-phosphatase from liver. *Methods Enzymol* 1956;2:541-543.
- Nishikimi M, Rao NA, Yagi K: The occurrence of superoxide anion in the reaction of reduced phenazine methosulphate and molecular oxygen. *Biochem Biophys Res Commun* 1972;46:844-853.
- Beutler E: *Red Cell Metabolism: A Manual of Biochemical Methods*, ed 3. New York, Grune & Stratton, 1982, pp 37-40.
- Akhtar MS, Ali MR: The study of hypoglycemic activity of *Cuminum nigrum* seeds in normal and alloxan diabetic rabbits. *Planta Med* 1984;2:81-85.
- Fayed T, El-Missiry MA, Emara H, El Saaad N: Effect of *Nigella sativa* or fish oil supplementation in alloxan diabetic rats. *J Union Arab Biol* 1998;9:237-250.
- Shanmugasundaram ER, Gopianth KL, Radha SK, Rajendram VM: Possible regeneration of the islets of Langerhans in streptozotocin diabetic rats given *Gymnema sylvestra* leaf extracts. *J Ethnopharmacol* 1990;30:265-279.
- Soto CP, Perez BL, Favari LP, Reyes JL: Prevention of alloxan-induced diabetes mellitus in the rat by silymarin. *Comp Biochem Physiol C Pharmacol Toxicol Endocrinol* 1998;119:125-129.
- El-Missiry MA: Enhanced testicular antioxidant system by ascorbic acid in alloxan diabetic rats. *Comp Biochem Physiol C Pharmacol Toxicol Endocrinol* 1999;124:233-237.
- Esterbauer H, Schaur RJ, Zollner H: Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. *Free Radic Biol Med* 1991;11:81-128.
- Grune T, Siems WG, Petras T: Identification of metabolic pathways of the lipid peroxidation product 4-hydroxynonenal in in situ perfused rat kidney. *J Lipid Res* 1997;38:1660-1665.
- Gerhauser C, You M, Liu J, Moriarty RM, Hawthorne M, Mehta RG, Moon RC, Pezzuto JM: Cancer chemopreventive potential of sulforamate, a novel analogue of sulforaphane that induces phase 2 drug-metabolizing enzymes. *Cancer Res* 1997;57:272-278.