Beneficial Effect of Substitution of Sesame Oil on Hepatic Redox Status and Lipid Parameters in Streptozotocin Diabetic Rats

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ABSTRACT
Sesame oil is being used a sole edible oil in South India. The aim of present study was to monitor the influence of sesame oil on blood glucose, and lipid peroxidation, antioxidants and lipid profile in the liver of control and streptozotocin (STZ) diabetic rats (Fifteen-week old Albino Wistar rats). Diabetes was induced in adult female albino Wistar rats weighing 180-200 g by administration of STZ (40 mg/kg of body weight) intraperitonially. Both control and diabetic rats were fed with a commercial diet containing 2% oil supplemented with 6% sesame oil for 42 days. Diabetic rats had elevated levels of blood glucose (322.61 ± 9.49 mg/dL), thiorbituric acid-reactive substances (TBARS), and lipid hydroperoxides (LH) and decreased activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX). When diabetic rats fed with sesame oil, were compared with diabetic rats, a significant reduction in levels of blood glucose (222.02 ± 8.27 mg/dL), TBARS, LP, and an elevation in SOD, CAT and GPX, were observed. Thus, sesame oil consumption beneficially influences blood glucose, lipid peroxidation, antioxidants and lipid parameters in diabetic rats.

KEY WORDS: Antioxidants; Blood glucose; Diabetes; Lipid parameters; Sesame oil; Streptozocin

INTRODUCTION
Diabetes mellitus is the metabolic disorder with the highest rates of prevalence and mortality world-wide (1). Many studies have found increased lipid peroxides or oxidative stress (or both) in different animal models of diabetes and in diabetic subjects (2 & 3). Oxidative stress, the prevalence of oxidant factors over antioxidant mechanisms, plays a central role in the pathogenesis and progression of diabetes and its complications (4). Earlier study has shown that treatment with antioxidant reduces diabetic complications (5). In this connection, minor dietary constituents, especially plant based foods have come under serious scrutiny (6). Sesame oil is used either fresh or fried for the sake of flavor in various food preparations. Sesame oil has been used as a domestic Ayurvedic remedy in India. Sesame oil should be considered as one of the more valuable foods for good health and for good quality of life in general (7). Enhanced formation of oxygen free radicals occurs in tissues during hyperglycaemia (8). Free radicals react with lipids and causes peroxidative changes that result in enhanced lipid peroxidation (9), which can be detected by the presence of peroxidation products (10), and results in alteration of antioxidant defense systems and lipid profile. There had been a reduction in peak plasma glucose concentration with the consumption of a diet high in monounsaturated fat (11). Sesame oil contains 40% and 43% monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA), respectively (12). As we reported antidiabetic and antioxidant effects of sesame oil in STZ-diabetic rats (13) and liver is the major organ for maintaining plasma glucose levels within narrow limits, the aim of the present study was to examine the effect of sesame oil on redox status and lipid profile in the liver of control and diabetic rats.

MATERIALS AND METHODS
Oil and treatment diet
Standard pellet diet and oil free diet were purchased from Pranav Agro Industries Ltd., Pune, India. Sesame oil (Idhayam gingelly oil) was purchased from the local market, and the oil was stored in a jar at 4 °C. The standard pellet diet contained 8% oil. Oil-free diet, which contained 2% oil, was mixed with sesame oil to make the oil content 8%.

Chemicals
STZ was purchased from Sigma-Aldrich (St. Louis, MO), and glibenclamide from Hoechst (Germany). All other chemicals used were of analytical grade obtained from E. Merck (Darmstadt, Germany) and HIMEDIA (Mumbai, India).

Experimental animals
Fifteen-week old adult albino female Wistar rats weighing 180-200g bred in the Central Animal House, Department of Experimental Medicine, Rajah Muthiah Medical College, Annamalai University, Annamalainagar, Tamil Nadu, India, were used in this study. The feed and water were provided ad libitum to the animals. Studies were carried out in accordance with Indian National Law on Animal Care and Use, and ethical clearance was provided by The Committee for the Purpose of Control and Supervision of Experiments on Animals of Rajah Muthiah Medical College and Hospital (Reg. No.: 160/1999/CPCSEA), Annamalai University, Annamalainagar, Tamil Nadu, India.

Experimental induction of diabetes
The rats were made diabetic with an intraperitoneal injection of STZ at a dose of 40 mg/kg of body weight dissolved in citrate buffer (0.1 M, pH 4.5). STZ-injected animals exhibited massive glycosuria and within a few days. Diabetes was confirmed

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in rats by measuring fasting blood glucose concentration, 96 hours after injection with STZ. The rats with a blood glucose level above 240 mg/dL were considered to be diabetic and used for the experiment.

**Experimental design**

The rats were randomly divided into five groups of six animals each.

- **Group I**: Normal
- **Group II**: Normal + sesame oil diet
- **Group III**: Diabetic control
- **Group IV**: Diabetic + sesame oil diet
- **Group V**: Diabetic + glibenclamide (600 μg/kg of body weight).

After 42 days of treatment, the over night animals were sacrificed by decapitation. Blood was collected in tubes containing a mixture of potassium oxalate and sodium fluoride (1:3 vol/vol) for the estimation of blood glucose. Liver tissue (250 mg) was sliced into pieces and homogenised in appropriate buffer in cold condition (pH 7.0) to give 20% homogenate (w/v). The homogenate were centrifuged at 1000 rpm for 10 min at 0 °C in cold centrifuge. The supernatant was separated and used for various biochemical estimations.

**BIOCHEMICAL ESTIMATIONS**

**Plasma glucose**

Plasma glucose was measured by the glucose oxidase method (14). To 1 mL of reagent 10 μL of plasma was added and kept at room temperature for 15 min. After incubation, the color developed was read at 530 nm against reagent blank.

**TBARS and HP**

The concentration of TBARS in the liver tissue was estimated (15). 0.5 mL of plasma was diluted to 1 mL with double distilled water and mixed well, and then 2.0 mL of TBA-TCA-HCl reagent was added. The mixture was kept in a boiling water bath for 15 min, after cooling, the tubes were centrifuged at 1000 g for 10 min and the supernatant was estimated. The absorbance of the chromophore was read at 535 nm against reagent blank.

Lipid hydroperoxide in the liver tissue was estimated (16). 0.9 mL of Fox reagent was mixed with 0.1 mL of the sample, incubated for 30 min at room temperature and the absorbance read in a Spectronic 20 at 560 nm.

**Enzymatic antioxidants**

The activities of SOD, CAT and GPx in the liver were measured (17-19).

The enzyme activity in the supernatant was determined as follows. The assay mixture contained 1.2 mL of sodium pyrophosphate buffer, 0.1 mL of phenazone methosulphate, and 0.3 mL of nitroblue tetrazolium and appropriately diluted enzyme preparation in a total volume of 3 mL. The reaction was started by the addition of 0.2 mL NADH. After incubation at 30 °C for 90 sec, the reaction was stopped by the addition of 1 mL glacial acetic acid. The reaction mixture was stirred vigorously and shaken with 4 mL n-butanol. The mixture was allowed to stand for 10 min; centrifuged and n-butanol layer was separated. The color density of the chromogen in n-butanol was measured in a Spectronic 20 at 520 nm.

Tissue homogenate was prepared by using phosphate buffer (0.01 M, pH 7.0). To 0.9 mL of phosphate buffer, 0.1 mL of tissue homogenate or 0.1 mL of serum and 0.4 mL of hydrogen peroxide were added. The reaction was arrested after 30 sec interval by adding 2.0 mL of dichromate-acetic acid mixture. The tubes were kept in a boiling water bath for 10 min, cooled and the color developed was read at 620 nm.

The tissue was homogenised using tris buffer. To 0.2 mL of tris buffer, 0.2 mL of EDTA, 0.1 mL of sodium azide, 0.5 mL of tissue homogenate were added. To the mixture, 0.2 mL of GSH followed by 0.1 mL of H₂O₂ was added. The contents were mixed well and incubated at 37 °C for 10 min, along with a control containing all reagents except homogenate. After 10 min, the reaction was arrested by the addition of 0.5 mL of 10% TCA. The tubes were centrifuged and the supernatant was assayed for GSH by the method of Ellman.

**Lipid parameters**

The levels of TC, TG, FFA and PL in the liver were estimated (20-23). To 10 μL of plasma or 10 μL of lipid extract, 1.0 mL of enzyme reagent was added, mixed well and kept at 37 °C for 5 min. 10 μL of cholesterol standard and distilled water (blank) were also processed similarly. The absorbance was measured at 510 nm.

To 10 μL of plasma or 10 μL of lipid extract, 1.0 mL of enzyme reagent was added, mixed well and incubated at room temperature for 10 min. 10 μL of triacylglycerol standard and distilled water (blank) were also processed similarly. The absorbance was measured at 510 nm. 0.5 mL of lipid extract was evaporated to dryness and dissolved in 6.0 mL chloroform-heptane-methanol solvent and 2.5 mL of copper reagent were added. All the tubes were shaken vigorously for 90 sec and were kept aside for 15 min. The tubes were centrifuged and 3.0 mL of the copper layer was transferred to another tube containing 0.5 mL of diphenyl carbazole and mixed carefully. The color developed was read at 540 nm against a reagent blank containing 3.0 mL solvent and 0.5 mL diphenyl carbazole. An aliquot of the lipid extract was evaporated to dryness. 1.0 mL of 5.0 N sulphuric acid was added and digested till light brown. Then 2 to 3 drops of concentrated nitric acid was added and the digestion was continued till it became colorless. After cooling, 1 mL of water was added and heated in a boiling water bath for about 5 min. Then, 1.0 mL of ammonium molybdate and 0.1 mL of ANSA were added. The volume was then made up to 10.0 mL with distilled water and the absorbance was measured at 680 nm within 10 min. Standards in the concentration range of 2-8 mg were treated in the similar manner. The values obtained were multiplied with a factor 25 to convert inorganic phosphorus to its phospholipids equivalents.

**Statistical analysis**

All quantitative measurements were expressed as means ± S.D. The mean value was from six rats in each group. The data were analyzed using one-way analysis of variance (ANOVA) on SPSS/PC (Statistical Package for Social Sciences, Personal Computer), and group means were compared by Duncan’s Multiple Range Test (DMRT). The
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liver of control and diabetic rats. A significant increase of TBARS and HP in diabetic rats and significant reduction (p<0.05) in the liver of diabetic rats fed with sesame oil were observed.

The effect of sesame oil on the activities of SOD, CAT and GPx in the liver of control and diabetic rats is given in the table 3. Diabetic rats had decreased activities of SOD, CAT and GPx in the liver, and sesame substitution significantly (p<0.05) improved the activities of these enzymes. The effect of sesame oil on the levels of TC, TG, FFA and PL in the liver of control and diabetic rats is given in the table 4. Diabetic rats had increased levels of TC, TG, FFA and PL in the liver, and sesame substitution significantly (p<0.05) decreased these levels.

DISCUSSION
STZ and alloxan are widely used chemicals for the induction of diabetes in animals. Alloxan not only destroys the pancreatic β-cells but also damages the kidney. The effect is however reversible. But STZ selectively destroys pancreatic insulin-secreting β-cells (24 & 25). In our study, the elevated level of blood glucose in STZ-diabetic rats is due to the destruction of pancreatic β-cells. In sesame oil fed rats, the reduction in blood glucose could be due to the presence of MUFAs as reported earlier by us (13), as MUFA rich diets are capable of lowering blood glucose as reported earlier (11). Liver is the main effector organ for maintaining plasma glucose levels within narrow limits. The increase of free radical mediated toxicity is well documented in clinical diabetes (26). Hyperglycemia can generate a redox imbalance inside the cells, especially in the liver (27). Free radicals result in the consumption of antioxidant defenses which may lead to disruption of cellular functions and oxidative damage to membranes and enhance susceptibility to lipid peroxidation (28). In our study, the lipid peroxidation markers (TBARS and LH) were elevated in the liver of diabetic rats as reported earlier by us (13). The increase in lipid peroxidation might be a reflection of ecrease in antioxidants of defense systems (29). Diabetic rats fed with sesame oil showed a significant reduction in TBARS and LH levels which could be associated with both the presence of vitamin E in the oil and improved glycemic control.

The efficiency of defense mechanism is altered in diabetes and, therefore, the ineffective scavenging of free radicals may play a crucial role in determining tissue damage (30). Normally, the SOD enzyme works in parallel with GPx, which plays an important role in the reduction of hydrogen peroxides in the presence of GSH-forming oxidized glutathione, (GSSG) thereby protecting cell protein and membrane from oxidative stress (31). In our study, the activities of SOD, CAT and GPx decreased in diabetic rats as reported earlier (32 & 33) which could be due to increased utilization for scavenging free radicals. Diabetic rats fed with sesame oil increased the activities of SOD, CAT and GPx significantly which could be associated with improved glycemic control.

Diabetes mellitus is a syndrome which is characterized by chronic hyperglycemia and disturbances of carbohydrate, fat and protein metabolism (34). The association of and altering of lipid parameters present is a major risk of cardiovascular diseases in diabetic patients (35 & 36). The lowering of lipid concentration through dietary or drugs therapy seems to be associated with a decrease in the risk of vascular disease (37). Since currently available hypolipidemic agents lack desired properties of an ideal drug, researchers are involved to find out an effective, safe and less expensive drug.

Diabetic rats fed with sesame oil showed a small but significant reduction in levels of TC and TG when compared with diabetic controls. This could be due to the presence of MUFA and PUFAs in the oil. There have been numerous studies in humans and animals that have demonstrated that oils containing saturated fatty acids raise serum TC and TG, while those enriched in unsaturated fatty acids lower TC and TG (38 &39). Diets high in monounsaturated fatty acids have been found to be relatively hypcholesterolemic and hypotriacylglycerolemic, respectively (40 & 41). The elevated level of FFA in diabetic rats was associated with increased mobilization of FFA from fat depots and treatment with sesame oil reduced FFA significantly. The decreased level of FFA in glibenclamide treated rats is associated with decreased actions of lipolytic hormones, which, in turn, decreased the activity of hormone sensitive lipases on fat deposits.

Phospholipids are vital components of biomembrane and play an important role in the transport of triglycerides (42). In STZ-diabetic rats, the elevated level of phospholipids may be due to the elevated levels of FFA (43) and TC, which can promote the synthesis of phospholipids (44). In diabetic rats fed with sesame oil, the decreased level of phospholipids may be due to decreased levels of TC and FFA.

Sesame oil supplementation in the diet gives better improvement in glycemic control (13) which maintains oscillation of redox status. As MUFA and FUFAs are hypocholesterolemic and hypotriacylglycerolemic, respectively (40 & 41), sesame oil supplementation has reduced the lipid parameters and improved antioxidant status owing to glycemic control and the presence of antioxidant and some active principles of sesame oil, in the liver of diabetic rats. This shows that sesame oil supplementation will be complementary to diabetic patients who are having antidiabetic drug. glibenclamide.

REFERENCES


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