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Ameliorative Influence of Sesame Lignans on Lipid Profile and Lipid Peroxidation in Induced Diabetic Rats

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Sesame lignans are working as antioxidants in various physiological functions. In the present study, the antioxidative effect of sesame lignans is examined in chemically induced diabetes mellitus (DM) in rats against lipid profile and lipid peroxidations. DM was induced in four groups of rats by injection of alloxan. The control groups (non-diabetic and diabetic) received a diet containing sunflower oil while the rest of the three experimental diabetic groups received a diet containing 0.25% α-tocopherol (D-Toc), 0.5% sesame lignan (D-SL), and 0.25% α-tocopherol + 0.25% sesame lignan (D-Toc-SL) in sunflower oil for 4 weeks. Lipid profile and lipid peroxidations of plasma, erythrocyte membrane (EM), and liver tissues were measured. The total cholesterol, non-HDL cholesterol, plasma lipid peroxidation, and also LDL-peroxidation decreased, and HDL cholesterol increased significantly ($P < 0.05$) in all the experimental groups as compared to the control diabetic sunflower oil group. The triacylglycerol (TAG) level in plasma decreased significantly in the D-SL and D-Toc-SL groups as compared to control diabetic group. Significant decrease in TAG level was observed in the D-SL group as compared to the D-Toc group. LDL peroxidation also decreased significantly in the D-Toc-SL group as compared to the D-Toc group. EM lipid peroxidation and liver lipid peroxidation decreased significantly in the D-Toc, D-SL, and D-Toc-SL groups as compared to the control diabetic group. Liver TAG level decreased more significantly in the D-SL and D-Toc-SL groups than in the control diabetic group. So, sesame lignans at 0.5% level and sesame lignan + α-tocopherol significantly ameliorate the alteration in lipid profile and the adverse free radical generative influence of DM induced by alloxan.

KEYWORDS: Antioxidant; diabetes mellitus; sesame lignan; peroxidations; α-tocopherol

INTRODUCTION

Sesame (*Sesamum indicum* L.) is one of the most important oil seed crops cultivated in Asia. India is the largest producer of sesame with approximately 27% of the total production in the world. The superior oxidative stability of sesame oil is due to sesamol, which is present in a very small amount in the natural oil. However, sesamolin, the natural constituent of sesame oil, is capable of generating sesamol by intermolecular transformation during the industrial bleaching process (*1*). Sesame lignans (sesamin and episesamin) are compounds commonly found in refined sesame oil. Episesamin, one of the important components of sesame lignans, is generated from an equivalent amount of sesamin by isomerization during the acid−clay bleaching of oil (*2*). Sesame lignans have multiple physiological functions including antioxidant activity (*3*), antihypertensive effects (*4*) in rats, and alleviation of hepatic injury caused by alcohol or carbon tetrachloride (*5*) in mice. Sesame lignans also affect lipid metabolism, inhibit cholesterol absorption from the intestine, and reduce 3-hydroxy-3-methyl-glutaryl CoA reductase activity in liver microsomes (*6*), thus reducing the cholesterol biosynthesis.

Dietary sesame seed and its lignans inhibit 2,7,8-trimethyl-2(2′-carboxyethyl)-6-hydroxychroman, a γ-tocopherol metabolite excretion into urine of rats fed γ-tocopherol (*7*) and increase the concentration of γ-tocopherol in tissues and serum. The synergistic effect of sesame lignans with tocopherols has also been observed (*8*, *9*). The antioxidant activity of sesamin and sesaminol triglucoside is enhanced by incorporating catechol functional moiety by culturing with genus *Aspergillus* (*10*). Sesaminol fed to rats also increased the α- and γ-tocopherol concentration of serum and tissue lipid. Sesaminol has been found to act as an antioxidant against *in vitro* oxidative damages of human low-density lipoprotein (LDL) (*11*).

Oxidant free radicals have been implicated in the pathogenesis of type I diabetes mellitus (DM) (*12*, *13*). Several biochemical

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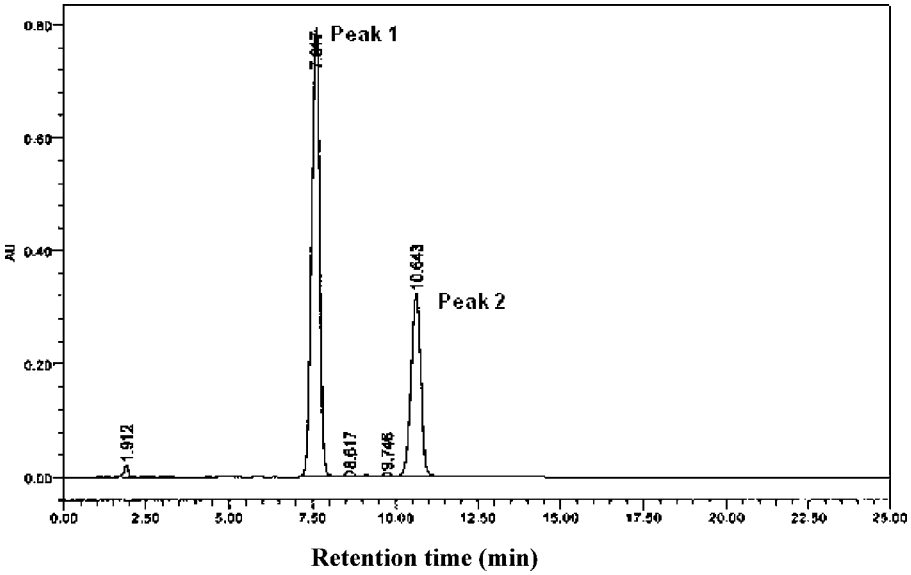

Figure 1. HPLC chromatogram of sesame lignans isolated from sesame oils. Column, Novapak bonded C18 (size: 4.6 × 150 mm); eluent, MeOH/H_2O = 65:35; flow rate 0.8 mL/min; detector, UV 290 nm. Peak 1, sesamin; peak 2, episesamin.

pathways are associated with hyperglycemia and toxic superoxide intermediates (*14*). Diabetic patients have significant defects of antioxidant protection (*15*). DM is also associated with the peroxidation of lipids and plasma lipoproteins (*16*). Oxidized lipoproteins lead to the development of atherosclerosis and diabetic vascular complications. Antioxidants scavenge free radicals and reduce the deleterious consequences within the lipid. Conjugated linoleic acid (CLA), garlic oil, and ferulic acid (*17*−*19*) each act as an antioxidant and reduce the pathological conditions of DM in rats.

A great deal of work has already been done on the best possible effect of sesame lignans in various stress conditions. The aim of the present study was to examine the antioxidative activity of sesame lignans in comparison with α-tocopherol as a protective agent against lipid peroxidation in alloxan induced DM rats.

MATERIALS AND METHODS

Preparation and Analysis of Sesame Lignans. Sesame lignans were prepared from refined sesame seed oil supplied by Vinayak Oils and Fats, Ltd., Kolkata, India, and purified by the method of Fukuda et al. *(2)*. Sesame lignans were analyzed by a high-performance liquid chromatography method. The instrument was provided with binary HPLC pump 1525 and Waters dual absorbance UV detector 2487. The column was Novapac bonded C18 (size: 4.6 × 150 mm) having microparticulate silica of particle size of 5 μm. A total of 20 μL of the sample solution was injected. The mobile phase was methanol/water (65:35, v/v) at a flow rate of 0.8 mL/min. The ultraviolet detection wavelength for sesamin and episesamin was 290 nm. Sesame lignans were 97.5% pure as observed in the HPLC chromatogram (**Figure 1**; 65.5:34.5 sesamin/episesamin, w/w).

Animal Treatment. The animal experiment was performed under the supervision of animal ethical committee of the Department of Chemical Technology, University of Calcutta. Male albino rats of Charles foster strain (selected for the authenticity of the strain) were housed in individual cages. The animals were divided into five groups, each consisting of 10 animals. There was one non-diabetic control group (NDC) without any treatment of alloxan. DM was induced in one control (DC) and three experimental groups of rats by single intraperitoneal injection of 200 mg/kg body weight of freshly prepared alloxan in sterilized water (*18*). After 48 h the blood glucose level was determined by collecting blood from their tail vain. The rats with glycemia > 230 mg/dL were considered diabetic.

The rats were fed experimental diets composed of fat free casein, 18%; fat, 20%; starch, 55%; salt-mixture 4% (composition of salt mixture No. 12 (in g) NaCl, 292.5; KH_2PO_4, 816.6; $MgSO_4$, 120.3; $CaCO_3$, 800.8; $FeSO_4 \cdot 7H_2O$, 56.6; KI, 1.66; $MnSO_4 \cdot 2H_2O$, 9.35; $ZnCl_2$, 0.5452; $CuSO_4 \cdot 5H_2O$, 0.9988; $CoCl_2 \cdot 6H_2O$, 0.0476) (*20*), cellulose, 3%; and one multivitamin capsule (vitamin A I.P. 10 000 units, thiamine mononitrate I.P. 5 mg, vit B I.P. 5 mg, calcium pantothenate USP 5 mg, niacinamide I.P. 50 mg, ascorbic acid I.P. 400 units, cholecalciferol USP 15 units, menadione I.P. 9.1 mg, folic acid I.P. 1 mg, vitamin E USP 0.1 mg) per kg of diet. The diets were adequate in all nutrients.

Sunflower oil (fatty acid composition $C_{16:0}$, 6.2; $C_{18:0}$, 3.4; $C_{18:1}$, 32.8; and $C_{18:2}$, 57.6%) was given as base oil in the diet. The two control groups non-diabetic control group (NDC) and diabetic control (DC) received sunflower oil free of sesame lignans as the dietary fat. The D-Toc group received sunflower oil containing 0.25% α-tocopherol. The D-SL group received a diet containing 0.5% sesame lignan in sunflower oil. The D-Toc-SL group received a diet containing 0.25% sesame lignan + 0.25% α-tocopherol in sunflower oil.

The rats were maintained on the above diets *ad libitum* for 4 weeks. The rats were sacrificed under anesthesia, blood was collected, and livers were immediately excised, blotted and stored frozen (−40 °C) for analysis.

Blood Glucose and Lipids Determination. According to the standard methods, the blood glucose level and the lipid components such as total cholesterol (*21*), high-density lipoprotein (HDL) cholesterol (*22*), and triacylglycerol (TAG) (*23*) of plasma were analyzed using enzymatic kits supplied by Ranbaxy Diagnostics, Ltd. (New Delhi, India).

Plasma lipid peroxidation was measured by the assay of thiobarbituric acid-reactive substances (TBARS) according to the standard method (*24*). The amount of malonedialdehyde (MDA) formed was calculated by taking the extinction coefficient of MDA to be 1.56×10^5 M^{-1} cm^{-1}.

Lipoprotein Oxidation Susceptibility (LOS) Test. A 500 μL plasma sample was treated with 50 μL of a solution containing 0.2 mM dextran sulfate (MW 50 000, Genzyme, Cambridge, MA) and 0.5 M $MgCl_2 \cdot 6H_2O$ to precipitate the apo B-containing lipoproteins (LDL and very low-density lipoprotein, VLDL) according to Bachorik and Albers (*25*). After centrifugation at 3000*g* at 20 °C for 10 min, the supernatant was removed, and 1 mL of 6% bovine serum albumin and another 50 μL of the dextran sulfate magnesium solution was added. The solution was briefly vortexed and recentrifuged as above to wash away any HDL or residual serum proteins (except, of course, albumin). The supernatant was removed, and washed precipitate (containing LDL and VLDL) was dissolved in 2.5 μL of 4% NaCl. A volume of redissolved precipitate containing 100 μg of non-HDL cholesterol was

Table 1. Plasma Lipid Profile and Blood Sugar Level of Rats in Different Groups[a]

group	blood glucose (mg/dL)	total cholesterol (mg/dL)	HDL cholesterol (mg/dL)	non-HDL cholesterol (mg/dL)	TAG (mg/dL)
NDC	81.7 ± 3.61	61.5 ± 2.79	35.6 ± 2.4	25.8 ± 3.70	33.6 ± 2.4
DC	261.3 ± 15.23[b]	80.9 ± 3.30[b]	13.9 ± 1.68[b]	66.9 ± 4.02[b]	46.4 ± 9.00[b]
D-Toc	218.6 ± 11.26[c]	54.7 ± 3.90[c]	20.5 ± 2.11[c]	33.9 ± 3.37[c]	39.5 ± 3.55
D-SL	229.9 ± 7.86[d]	59.6 ± 2.36[d]	20.0 ± 2.58[d]	39.6 ± 2.87[d]	24.5 ± 3.3[d,f]
D-Toc-SL	219.2 ± 8.72[e]	53.9 ± 2.84[e]	21.3 ± 1.23[e]	33.9 ± 3.37[e]	28.5 ± 0.32[e]

[a] NDC, non-diabetic control group fed sunflower oil; DC, diabetic control group fed sunflower oil; D-Toc, diabetic group fed sunflower oil containing 0.25% α-tocopherol; D-SL, diabetic group fed sunflower oil containing 0.5% sesame lignans; D-Toc-SL, diabetic group fed sunflower oil containing 0.25% α-tocopherol + 0.25% sesame lignans. All values are mean ± SEM of eight rats. Significant *F* ratios for total cholesterol (*p* value: 0.0001), HDL cholesterol (*p* value: 0.0001), non-HDL cholesterol (*p* value: 0.0001), TAG (*p* value: 0.004), and blood sugar (*p* value: 0.0001). [b] DC vs NDC group. [c] D-Toc vs DC group. [d] D-SL group vs DC group [e] D-Toc-SL group vs DC group [f] D-SL group vs D-Toc group.

combined with sufficient 4% NaCl to give a total volume of 500 μL (approximately a 1:5 dilution). A total of 50 μL of a 0.5 mM $CuCl_2 \cdot 2H_2O$ solution was added (final copper concentration was 46 μM), and then the samples were incubated at 37 °C in a shaking water bath for 3 h. Next TBARS was measured by adding 2 mL of the TBARS reagent (26 mM thiobarbituric acid (TBA) in 0.25 N HCl containing 15.03 g trichloroacetic acid (TCA) in 100 mL) to each tube. The mixture was heated at 100 °C in a water bath for 15 min. After removing and cooling the tubes, 2.5 mL of *n*-butanol was added and the tubes were vortexed and then centrifuged for 15 min at 3000 rpm at room temperature. The pink upper layer was removed, and the optical density was determined in a spectrophotometer at 532 nm according to the method described by Phelps and Harris (*26*).

Preparation and Oxidative Sensitivity of Erythrocyte Membrane (EM) Ghost (*27*). After plasma separation, the red blood cells (RBC) were washed three times by centrifugation at 3000*g* for 10 min with three volumes of a cooled isotonic solution containing 0.15 M NaCl and 10^{-5} M ethylenediamine tetraacetic acid. RBC was hemolized using hypotonic solution and centrifuged at 20 000*g* for 40 min in a cold centrifuge at 4 °C. The supernatant was removed carefully with a Pasteur pipet. The process was repeated two more times. After the last wash step, the supernatant was removed as much as possible, and the loosely packed milky-looking membrane pellet was re-suspended at the bottom of the tube using 0.89% NaCl solution. The concentrated membrane solution was taken in 2 mL screw cap vial and stored at −40 °C.

A modification of the 2-thiobarbituric acid test (*28*) was used to measure the lipid peroxides. A 0.5 mL aliquot of the red blood corpuscle membrane suspension was mixed with 1.0 mL of 10% TCA and 2.0 mL of 0.67% of 2-thiobarbituric acids. The mixture was heated at 95 °C for 15 min, cooled, and centrifuged. The absorbance of the supernatant was measured at 534 nm in a spectrophotometer (Shimadzu, Tokyo, Japan), and the amount of MDA formed was calculated by taking the extinction coefficient of MDA to be 1.56×10^5 M^{-1} cm^{-1}.

Liver Tissue Lipid Extraction and Peroxidation. Total lipids were extracted from an aliquot of tissue homogenate by the method of Bligh and Dyer (*29*). For lipid peroxide measurement, a TBA test in the chloroform phase was performed according to the method described by Schmedes and Holmer (*30*). Chloroform (2.5 mL) containing liver lipids from the Bligh and Dyer extraction was pipetted into a 30 mL autoclavable glass culture tube (Kimax; Kimble/Kontes, Vineland, NJ) having a Teflon lined screw cap; 4 mL of the TBA reagent (0.67% TBA in water) was added, and the tube was capped tightly. Safety-shielded tubes were heated for 30 min in a boiling water bath and cooled in tap water. After cooling, 3.5 mL of TCA (5%) was added to each tube, mixed thoroughly, and the tubes were then centrifuged at 3000*g* for 5 min. The absorbance was measured at 534 nm, and relative amount of lipid peroxidation was calculated by taking the extinction coefficient of MDA to be 1.56×10^5 M^{-1} cm^{-1}.

Assay of Protein. EM protein was estimated by the method of Lowry et al. (*31*).

Total Phospholipid Content in Liver Lipids. Phospholipid content in liver lipids was measured by the method of Chen et al. (*32*)

Statistical Analysis. The data were expressed as mean ± standard error of mean (SEM). A one-way ANOVA was also used for statistical analysis between groups. The *F* ratio of one-way ANOVA is significant when $P < 0.05$. Tukey's multiple range method (*33*) was used for comparison. The statistical program was MINITAB release 13.31 (MINITAB, State College, PA).

Table 2. Lipid Profile of EM Ghost of Rats[a]

diet	phospholipid mg/mg of protein	cholesterol mg/mg of protein	phospholipid/cholesterol ratio
NDC	1.33 ± 0.17	0.33 ± 0.02	5.32 ± 0.47
DC	1.79 ± 0.19	0.32 ± 0.03	4.72 ± 0.41
D-Toc	1.55 ± 0.25	0.38 ± 0.05	4.85 ± 0.81
D-SL	1.93 ± 0.25	0.31 ± 0.03	5.83 ± 0.49
D-Toc-SL	1.73 ± 0.19	0.25± 0.03	5.56 ± 0.53

[a] All values are mean ± SEM of eight rats. *F* < critical *F* for phospholipid, cholesterol, and phosoholipid/cholesterol ratio.

RESULTS

Table 1 summarizes the lipid profile and blood sugar level of the rats (NDC and DC groups), raised on sunflower oil and sunflower oil containing 0.25% α-tocopherol (D-Toc), 0.5% sesame lignan (D-SL), and 0.25% α-tocopherol + 0.25% sesame lignan (D-Toc-SL). Diabetes induced by alloxan increased total cholesterol, non-HDL cholesterol, TAG, and blood sugar levels and decreased HDL cholesterol significantly in comparison with the corresponding values of the control non-diabetic rats. Total cholesterol and non-HDL cholesterol decreased, and HDL cholesterol increased in all the experimental groups significantly ($P < 0.05$) compared to the control diabetic sunflower oil group (DC). The TAG level in plasma decreased significantly in the D-SL and D-Toc-SL group compared to that in the control DC group. The TAG level also decreased significantly in the D-SL group compared to that in the D-Toc group.

Lipid profiles (**Table 2**) in EM ghost of five dietary groups of rats were analyzed. Phospholipid and cholesterol content in the DC group did not increase significantly compared with the NDC group. The phospholipid and cholesterol content also showed no significant difference in DC and all the experimental groups of rats.

Table 3 shows the liver lipid profile of rats. No significant difference was noted in the content of total lipid, total cholesterol, and phospholipid between the DC group and the NDC group. But the TAG level increased significantly in the DC group compared with the NDC group. The TAG content decreased significantly in the D-SL and D-Toc-SL group compared to the control diabetic group DC.

Lipid peroxidation of plasma, LDL, EM ghost, and liver lipids was measured in five dietary groups (**Table 4**). Plasma lipid peroxidation increased significantly in the DC group compared

Table 3. Liver Lipid Profile of Rats (per g of Tissue)[a]

diet	total lipid (mg)	total cholesterol (mg)	phospholipid (mg)	TAG (mg)
NDC	80.42 ± 1.22	2.12 ± 0.27	16.11± 0.51	7.22 ± 0.54
DC	61.13 ± 2.41	2.19 ± 0.21	15.83 ± 1.33	11.41 ± 1.23[b]
D-Toc	81.32 ± 2.24	3.05 ± 0.28	14.28 ± 1.23	8.56 ± 1.13
D-SL	72.52 ± 2.43	2.38 ± 0.28	16.27 ± 1.53	6.79 ± 0.69[c]
D-Toc-SL	71.63 ± 2.71	2.36 ± 0.13	16.89 ± 1.15	6.65 ± 0.58[d]

[a] All values are mean ± SEM of eight rats. Significant *F* ratios for liver TAG (*p* value: 0.003). *F* < critical *F* for total lipid, phospholipid, and total cholesterol. [b] DC vs NDC. [c] D-SL vs DC. [d] D-Toc-SL vs DC.

Table 4. Plasma Lipid, Lipoprotein, EM, and Liver Lipid Peroxidation of Rats of Different Groups[a]

diet	plasma peroxidation (nmol MDA/mL of plasma)	LDL peroxidation (nmol of MDA/mg of non-HDL cholesterol)	EM lipid peroxidation (nmol of MDA/mg of protein)	liver lipid peroxidation (nmol of MDA/mg of tissue lipid)
NDC	2.89 ± 0.06	0.18 ± 0.02	4.93± 0.36	1.48 ± 0.21
DC	6.13 ± 0.08[b]	0.25 ± 0.01[b]	12.81 ± 0.64[b]	3.05 ± 0.24[b]
D-Toc	1.15 ± 0.05[c]	0.13 ± 0.02[c]	3.77 ± 0.56[c]	1.58 ± 0.18[c]
D-SL	1.82 ± 0.03[d]	0.08 ± 0.02[d]	4.38 ± 0.44[d]	1.51 ± 0.28[d]
D-Toc-SL	1.36 ± 0.02[e]	0.07± 0.01[e,f]	2.29 ± 0.21[e,f,g]	1.47 ± 0.20[e]

[a] All values are mean ± SEM of eight rats. Significant *F* ratios for plasma lipid peroxidation, LDL peroxidation, EM lipid peroxidation, and liver lipid peroxidation (*p* value: 0.0001). [b] DC vs NDC. [c] D-Toc vs DC. [d] D-SL vs DC. [e] D-Toc-SL vs DC. [f] D-Toc-SL vs D-Toc. [g] D-Toc-SL vs D-SL.

to the NDC group. But plasma lipid peroxidation decreased significantly in the D-Toc, D-SL, and D-Toc-SL groups compared to the control DC group. LOS induced by copper decreased significantly in the D-Toc, D-SL, and D-Toc-SL group compared to the control DC group (**Table 4**). It also decreased significantly in the D-Toc-SL group compared to the D-Toc group.

EM lipid peroxidation increased significantly in the DC group (**Table 4**). The TBARS production in the D-Toc, D-SL, and D-Toc-SL groups decreased significantly compared to the control DC group. EM lipid peroxidation also decreased significantly in the D-Toc-SL group compared to the D-SL and NDC group. In the case of liver lipids (**Table 4**), lipid peroxidation increased significantly in the DC group compared to the NDC group, whereas the production of TBARS *in vitro* in liver lipids decreased significantly in the D-Toc, D-SL, and D-Toc-SL group compared to the DC group.

DISCUSSION

As one of the several targets in the management of diabetes is to control the atherogenic lipid content, the plasma lipid profile of DC and NDC rats after sesame lignans and α-tocopherol supplementation was determined. Besides effects on blood sugar levels, DM induced by alloxan also changed the lipid profiles dramatically. The present study demonstrates that dietary supplementation with 0.25% α-tocopherol (D-Toc), 0.5% sesame lignan (D-SL), 0.25% sesame lignan + 0.25% α-tocopherol (D-Toc-SL) ameliorates the atherogenic lipid profile associated with alloxan-induced diabetes.

The plasma TAG level in the DC group was increased significantly compared to the NDC group. Insulin acts as a potent inhibitor of lipolysis in the liver and adipose tissue. Thus it is speculated that lipolysis is accelerated in DM rats with a concurrent increase in plasma TAG level. The 0.5% sesame lignan and 0.25% sesame lignan + 0.25% α-tocopherol for 28 days significantly decreased plasma TAG levels in the diabetic rats. Sesame lignans profoundly increases the hepatic fatty acid oxidation rate, lower the fatty acid synthesis (*34*), and thus decrease the serum TAG level of the diabetic rats. Plasma total cholesterol and non-HDL cholesterol increased in the DC group of rats compared to the NDC group. But 0.5% sesame lignan and 0.25% sesame lignan + 0.25% α-tocopherol supplementation in the diet decreased plasma total cholesterol and non-HDL cholesterol and increased the HDL cholesterol in diabetic induced rats. Earlier, the report on the effects of sesamin on lipid metabolism revealed that it inhibits Δ5-desaturase activity (*35*), reduces cholesterol absorption in the intestinal tract, increases excretion of cholesterol into bile, and reduces HMG-CoA reductase activity, a rate-limiting enzyme of the cholesterol synthesis (*6*). In cultured liver cells it has been observed that sesamin inhibits acyl-CoA/cholesterol acyltransferase (ACAT) activity (*36*). In the present study we also observed the reduction of hepatic cholesterol and TAG levels in diabetic rats when more sesame lignan is supplemented in the diet compared to the control groups.

In alloxan induced DM, a large amount of free radicals are generated (*37*) and cause oxidative damage. The effects of different synthetic and natural antioxidants as inhibitors of LDL peroxidation and their possible therapeutic effects to counteract atherogenesis are well documented (*38*, *39*). In the present study, the D-Toc, D-SL, and D-Toc-SL group rats showed significant decrease in MDA formation compared to the control DC group both in plasma and LDL peroxidation. The formation of MDA was completely inhibited when α-tocopherol was supplemented along with sesame lignan. The reduction of MDA formation was significantly greater compared to the D-Toc group. Previous workers have investigated that sesaminol inhibited the Cu^{+2} induced LDL peroxidation. Probucol and α-tocopherol at the same concentration exhibited a lesser inhibitory effect (*40*). Our findings suggest that dietary sesame lignans and sesame lignans along with α-tocopherol is a potentially effective antioxidant that can protect LDL against oxidation in the diabetic condition.

DM is associated with a wide variety of toxicological effects, including decreased membrane fluidity and function. The RBC membrane is rich in polyunsaturated fatty acids that are very susceptible to free radical mediated lipid peroxidation. Alloxan induced DM generated a very high amount of free radicals (*41*). Sesame lignans along with α-tocopherol supplementation reduced the MDA formation in EM ghost significantly compared to the DC and the D-SL groups. Thus sesame lignans + α-tocopherol showed a synergistic role in scavenging free oxygen radicals and in stabilizing the cell membrane, thus maintaining its permeability. This observation is supported by the previous observation that sesamin and α-tocopherol synergistically suppress lipid peroxide in rats fed a diet containing a high dose of docosahexaenoic acid (*42*).

The liver lipid peroxidation also showed a marked increase in the DC group of rats. Dietary intake of sesame lignans, α-tocopherol, and sesame lignans + α-tocopherol significantly reduced the MDA formation in liver lipid peroxidation. According to our observation the dietary antioxidants brought the level of MDA formation to the non-diabetic condition. The possible mechanism of the antioxidative effect of sesame lignans may raise the α-tocopherol concentrations in liver and plasma. Previous studies have shown that sesame lignans added to rat diet resulted in significantly greater plasma and tissue concen-

trations of α- and γ-tocopherol in supplemented rats compared to rats without supplementation (*11*).

In the experimental rats sesame lignans with the 0.5% level in the diet significantly decreased the cholesterol levels which may be due to reduction of 3-hydroxy-3-methyl-glutaryl CoA reductase activity in liver microsomes as observed by others (*6*). Sesame lignans also affects lipid metabolism as reflected by reduction of TAG level.

In alloxan induced DM, a large amount of free radicals are generated that cause oxidative damage. Sesame lignans present in the diet of the D-SL and D-Toc-SL groups of rats have significantly reduced the peroxidations in plasma lipid, LDL lipid, EM lipid, and liver lipid. The antioxidative effect of sesame lignans is enhanced in the presence of α-tocopherol as observed in EM lipid peroxidation. So sesame lignans can play a definite role in regulating the lipid abnormalities and lipid peroxidation caused by non-insulin dependent diabetes mellitus.

The results of the present study demonstrated the beneficial effect of sesame lignans at the 0.5% level by significantly ameliorating the adverse free radical generative influence of alloxan. The present report measuring the effect of sesame lignans as an *in vivo* antioxidative agent on lipid peroxidation and lipid profile in diabetic rats is first of its kind.

ABBREVIATIONS USED

DM, diabetes mellitus; EM, erythrocyte membrane; LDL, low-density lipoprotein; VLDL, very low-density lipoprotein; TAG, triacylglycerol; CLA, conjugated linoleic acid; HPLC, high-performance liquid chromatography; TBA, thiobarbituric acid; TCA, trichloroacetic acid; MDA, malonedialdehyde; NIDDM, non-insulin dependent diabetes mellitus; NDC, non-diabetic control group; DC, diabetic control group

LITERATURE CITED

(1) Tashiro, T.; Fukuda, Y.; Osawa, T.; Namiki, M. Oil and minor components of sesame (*Sesamum indicum* L.) strains. *J. Am. Oil Chem. Soc.* **1990**, *67*, 508−511.

(2) Fukuda, Y.; Nagata, M.; Osawa, T.; Namiki, M. Contribution of lignan analogues to antioxidative activity of refined unroasted sesame seed oil. *J. Am. Oil Chem. Soc.* **1986**, *63*, 1027−1031.

(3) Hirose, N.; Doi, F.; Ueki, T.; Akazawa, K.; Chijiiwa, Sugano, K.; M.; Akimoto, K.; Shimizu, S.; Yamada, H. Suppresive effect of sesamin against 7, 12-Dimethyl benzen[a]anthracene induced rat mammary carcinogenesis. *Anticancer Res.* **1992**, *12*, 1259−1266.

(4) Matsumura, Y.; Kita, S.; Morimoto, S.; Akimoto, K.; Furua, M.; Oka, N.; Tanaka, T. Antihypertensive effect of sesamin I. Protection against deoxycorticosterone acetate salt-induced hypertension and cardiovascular hypertrophy. *Biol. Pharm. Bull.* **1995**, *18*, 1016−1019.

(5) Akimoto, K.; Kitagawa, Y.; Akamatsu, T.; Hirose, N.; Sugano, M.; Shimizu, S.; Yamada, H. Protective effects of sesamin against liver damage caused by alcohol or carbon tetrachloride. *Ann. Nutr. Metab.* **1993**, *37*, 218−224.

(6) Hirose, N.; Inoue, T.; Nishihara, K.; Sugano, M.; Akimoto, K.; Shimizu, S.; Yamada, H. Inhibition of cholesterol absorption and synthesis in rats by sesamin. *J. Lipid Res.* **1991**, *32*, 629−638.

(7) Ikeda, S.; Tohyama, T.; Yamashita, K. Dietary sesame seed and its lignans inhibit 2,7,8-Trimethyl-2(2′-carboxyethyl)-6-hydroxychroman excretion into urine of rats fed γ-tocopherol. *J. Nutr.* **2002**, *132*, 961−966.

(8) Ghafoorunissa; Hemalatha, S.; Rao, M. V. Sesame lignans enhance antioxidant activity of vitamin E in lipid peroxidation systems. *Mol. Cell Biochem.* **2004**, *262*, 195−202.

(9) Hemalatha, S.; Raghunatha, M.; Ghafoorunissa. Dietary Sesame oils inhibits iron-induced oxidative stress in rats. *Br. J. Nutr.* **2004**, *92*, 581−587.

(10) Miyake, Y.; Fukumoto, S.; Okada, M.; Sakaida, K.; Nakamura, Y.; Osawa, T. Antioxidative catechol lignans converted from sesamin and sesaminol triglucoside by culturing with *Aspergillus*. *J. Agric. Food Chem.* **2005**, *53*, 22−27.

(11) Yamashita, K.; Ikeda, S.; Lizuka, Y.; Ikeda, I. Effect of sesaminol on plasma and tissue tocopherol and tocotrienol concentrations in rats fed a vitamin E concentrate rich tocotrienols. *Lipids* **2002**, *37*, 351−358.

(12) Oberley, L. W. Free radicals and diabetes. *Free Radical Biol. Med.* **1998**, *5*, 113−124.

(13) Wolff, S.P. Diabetes mellitus and free radicals. *Br. Med. Bull.* **1993**, *49*, 642−652.

(14) Guiugliano, D.; Cericilo, A.; Paaolisso, G. Oxidative stress and diabetic vascular complications. *Diabetes Care* **1996**, *19*, 257−267.

(15) Baynes, J. W. Role of oxidative stress in development of complications in diabetes. *Diabetes* **1991**, *40*, 405−412.

(16) Gallou, G.; Ruelland, A.; Legras, B.; Maugendre, D.; Allannic, H.; Cloarec, L. Plasma malonyldehyde in type I and type II diabetic patients. *Clin. Chem. Acta* **1993**, *214*, 227−234.

(17) Parthsarathy, S.; Young, S. G.; Witztum, J. L.; Pitman, R. C.; Steinberg, D. Probucol Inhibits Oxidative Modification of Low Density Lipoprotein, *J. Clin. Invest.* **1986**, *77*, 641−644.

(18) Ohaeri, O. C.; Odoja, G. I. Effects of garlic oil acidity and electrolytes in experimental diabetic rats. *Med. Sci. Res.* **1998**, *26*, 487−488.

(19) Anuradha, C. V.; Ravikumar, P. Anti-lipid peroxidative activity of seeds of fenugreek (*Trigonella foenum graecum*). *Med. Sci. Res.* **1999**, *27*, 487−489.

(20) Joanes, J. H.; Foster, C. A. Salt mixture for use with basal diet either low or high in phosphorus. *J. Nutr.* **1942**, *24*, 245−256.

(21) Allain, C. C.; Poon, L. S.; Chan, C. S. G.; Richmond, W.; Fu, P. C. Enzymatic determination of total serum cholesterol. *Clin. Chem.* **1974**, *20*, 470−475.

(22) Warnick, G. R.; Nguyen, T.; Albers, A. Comparison of improved methods for quantification of high-density lipoprotein. *Clin. Chem.* **1985**, *31*, 217−222.

(23) Bucolo, G.; David, M. Quantitative determination of serum triglyceride by the use of enzymes. *Clin. Chem.* **1973**, *19*, 476−482.

(24) Wills, E. D. Evaluation of lipid peroxidation in lipids and biological membranes. In *Biological Toxicology: A Practical Approach*; Snell, K., Mullock B., Eds.; IRL Press: Oxford, 1987; pp 127−151.

(25) Bachorik, P. S.; Albers J. J. Precipitation methods for quantification of lipoproteins, *Methods Enzymol.* **1986**, *129*, 78−100.

(26) Phelps, S.; Harris, W. S. Garlic supplementation and lipoprotein oxidation susceptibility. *Lipids* **1993**, *28*, 475−477.

(27) Rose, H. G.; Oklander, M. J. Improved procedures for the extraction of lipids from human erythrocytes. *Lipid Res.* **1965**, *6*, 428−431.

(28) Mezick, J. A.; Settlemire, C. T.; Brierley, G. P.; Barefield, K. P.; Jensen, W. N.; Cornwell, D. G. Erythrocyte membrane interactions with menadione and menadione−induced hemolysis. *Biochim. Biophys. Acta* **1970**, *219*, 361−371.

(29) Bligh, E. H.; Dyer, W. J. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* **1959**, *37*, 911−916.

(30) Schmedes, A.; Holmer, G. New thiobarbituric acid (TBA) method for determining free malondialdehyde (MDA) and hydroperoxides selectivity as a measure of lipid peroxidation. *J. Am. Oil Chem. Soc.* **1989**, *66*, 813−817.

(31) Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. Protein measurement with the Foline phenol reagent. *J. Biol. Chem.* **1951**, *193*, 265−275.

(32) Chen, P. S.; Toribara, T. Y.; Warner, H. Micro determination of phosphorous. *Anal. Chem.* **1956**, *28*, 1756−1758.

(33) Pearson, S., Hartley, H. O., Eds. *Biometrika Tables for Statisticians*; Cambridge University Press: Cambridge, 1966; Vol. 1, Table 12.

(34) Sirato-Yasumoto, S.; Katsuta, M.; Okuyama, Y.; Takashashi, Y.; Ide, T. Effect of sesame seeds rich in sesamin and sesamolin on fatty acid oxidation in rat liver. *J. Agric. Food Chem.* **2001**, *49*, 2647−2651.

(35) Shimizu, S.; Akimoto, K.; Kawashima, H.; Sugano, M.; Yamada, H. Sesamin is a potent and specific inhibitor of Δ-5 desaturase in polyunsaturated fatty acid biosynthesis. *Lipids* **1991**, *26*, 512−516.

(36) Sawada, R. U.; Fujiwara, Y.; Igarashi, O. Effect of sesamin on cholesterol synthesis and on the distribution of incorporated linoleic acid in lipid subfractions in cultured rat cells. *Biosci. Biotechnol. Biochem*. **1994**, *58*, 2114−2115.

(37) Takasu, N.; Komiya, I.; Asawa, T.; Nagasawa, Y.; Yamada, T. Streptozotocin and alloxan induced H_2O_2 as mediator for DNA fragmentation. *Diabetes* **1991**, *40*, 1141−1145.

(38) Osawa, T. Phenolic antioxidants in dietary plants as antimutagens. In *Phenolic Compounds in Food and Health*; Ho, C. T., Lee, C. Y., Hung, C. Y., Eds.; American Chemical Society: Washington, DC, 1992; pp 135−149.

(39) Osawa, T.; Yoshida, S.; Yamashida, K.; Ochi, H. Protective role of dietary antioxidants in oxidative stress. In *Oxidative Stress and Aging*; Cutler, R. G., Packer, L., Bertram, J., Mori, A., Eds.; Birkhauser-Verlag: Basel, Switzerland, 1995; pp 367−377.

(40) Kang, M. H.; Naito, M.; Sakai, K.; Uchida, K.; Osawa, T. Mode of action of sesame lignans in protecting low density lipoprotein against oxidative damage *in vitro*. *Life Sci.* **1999**, *66*, 161−171.

(41) Dhar, P.; Bhattacharyya, D.; Bhattacharyya, D. K.; Ghosh, S. Dietary comparison of conjugated linolenic acid (9 cis, 11 trans, 13 trans) and α-tocopherol effects on blood lipids and lipid peroxidation in alloxan-induced diabetes mellitus in rats. *Lipids* **2006**, *41*, 49−54.

(42) Yamashita, K.; Kagaya, M.; Ahiguti, N.; Kiso, Y. Sesamin and α-tocopherol synergistically suppress lipid-peroxide in rats fed a high docosahexaenoic diet. *Biofactors* **2000**, *11*, 11−13.

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