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Antioxidative Effect of Sesame Lignans in Diabetes Mellitus Blood: an *in vitro* study

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Abstract: Refined and bleached sesame oil (*Sesamum indicum* L.) contains sesame lignans, mainly sesamin and episesamin, and they are isolated from refined sesame oil. The *in vitro* antioxidant properties of sesame lignans and sesamol are investigated on oxidative modification of human plasma, low-density lipoprotein (LDL) and erythrocyte membrane lipid. Blood samples are collected from diabetic and non-diabetic (normal) healthy individuals. Sesame lignans and sesamol are added at 0.05% and 0.1% concentrations to plasma, LDL and erythrocyte membrane isolated from the respective blood samples and peroxidations are determined against control samples. A significant increase of peroxidation levels was observed consequently in diabetic control blood and the non-diabetic control blood. Sesamol and sesame lignans decreased lipid peroxidation. The findings suggest that sesamol and sesame lignans (mainly sesamin and episesamin) are potentially effective antioxidants that can protect plasma, LDL and erythrocyte membrane from oxidation which may be effective in reducing the risk of coronary heart disease in diabetes.

Key words: antioxidant, diabetes mellitus, sesame lignan, sesamol

1 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 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 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, reduce 3-hydroxy-3-methyl-glutaryl CoA reductase activity in liver microsomes (6) thus reducing the cholesterol biosynthesis.

Diabetes is a heterogeneous metabolic disorder characterized by an elevated blood glucose level and inefficient insulin action. There is evidence that the development of diabetes, primarily non-insulin dependent diabetes mellitus (NIDDM) is strongly associated with

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dietary factors. The diabetic persons with NIDDM are at high risk for development of atherosclerosis as a result of cardiovascular diseases and the cause of approximately 80% of mortalities in diabetic persons. Lipid abnormalities such as hyper triglyceridemia (7) and fatty acid distribution changes (8) could participate in the development of vascular lesions in diabetes.

Free-radical mechanisms have been implicated in the pathology of several human diseases, including cancer, atherosclerosis, malaria, rheumatoid arthritis, neuro degenerative diseases and the most killer disease, diabetes mellitus. Free radicals of importance in living organisms include hydroxyl (OH·), superoxide (O_2·-), nitric oxide (NO·), and peroxyl (RO_2·). Peroxynitrite (ONOO-), hypochlorous acid (HOCl), hydrogen peroxide (H_2O_2), singlet oxygen and ozone (O_3) are not free radicals but can easily lead to free-radicals reactions in living organisms and are included in the term reactive oxygen species (ROS).

Diabetes mellitus leads to the production of ROS, which leads to tissue damages, and coronary heart disease. Sesame lignans acting as antioxidants may protect from ROS produced during diabetes mellitus.

The main objective of the work is to study the *in vitro* antioxidative role of sesame lignans (sesamin and episesamin), and sesamol by determining the thiobarbituric acid reactive substance (TBARS) in plasma, LDL and erythrocyte membrane ghost lipid peroxidation induced by copper of diabetic and non-diabetic human blood collected from male individuals.

2 Materials and Methods

2·1 Preparation and Analysis of Sesame Lignans

Sesame lignans are prepared by the method of Fukuda *et al*. (2) from refined sesame seed oil. Sesame lignans were analyzed by HPLC method and it was 97.5% pure (sesamin : episesamin −51.4:48.6, w/w). Sesamol was purchased from Sigma Chemical Company, St. Louis, MO, USA.

2·2 Collection of Blood

Normal (non-diabetic) blood was collected from human volunteers of the age group 40−50 years of fasting blood glucose level 90 ± 10 mg/dL. Diabetic blood was collected from patients suffering from diabetes mellitus (oral drug dependent) for at least 5 years with high fasting blood glucose level (200 ± 20 mg/dL) in an uncontrolled manner. Plasma was separated and glucose, total-cholesterol and HDL-cholesterol were measured by using enzymatic kit (Ranbaxy Diagnostics Limited, New Delhi, India).

2·3 Plasma Peroxidation

Plasma peroxidation was measured by the assay of thiobarbituric acid-reactive substances (TBARS) according to the standard method (9). The antioxidants dissolved in methanol were added to the plasma samples. The amount of malone-di-aldehyde (MDA) formed was calculated by taking the extinction coefficient of MDA to be $1.56 \times 10^5 M^{-1}cm^{-1}$.

2·4 Lipoprotein Oxidation Susceptibility (LOS) Test

Non-HDL Cholesterol oxidation was carried out by precipitating apo-B lipoprotein (LDL and VLDL) according to *Bachorik* and *Albers* (10). The antioxidants dissolved in methanol were added to the plasma samples. The LOS test was carried out according to the method described by *Phelps* and *Harris* (11).

2·5 Preparation and Oxidative Sensitivity of Erythrocyte Membrane Ghost

After plasma separation, the erythrocytes were washed three times with three volume of a cooled isotonic solution containing 0.15 M NaCl and 10^{-5}M EDTA. The buffy coat was removed by aspiration after each wash. Finally the erythrocyte membrane ghosts were prepared by using hypotonic solution of NaCl according to the method of Rose and *Oklander* (12).

2·6 Modified 2-thiobarbituric Acid Test

A modification of the 2-thiobarbituric acid test (13) was used to measure the lipid peroxides. A 0.5 mL aliquot of the erythrocyte membrane suspension was mixed with 1.0 mL of 10% trichloroacetic acid and 2.0 mL of 0.67% of 2-thiobarbituric acid. The mixture was heated at 95 ℃ for 15 min, cooled, and centrifuged. The absorbance of the supernatant was measured at 534 nm in a spectrophotometer (Shimadzu, Tokyo, Japan), and the relative amounts of lipid peroxides were expressed in absorbance units, A_{534} nm (14).

2·7 Assay of Protein

Erythrocyte membrane protein was estimated by the

method of Lowry *et al*. (15).

2·8 Statistical Analysis

The results are given as the mean ± standard error of mean. For statistical analysis of result, student's t test (16) was performed.

3 Results and Discussion

The blood was collected from non-diabetic and diabetic male individuals. The extent of plasma lipid and LDL lipid peroxidations induced by 10 μM $CuSO_4$ was assessed by measuring the formation of TBARS. **Table 1** shows that different doses of sesame lignans and sesamol strongly inhibited the oxidative modification of plasma lipid and LDL. Peroxidations are reduced more in the sesamol and sesame lignan added groups than the control group. There is no significant change of plasma lipid peroxidation with the increase of sesamol and sesame lignan concentrations in case of non-diabetic group. In diabetic group 0.1% concentration of sesamol and sesame lignans is more effective than 0.05% concentration in reducing plasma lipid peroxidation. Both sesamol and sesame lignans exhibited similar extend of reduction of MDA formation in diabetic group compared to control group. Copper (Cu) induced lipoprotein oxidation susceptibility (LOS) decreased significantly both in diabetic and non-diabetic group than control group. With the increase of sesamol and sesame lignan concentration there is no significant change of LDL peroxidation level in non-diabetic group, where as in diabetic group 0.1% sesamol is more effective than 0.1% concentration of sesame lignan in reducing MDA formation. Erythrocyte membrane (Ghost) lipid peroxidations of the diabetic and non-diabetic groups are given in **Table 2**. The erythrocyte membrane lipid peroxidations are significantly restricted in the experimental groups where sesamol and sesame lignans are added at 0.05% and 0.1% concentrations. But there is no significant difference of MDA formation both in diabetic and non-diabetic group with the variation of sesamol and sesame lignan concentration.

Endogenous antioxidants may be important for defense against oxidative stress (17). A considerable amount of study has been conducted to the different synthetic and natural antioxidants as inhibitors of LDL peroxidation and their possible therapeutic effects to counteract atherogenesis (17,18). Phenolic compounds in sesame seed exhibited strong antioxidant properties (19). Plasma lipid, LDL and erythrocyte membrane lipid are susceptible to peroxidation. LDL-peroxidation and its inhibition by antioxidants are important in the molecular mechanism of atherosclerosis and in identifying foods that may prevent the disease. Glucose autooxidation, polyol pathway and protein glycation are biochemical pathways associated with hyperglycaemia and toxic superoxide intermediates of type I diabetes mellitus (20). This lead to the peroxidation of lipids of plasma, lipoprotein and erythrocyte membrane (21). Oxidized lipoproteins lead to the development of atherosclerosis and diabetic vascular complications. Erythrocyte membranes also contain high amount of polyunsaturated fatty acids, which are prone to oxidation by free radicals generated in diabetes mellitus.

In the present study, overall plasma, LDL and erythrocyte membrane obtained from blood of diabetic and non-diabetic individuals were used to investigate the lipid peroxidations and also to elucidate the antioxida-

Table 1 Effect of Sesame Lignans on *In Vitro* Plasma and LDL Peroxidation In Non-Diabetic and Diabetic Blood.

Groups	Plasma Lipid peroxidation (nmole of MDA/ml of Plasma)		Lipoprotein Oxidation Susceptibility (LOS) (nmole of MDA/mg of non-HDL Cholesterol)	
	Non-Diabetic	Diabetic	Non-Diabetic	Diabetic
Control	10.64 ± 0.132$^{\#\#}$	34.46 ± 5.38$^{\#}$	0.082 ± 0.023$^{\#}$	0.080 ± 0.01$^{\#\#}$
Sesamol (0.05%)	3.64 ± 1.072$^{\#\#}$	5.36 ± 0.979$^{\#}$	0.035 ± 0.001$^{\#}$	0.026 ± 0.005$^{\#\#}$
Sesamol (0.1%)	2.51 ± 0.905$^{\#\#}$	3.09 ± 0.939$^{\#}$	0.031 ± 0.005$^{\#}$	0.012 ± 0.005$^{\#\#}$
Sesame Lignans (0.05%)	2.07 ± 0.56$^{\#\#}$	5.02 ± 1.356$^{\#}$	0.022 ± 0.002$^{\#}$	0.024 ± 0.007$^{\#\#}$
Sesame Lignans (0.1%)	2.41 ± 0.715$^{\#\#}$	2.66 ± 0.378$^{\#}$	0.014 ± 0.002$^{\#}$	0.022 ± 0.006$^{\#\#}$

Values are Mean ± SEM, n = 6

$^{\#}p < 0.05$

$^{\#\#}p < 0.001$

Table 2 Effect of Sesame Lignans on *In Vitro* Erythrocyte Membrane Lipid Peroxidation In Non-Diabetic and Diabetic Blood.

Groups	Erythrocyte Membrane Lipid Peroxidation (nmole of MDA/mg of protein)	
	Non-Diabetic	Diabetic
Control	$4.41 \pm 0.24^{\#}$	$6.91 \pm 0.298^{\#\#}$
Sesamol (0.05%)	$1.34 \pm 0.25^{\#}$	$2.81 \pm 0.455^{\#\#}$
Sesamol (0.1%)	$1.41 \pm 0.227^{\#}$	$2.68 \pm 0.379^{\#\#}$
Sesame Lignans (0.05%)	$1.94 \pm 0.34^{\#}$	$3.53 \pm 0.63^{\#\#}$
Sesame Lignans (0.1%)	$1.88 \pm 0.46^{\#}$	$2.78 \pm 0.33^{\#\#}$

Values are Mean $\pm$ SEM, $n = 6$, $^{\#}p < 0.05$, $^{\#\#}p < 0.001$

tive activity of sesame lignans and sesamol at two different concentrations. The present study has verified the remarkable ability of sesamol and sesame lignans to function as a phenolic antioxidant. Sesamol not only protect the sesame oil from aerial oxidation but also it is equally effective as an antioxidant when present in a small concentration in human blood.

Umeda Sawada *et al*. (22) have shown that sesame lignans when present in the diet at first be incorporated into the liver, after which they are transported into the peripheral tissues through the blood stream. Sesame lignans are lost from the body within 24 h. So presence of sesame lignans in a very small concentration in the blood stream will be very much effective in reducing the risk of CHD not only in normal conditions but also in diabetic conditions.

4 Conclusions

Sesamol and sesame lignans (mainly sesamin and episesamin) act as effective antioxidants in *in vitro* study. In *in vivo* condition sesame lignans may possibly function in reducing the risk of coronary heart disease in diabetes mellitus.

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