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Protective Effect of Linseed Oil on Hyperlipidemia in Experimental Animals

George Emad Rasmy

Biochemistry Department, National Research Center, Dokki, Egypt.

ABSTRACT

The aim of this study was to investigate the effect of Linseed oil (LO) rich in α-linolenic acid (ALA, C_{18}: 3 n-3), on lipid profile and some growth parameters in rats fed high saturated fat diet (HSF). Thirty two rats were divided into 4 groups control, HSF, HSF + LO and LO. Methods: Measured parameters were nutritional (liver and body wt), biochemical in serum (T. chol, LDL-C, HDL-C, TAG, total lipids) and liver (total lipids, total and free cholesterol, TAG, phospholipids) and histolological sections of hepatic tissues. Result showed that LO supplementation significantly prevent the marked increase body and liver weight gain, hepatic lipids as well as it caused limitation of the negative effect on lipoprotein parameters caused by HSF supplementation. Histological examinations of rats' livers revealed hepatic protection against fatty liver by LO ingestion. No adverse effect of LO on growth parameters and plasma lipids was noticed in rats fed with normal diet. These data suggest that LO participate in the normal regulation of plasma lipid and cholesterol levels in liver, demonstrating that LO may be developed as useful popular and commercial oil for protection against hyperlipidemia.

Key Words: Linseed oil, alpha linolenic acid, hyperlipidemia

Corresponding Author: George Emad Rasmy
E-mail: gefahmy@gmail.com

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INTRODUCTION

Coronary heart disease (CHD) is the most common cause of death in the United States, the United Kingdom and other Western industrialized countries *(American Heart Association, 2003)*. There is a consensus among scientists that dietary changes during the last century, which have included an increased intake of total lipids, Trans fatty acids and saturated and polyunsaturated n-6 fatty acids, have led to the high incidence of CHD *(Simopoulos, 1999, Mozaffarian et al., 2007 and Volk, 2007)*.

Abnormal lipid metabolism is a main cause of dyslipidemia, which is a major risk factor for cardiovascular disease, obesity and overall mortality *(Rizvi et al., 2003 and Garg and Simha, 2007)*. The concentration of plasma cholesterol can be regulated by cholesterol biosynthesis, removal of cholesterol from the circulation, absorption of dietary cholesterol and excretion of cholesterol via bile and feces *(Choi et al., 2001 and Xu et al., 2006)*. In liver, such lipid accumulation initially results in fatty liver that develops fatty infiltration and in chronic stages results in damage of hepatocytes, that causes gross fatty infiltration in parenchyma cells of liver *(Jayasooriya et al., 2000)*. Generally the therapeutic purpose of using hypolipidemic drugs is to reduce the elevated levels of plasma lipids, notably cholesterol *(Lipid Research Clinics Program, 1984, Simons, 2002 and Garg and Simha, 2007)*.

It is well known that diet plays an important role in the control of cholesterol homeostasis *(Xu et al., 2006)*. Many plants have been used for medicinal purpose in hyperlipidemia that may be useful adjuncts in reducing the risk of cardiovascular disease and alterations in liver metabolism *(Craig, 1999)*. Recent studies have demonstrated that ingestion of polyunsaturated fatty acids (PUFA) especially ω-3, present in fish oil and vegetable oils, is inversely related to the incidence of heart disease by decreasing cholesterol and triacylglycerol plasmatic levels *(Simopoulos, 1999)*. α-Linolenic acid (ALA, C_{18}: 3 n-3) is the shorter-chain ω-3 PUFA found in various plant sources, mainly Linseeds. Linseed (Linum usitatissimum) contains 32–45% of its mass as oil of which 51–55% is alpha-linolenic acid (ALA). So, LO is considered as one of the good vegetable sources of ALA and its content ranges from approximately 40% to 60% of the total fatty acids *(Prasad, 2000)*.

ALA, found in LO desaturates and elongates in the human body to eicosapentanoic acid (EPA) and docosahexanoic acid (DHA) and by itself may have beneficial effects in health and in control of chronic diseases *(Mantzioris et al., 1994)*. It has been suggested that the degree of ALA conversion to EPA and DHA is 5–10% and 2–5%, respectively *(Davis and Kris-Etherton, 2003)*. Clinical conditions such as cardiovascular

disease, blood pressure, cancer, skin diseases and immune disorders such as renal failure, rheumatoid arthritis and multiple sclerosis may be prevented by ALA in Linseed oil *(Kelley et al., 1991)*. The n-3 PUFAs in LO have anti-inflammatory properties that are mediated by the production of anti-inflammatory eicosanoids *(Cohen et al. 2005)*.

Cunnane et al. *(1993), Craig (1999) and Pellizzon et al. (2007)* reported that n-3 fatty acids, including ALA, are reported to lower serum cholesterol and triacylglycerol levels. In addition, beneficial effects of ALA on plasma lipid and lipoproteins are more controversial; it has been reported to decrease in total cholesterol (TC), LDL-cholesterol (LDL-C), LDL-C / HDL-C *(Cunnane et al., 1993)*. Recently research has been conducted on the effect of dietary ALA on hepatic cholesterol metabolism in the hamster model *(Morise et al., 2004 and Haliga et al., 2007)*. However, little is known about the effects of LO on hepatic lipid metabolism in rat model, it is therefore of interest to conduct research in this line *(Basch et al., 2007)*.

The objective of the current study was to examine the effects of LO on hyperlipidemia in rats; by analyzing the changes of plasma lipid levels, lipoprotein cholesterol levels and hepatic lipids in high saturated fat diet (HSF) fed rats.

MATERIALS AND METHODS

Chemical sources:
Linseed oil was obtained from commercial (hot oil) available in the Egyptian market. Cholesterol, fatty acids standards were purchased from Sigma Chemical Company, St. Louis Missouri, USA.

Esterification of oil:
Esterification of Linseed oil to methyl ester was carried out according to the method described by (*Lepage and Roy, 1986)*.

Gas liquid chromatographic analysis:
Fatty acid methyl esters, dissolved in benzene, were chromatographic at the central lab for services, National Research Center, using an HP Innowax cross-linked polyethylene glycol on a capillary HP-6890 GC system. GLC peaks were identified by comparison with those of known esterifies fatty acid standards, on the basis of their retention times.

Animals and diet:
Adult male albino rats of Wister strain weighing about 120–150 g were used for the present study. The animals were obtained from and kept in the animal house of the National Research Center, Cairo, Egypt. The animal room was well ventilated with a 12 h light/dark cycle throughout the experimental period. They were maintained in clean, sterile, polypropylene cages and had free access to food and water. The control and high saturated fat diet composition are illustrated in (Table 1).

Table 1: Composition of diets.

Dietary Composition, g/100 g	Control Diet	High Saturated Fat Diet
Carbohydrate	63.2	41.0
protein (Casein)	22.0	20.6
DL-Methionine	0.2	0.4
Fat	9.6	33.0
Vitamin and mineral mix	5.0	5.0

Experimental design:
Rats were then divided into 4 groups (8 animals in each). All groups received normal basal diet.

Group I: Control (rats were fed normal diet).
Group II: Rats were supplemented with high saturated fat diet (HSF).
Group III: Rats were fed with HSF as mentioned above and treated simultaneously with linseed oil (LO; 1g/kg B.Wt.) for 60 days orally (group HSF+LO).
Group IV: Rats were orally administered LO (1g/kg b.w.) alone for 60 days (group LO).

Body weights were recorded once a week and at the end of the stipulated period, the animals were kept for overnight fasting. Blood samples were collected by jugular vein puncture, under slight 1% anesthesia. Plasma was obtained by centrifugation at 3000rpm.and stored at − 20°C until analysis. Liver was isolated, weighed. A portion of liver tissue was homogenized in 0.1 M Tris–Hcl buffer, pH 7.4 at 4°C for analysis of biochemical parameters.

Biochemical analysis:
Liver lipids were extracted according to *Folch et al. (1957)*. Serum total lipids were estimated according to the method of *Knight et al. (1972)*, using kits from Cal-Test Diagnostic Inc., USA.

Serum cholesterol was estimated by the method of Trinder, 1969, using kits from Boehringer Mannheim, Germany. HDL-C was determined according to the method of *Warnick et al. (1982)*, using kits from Stanbio, USA. Serum LDL-C content was determined according to the method of *Bergmenyer (1985)*, using kits from Quimica Clinica Aplicada, Spain. Liver free cholesterol level was

estimated according to the method of *Trinder (1969) and Wybenga and Inkpen, (1974)*, using kits from Boehringer Mannheim, Germany. Liver cholesterol ester was calculated by subtracting the free cholesterol conc from the total cholesterol concentration.

Triacylglycerols (TAG) were estimated by the method of *Wahlefeld (1974)*, using kits from Stanbio, USA. Phospholipid levels were estimated in plasma and liver according to the method of *Takayama et al. (1977)*, using kits from bioMérieux, France.

Histological studies:

Liver tissue from experimental and control animals were fixed in 10% neutral buffered formalin processed by standard procedure for paraffin embedding and serial sections were cut (5 μ). The sections were stained with hematoxylin and eosin dycs.

Statistical analysis:

The results are presented as mean ± S.D. The statistical analysis was performed using student's T test for all parameters. The values were considered significant at the levels of $P < 0.05$ and $P < 0.01$.

RESULTS

Gas liquid chromatographic analysis of LO:

GLC of Linseed fatty acids methyl esters revealed the presence of these fatty acids.

Palmitic	$C_{16:0}$	4.5%
Stearic acid	$C_{18:0}$	7.3 %
Oleic acid	$C_{18:1}$ n-9	21.3%
Linolic	$C_{18:2}$ n-6	13.8%
α-Linolenic	$C_{18:3}$ n-3	52.9%

Effect of LO on body weight gain and relative liver weight:

Body weight gain and liver weight of rats fed with HSF significantly increased as compared with normal basal diet. LO administration reduces the body weight gain and liver weight in HSF fed rats (Group III). No statistically significant changes were noted in LO alone administered rats in comparison with rats feding normal diet (Figure 1).

Effect of LO on plasma lipid profile concentration:

Results revealed that there were an increase in plasma levels of total lipids, TC, TAG and phospholipid in hyperlipidemic rats as compared with those feding normal diet (Figure 2).

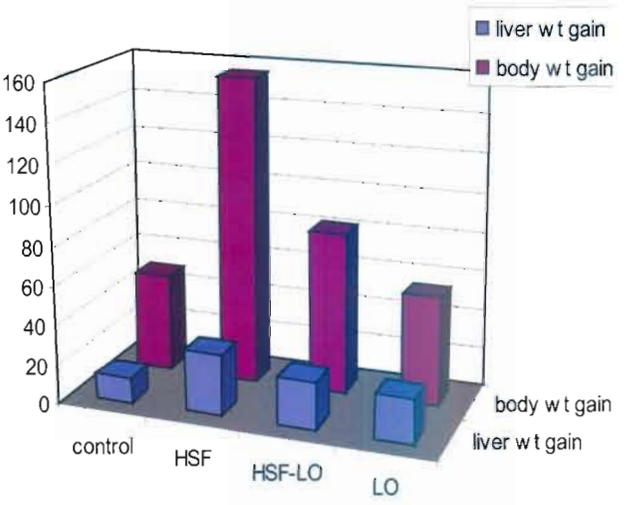

Figure 1: Effect of Linseed oil (LO) on body weight gain and liver weight in high saturated fat diet (HSF) fed rats. Values are expressed as mean mean of 7 rats. (*) statistically significant from HSF group at $P < 0.05$.

The LO consistently lowered the plasma total cholesterol in the presence of dietary fat; while the reduction of TAG and phospholipid was significant in HSF + LO rats. No significant changes in these lipid components were observed between control groups and LO rats.

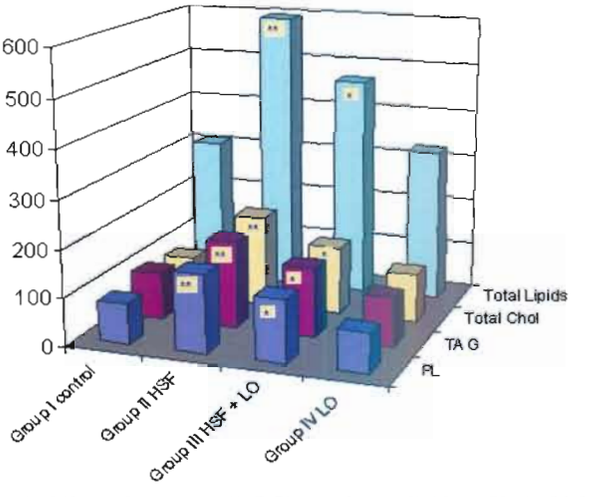

Figure 2: Effect of Linseed oil (LO) on plasma lipid profile in high saturated fat diet (HSF) fed rats. Values are expressed as mean mean of 7 rats. (* and **) statistically significant from HSF group and control at $P < 0.05$ and <0.01, respectively.

Effect of LO hepatic lipids:

The concentrations of hepatic lipids in control and experimental rats are shown in (Table 2). High saturated fat diet resulted in a significant increased concentration of hepatic total, free cholesterol and cholesterol esters, phospholipids and TAG as compared with control rats. LO consistently lowered the hepatic lipid levels of rats fed with saturated fat-enriched diet. Although administration of LO tended to decrease total lipids, cholesterol, TAG and phospholipids levels than in those fed with high saturated fat diet.

Table 2: Effect of Linseed oil (LO) on total lipids, total cholesterol, free cholesterol, ester cholesterol, triacylglycerols and phospholipids in liver tissue of high saturated fat diet (HSF) fed rats.

Parameters mg/g tissue	Group I control	Group II HSF	Group III HSF + LO	Group IV LO
Hepatic Total lipids	53.31 ± 4.22	115.56 ± 10.15	62.61 ± 7.83*	57.11 ± 4.27
Hepatic Total cholesterol	11.1 ± 0.87	35.4 ± 2.91	13.25 ±1.06*	14.71 ± 0.93
Hepatic Free cholesterol	6.42 ± 0.99	15.49 ± 1.15	8.0 ± 0.55	7.83 ± 0.87
Hepatic cholesterol Ester	4.68 ± 0.28	19.91 ± 1.77	5.25 ± 0.02*	6.88 ± 0.41
Hepatic Triacylglycerols	8.16 ± 0.64	28.25 ± 2.17	11.33 ± 1.96*	8.33 ± 0.73
Hepatic Phospholipids	33.52 ± 4.32	46.32 ± 6.78	36.34 ± 4.35**	32.47 ± 3.13

Results are expressed as mean ± SD for six animals in each group. * and **, statistically significant from HSF rats at $P < 0.01$ and $P < 0.05$, respectively.

Effect of Linseed oil (LO)on plasma lipid profile:

The plasma lipoproteins levels in the control and experimental animals are presented in (Figure 3). The levels of HDL-C were significantly decreased ($P < 0.01$) in HSF rats with a significant increase ($P < 0.01$) in LDL-C, VLDL-C, LDL-C:HDL-C and TC: HDL-C ratios when compared to control group. However, in HSF + LO rats the levels of LDL-C, VLDL-C, TC: HDL-C and LDL-C: HDL-C ratio were significantly decreased with no alteration in HDL-C levels when compared to HSF rats. No significant alterations were seen between normal and LO alone treated rats.

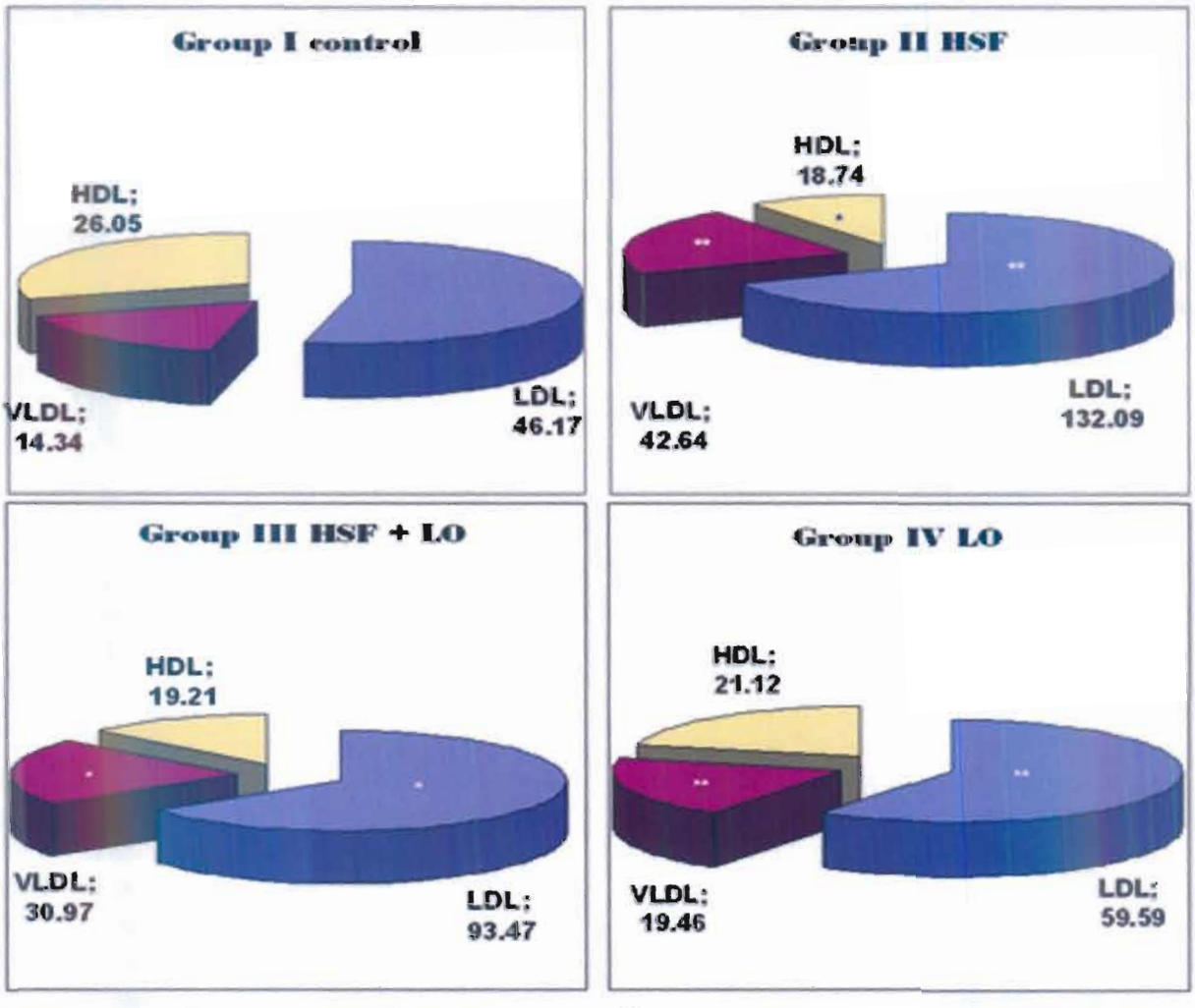

Figure 3: Effect of Linseed oil (LO) on plasma total cholesterol (LDL-C, VLDL-C, HDL-C) in high saturated fat diet (HSF) fed rats.

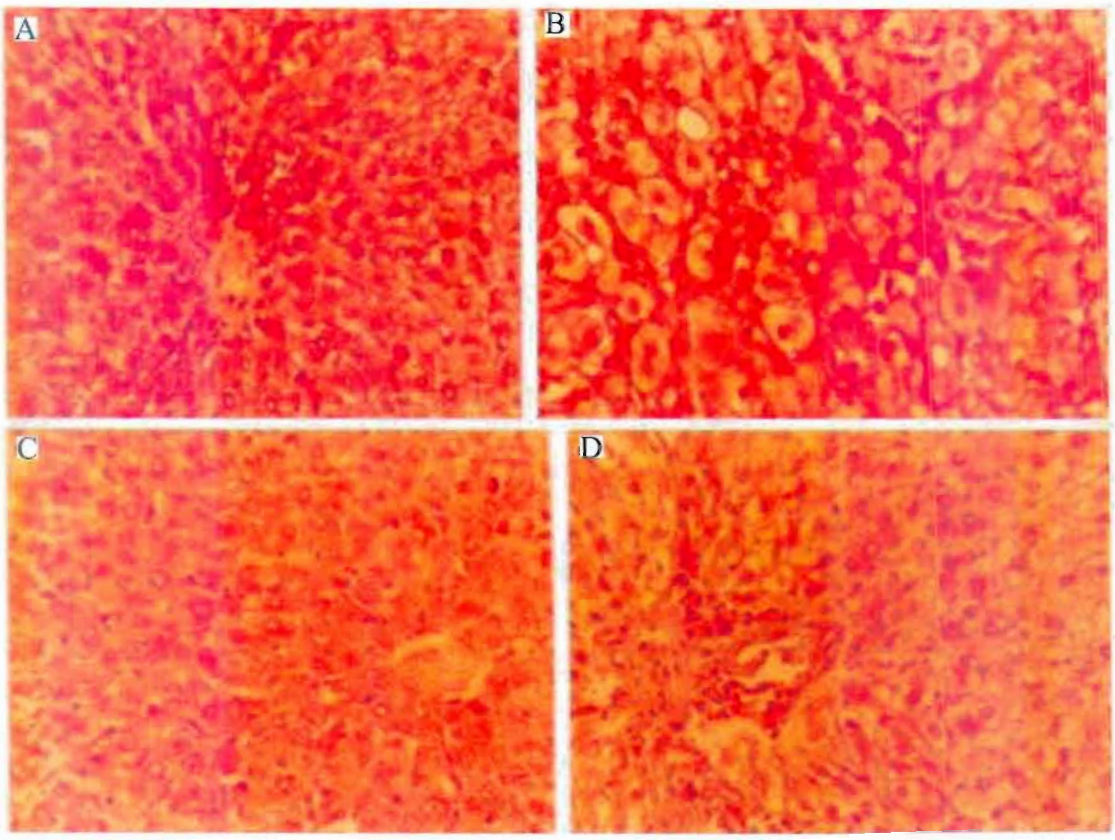

Figure 4: Histological examinations of liver tissue section stained with hematoxylin and eosin dye (100×) are shown in this Figure. A section of liver of normal basal diet fed rats (control) showed normal vascular architecture (A). Rats fed with HSF caused fatty deposits in the liver tissue section (B). In HSF + LO rats showed reduction of fatty deposition and recovery of parenchymal architecture of cells (C). Absence of any pathologic changes with normal hepatocytes and central vein in the liver tissues in LO alone treated rats (D).

Histological examination of liver tissue:

Histological examination of the control liver (Figure 4-A) depicted intact cell architecture that was also evident in LO rats (Figure 4-D). Significant morphological changes were evident in HSF rats. Hepatic lobes showed poor cellularity due to fatty change in the centrilobular region indicating fatty liver and portal inflammation with large hepatocytes (Figure 4-B), whereas, HSF + LO rats (Figure 4-C) showed minimal alterations with reduced fat deposition as compared to HSF rats. These findings confirmed the protective effect of LO against the histological changes in HSF group.

DISCUSSION

Saturated fat enriched diet is regarded as an important factor in the development of cardiac diseases since it leads to the development of hyperlipidemia, atherosclerosis and abnormal lipid metabolism *(Onody et al., 2003 and THUSA study, 2007)*. In the present study, the effects of LO on the alterations of lipid and lipoprotein profile in high saturated fat (HSF) rats were investigated. *Jayasooriya et al. (2000)* have reported that the rats fed with HSF showed significant increase in body weight and liver weight, which leads to secondary complications clinically. In this study, body and liver weight gain in HSF rats were decreased significantly upon treatment with LO. The hypolipidemic *(Cunnane et al., 1993)* and antioxidant *(Prozorovskaia et al. 2003)* effects may be responsible for the beneficial action of LO on body weight gain and liver weights.

The liver plays an important role in the synthesis and net excretion of cholesterol either directly as free cholesterol in the bile or after conversion into bile acid *(Choi et al., 2001)*. The rise in cholesterol in liver and/or plasma may be due to increased uptake of exogenous cholesterol and subsequent deposition and decreased cholesterol catabolism as evidenced by a reduction in bile acid production and turnover of bile acids *(Jaganathan et al., 1974)*. The metabolism of free and esterified cholesterol are impaired in liver, spleen and thymus tissue and the rate of turnover was specifically decreased in all tissues of hyperlipidemic rats *(Feoli et al., 2003)*. The major clinical complications of hyperlipidemia are lipid deposition, mostly cholesterol esters and cholesterol *(Wissler 1991)* which is consistent with the present report.

The decrease of plasma cholesterol by administration of LO was ascribed to the decrease of both free and esterified cholesterol. It is clear that lignin, fiber and vegetable proteins present in the Linseed could have play major roles in reducing serum cholesterol in animal models and/

or in humans *(Wiesenfeld et al., 2003)*. ALA rich LO results in a higher cholesterol secretion into bile, leading to a depletion of the intra-hepatic pool of cholesterol and thus to an increase in cholesterol synthesis and turnover *(Morise et al., 2004)*. Moreover, ALA rich diet reduces hepatic lipid accumulation both by stimulating β-oxidation and by suppressing fatty acid synthesis *(Murase et al., 2005)*. *Ide et al. (2000)* reported that LO could have exerted its protective effect probably as a better substrate for mitochondrial and peroxisomal β-oxidation. All these mechanisms may account for the better regulation of hepatic lipid metabolism by LO.

Administration of saturated fat increases the biosynthesis of phospholipids *(Whereat and Rabinowitz, 1975)* possibly by a decrease in phospholipase activity or increased phospholipid turnover due to an onset of inflammatory process. Jayakumar et al. *(1991)* found an increase in the synthesis of phospholipids and cholesterol esters in rats fed HSF which is in agreement with the present study. Treatment with LO showed decreased phospholipid concentration, which might be due to an inhibitory effect of LO on the lipogenic enzymes *(Hansen et al., 2002)*.

Like cholesterol, triacylglycerol (TAG) in the blood tends to damage vascular endothelial cells, leading to heart disease *(Suprijana et al., 1997)*. High saturated fat diet produces an increase in TAG levels due to lipoprotein lipase triacylglycerol hydrolysis, so that the accumulation in the liver becomes more evident *(Feoli et al., 2003)*. In contrast, the effect of ALA rich-LO can be attributed to a reduction in the hepatic synthesis of fatty acid, which decreases the concentration of triacylglycerols in the liver. In another study, healthy adults who consumed Linseed had a decrease in plasma TC and LDL-C but no change in TAG *(Cunnane et al., 1993)*. *Prasad, (2000)* have reported that LO lowers TAG levels in HSF fed rats, which might be due to the increased activity of lipoprotein lipase. Morise et al. *(2004)*, reported a hypotriglyceridemic effect of LO in the male hamster model. This view is paralleled in our study also by decreased TAG levels in HSF + LO rats which could be due to lipid lowering effect of Linseed *(Chan et al., 1991)*

Increasing LDL-C levels may cause deposition of cholesterol in the arteries and aorta leading to increase the risk factor for CHD *(Gutteridge, 1995)*. Plasma levels of HDL are important predictors for the development of premature CHD. HDL-C has been indicated as a positive factor in determining the development of atherosclerosis *(Miller and Miller, 1997)*. In two different studies, hyperlipidemic subjects fed with Linseed had a significant reduction in TC, LDL-C without a reduction in HDL-C *(Bierenbaum et al., 1993 and Jenkins et al., 1999)*. Similar findings are reported in the present study in HSF + LO group. In HSF group, hyperlipidemia is accompanied by an increase in the secretion of β-VLDL which leads to increase in cholesterol and triacylglycerol synthesis *(Mahfouz and Fred, 2000)*. The hypolipidemic effect of ω-3 polyunsaturated fatty acid decreases the VLDL hepatic secretion that leads to an accumulation of triacylglycerols in the rat liver *(Kawahara et al., 1997)*. The decrease in VLDL-C level in HSF + LO rats is in accordance with the previous reports *(Kaminskas et al., 1991)*.

Total cholesterol / HDL-C and LDL-C:HDL-C ratios are also well known predictors of coronary risk (National Cholesterol Education Program Expert (*Panel, 1994*). A significant increase in TC / HDL-C and LDL-C:HDL-C ratios were observed in rats fed high saturated fat. LO may cause inhibition of the apo lipoprotein B synthesis or increase its catabolism which explains the reduction of these ratios in HSF + LO rats. ALA-rich diet resulted in the significant decrease of a number of atherogenic factors, such as TC, LDL-C and LDL-C / HDL-C *(Morise et al., 2004)*. Linseed mucilage has been reported to be a hypolipidemic agent *(Cunnane et al., 1993)*, which may be responsible for the altered levels of lipoproteins by LO in hyperlipidemic condition.

Esterified cholesterol formed in the liver through the action of acyl-CoA cholesterol acyl transferase, which catalyzes the conversion of cholesterol into more hydrophobic form that is transported in secreted lipoprotein particles to other tissues that use cholesterol or is stored in the liver *(Yokozawa et al., 2003)*. The variations in the processes of digestion and absorption in the small intestine affect the nature of chylomicron and thus, lipid metabolism in the liver *(Murase et al., 2005)*. The present results indicate that hepatic cholesterol lowering effect resulted from the reduction of cholesterol synthesis in liver tissues.

Thus, the reduction of esterified cholesterol level by LO may indicate that the cholesterol was used for the synthesis of vital molecules in tissues, including the liver. Dietary ALA results in higher cholesterol secretion into bile, leading to a depletion of the intrahepatic pool of cholesterol, which indicated reduced cholesterol content of the liver *(Kim et al., 1999)*. Supplementation with ALA significantly inhibited the hepatic triacylglycerol accumulation and fatty liver formation *(Murase et al., 2005)*. There was a consistent reduction of hepatic TAG and PL concentrations in HSF + LO rats in comparison with HSF rats which might be due to hypocholesterolemic activity of ALA in FO. These observations may suggest that LO is incorporated into the liver cells and influence metabolism of serum and liver lipids. Histologic studies of liver tissue have confirmed the hypolipidemic efficacy of LO that might be useful for

the treatment of fatty liver disease. These results suggest that ALA rich LO produce an alteration in serum lipids and hepatic fat content, emphasizing the protective role on hyperlipidemia.

CONCLUSION

It could be suggested that LO afforded substantial protection against disorders obtained by consumption of hyperlipidemia and these effects are mainly mediated by the ALA present in it. Patients should be encouraged to replace low ALA oils (corn oils and safflower oil) with flax oil into their diet. Further research is needed to elucidate the mechanisms of action and the relative influences of the components of LO on cholesterol concentrations.

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