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COMPARATIVE STUDY OF THE POSSIBLE PROPHYLACTIC AND CURATIVE EFFECTS OF FLAXSEED OIL ON LIPID PROFILE AND ANTIOXIDANT STATUS IN HYPERLIPIDEMIC RATS

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ABSTRACT:

Background:

Globally, the prevalence of overweight and obesity is increasing, predisposing both sexes to health hazards including cardiovascular diseases and death.

Objective:

This study aimed to evaluate and compare the possible prophylactic and curative effects of flaxseed oil on vascular health in hyperlipidemia.

Material and methods:

Forty rats were divided into four equal groups: Group I (control group), Group II (hyperlipidemic group), Group III (flaxseed oil- pretreated group), Group IV (flaxseed oil-treated group). At the end of the experiment, the body weight, serum levels of [lipid profile, malondialdehyde (MDA), reduced glutathion (GSH), Interleukin 6 (IL-6), tumor necrosis factor alpha (TNF-α), and vascular cell adhesion molecule 1 (VCAM1)] were determined for all groups. **Results:** Flaxseed oils pretreatment and treatment significantly decreased body weight by 28% and 19% respectively. Serum cholesterol, triglycerides and LDL significantly decreased, while HDL significantly increased. Furthermore, flaxseed oil suppressed the increase in MDA, serum IL-6, TNF-α, and VCAM1 levels and elevated the serum GSH level significantly.

Conclusion:

Flaxseed oil possessed anti-hyperlipidemic and anti-inflammatory actions and may contribute to reduce the risk factors of cardiovascular disease. Flaxseed oil pretreatment was more effective in hyperlipidemia than its use as a treatment. Thus, flaxseed oil may be a novel therapeutic strategy for prevention of atherosclerosis.

Key words: *Flaxseed oil, Hyperlipidemia, Atherosclerosis, Cardiovascular diseases*.

INTRODUCTION:

Cardiovascular diseases are a leading cause of death and a major economic challenge that faces the health care system **(Rosamond et al., 2007)**. It has become one of the most serious threats to global public health. The causes of cardiovascular diseases are various, but hypertension and atherosclerosis are the most common **(Dantas et al., 2012**). Physiologically, lipids play an essential role in the appropriate functioning of the cardiovascular system. Hyperlipidemia is an extremely predisposing factor for arteriosclerosis and cardiovascular diseases (**Canto and Iskandrian, 2003**). Scientific evidences indicate that a diet with high intake of saturated fat, trans-fat, salts and low intake of vegetables, fruits and fish has been associated with cardiovascular risk factors **(Joshipura et al., 1999; Paradis and Fodor, 1999**). Both saturated fats and trans-fat tend to block low density lipoprotein (LDL) receptors, thus preventing their uptake from the bloodstream. When LDL gets oxidized, it induces endothelial cell injury as well as foam cell formation, thus development of atherosclerosis. Frequent consumption of energy-dense foods, such as foods that are rich in fats and sugars, promotes obesity and increases the risk of atherosclerosis and cardiovascular diseases (**Buttar et al., 2005**).

Medicinal plants have been used as possible sources and as a traditional treatment to many human diseases for the past thousands of years in many places of the world. In rural regions of the developing countries, they continue to use them as the primary source of medicine, sometimes the only source of health care. It is estimated that up to four billion people, living in the developing world, rely on medicinal plants **(Ekor, 2014**). Pharmacologically and biologically active compounds have been extracted from the medicinal plants. Many of these compounds have been the basis for the development of potentially therapeutic drugs to target a specific disease **(Kong et al., 2003; Pal and Shukla, 2003**). Flax, pumpkin and purslane seeds are medicinal plants which are productive sources of unsaturated fatty acids, antioxidants and fibers.

Flax (***Linum usitatissimum)*** is a blue flowering crop that produces small flat seeds (Flaxseed or Linseed). These seeds are ranging in color from golden yellow to reddish brown. Flaxseed was recognized as a substitute plant source of ω-3 fatty acids. However, the ω-3 fatty acids (**α–linolenic acid, ALA**) found in flaxseed are different from those in fish. The seeds are usually consumed in one three ways: whole seed, ground seed or flaxseed oil. ALA has higher bioavailability in flaxseed oil than in ground or whole seed (**Austria et al., 2008**). Flaxseed has gathered attention due to its health benefits related to the presence of three important components found in it as ALA, lignans and fibers. The ALA content of flaxseed was suggested to be the principal constituent that provided the antiarrhythmic effect (**Ander et al., 2004**). Also flaxseed supplementation in the hypercholesterolemic rabbit prevented atherosclerosis suggesting that flaxseed has significant anti-atherogenic effects **(Dupasquier et al., 2006**). Lignans are one of the main groups of phytoestrogens that have antioxidant and antitumorigenic properties (**Thompson et al., 2005**). The consumption of flaxseed has been beneficial to health in different situations, such as preventing cardiovascular disease and reduction of total plasma cholesterol and triglycerides **(Lucas et al., 2004)**. Also it has been reported that flaxseed consumption is beneficial in minimizing menopause symptoms. This effect may represent tissue-specific responses to lignans enclosed in flaxseed (**Dalais et al., 1998**). Furthermore, certain types of cancer that are developed under the influence of hormones, breast and uterus cancer,

were prevented by the consumption of flaxseed. The inhibitory effect of flaxseed on the growth and metastasis of breast cancer xenografts is assigned to its lignans and oils components (**Wang et al., 2005**).

Despite the benefits attributed to the consumption of flaxseed, its use in stages of cardiovascular disease has sparked interest in the scientific research. Little is known about the association of flaxseed and vascular function. The aim of the present study was to evaluate and compare the possible prophylactic and curative effects of flaxseed oil on hyperlipidemia induced in male albino rats.

MATERIAL AND METHODS:

ANIMALS:

Forty male Albino rats of local strain (**7-8-weeks old, weighing 130-150 g**) were purchased from the Nile Pharmaceuticals Company (**Cairo, Egypt**). They were housed in standard cages (**5 rats/25X30X30 cm cage**), under specific pathogen-free conditions in facilities maintained at controlled room temperature with a 40-60% relative humidity, and a natural light-dark cycle. All animals had free access to rat chow diet (see below) and water provided *ad libitum* and were left to adapt to new environmental conditions for one week. All procedures were approved by the Animal Care Committee of Al Azhar University. The "Principles of laboratory animal care" as well as specific national laws were followed, where applicable.

MATERIALS:

Commercial rat chow diet (**balanced diet**), containing 67% carbohydrates, 23% protein, and 10% fat as the energy sources (total calories: 3.6 kcal/g), was purchased from El Gomhorya company (**Cairo, Egypt**). High fat diet (**HFD**), consisting of 88% of standard pellet animal diet, 10% lard and 2% cholesterol was prepared and used to induce hyperlipidemia. The major component of the diets used in this study was previously characterized by **(Hussain et al., 2016; Xu et al., 2010**). The HFD was composed of the following energy sources: 52% carbohydrates, 30% fats and 18% proteins (**total calories: 4.8 kcal/g**) **(Hussain et al., 2016)**. Natural cold pressed flaxseed oil was purchased from Imtenan Company (**Cairo, Egypt**).

Experimental Design:

After one-week acclimatization period, Rats were divided into 4 equal groups.

Group I: Rats were assigned to control group and given normal balanced chow and supplemented with saline using a gastric gavage tube for 12 weeks.

Group II: HFD group, rats were given normal balanced chow for the first 4 weeks, then the normal diet was replaced with HFD for another 8 weeks **(Hussain et al., 2016; Xu et al., 2010).** The rats also were supplemented with saline using a gastric gavage tube.

Group III: HFD-flaxseed prophylactic-group, flaxseed oil was given orally at a daily dose of 1.8 mg/kg for 4 weeks with normal balanced chow **(Tanna et al., 2012)** and then rats received HFD for the following 8 weeks.

Group IV: HFD-flaxseed-treated- group, rats were kept on HFD for 8 weeks, and then rats

were given flaxseed oil orally at a daily dose of 1.8 ml/kg for another 4 weeks with normal balanced diet.

The body weight of each rat was measured and recorded weekly for all groups. Hyperlipidemia was confirmed by measuring serum lipids and lipoproteins levels in HFD group.

At the end of the experiment, after overnight fasting, rats were anesthetized in the morning, and blood samples were collected from retro-orbital venous plexus under light ether anesthesia. 0.2 ml of the blood was hemolyzed by addition of 1.8 ml H_2O, and the hemolysate was used for assessment of GSH level. The rest of the blood was then centrifuged at 3000 rpm for 15 minutes for serum collection. Serum was separated in aliquots in Eppendorf tubes and kept frozen at -80°C until analysis. The separated serum was analyzed for estimation of the levels of lipid profile, oxidative stress markers, inflammatory markers and cell adhesion molecule marker.

BIOCHEMICAL ANALYSIS:

1. The total serum cholesterol and HDL were measured by quantitative enzymatic colorimetric determination of total and HDL cholesterol in serum using biomed diagnostic assay kits (**MacLachlan et al., 2000**).
2. Serum triglycerides were measured by quantitative enzymatic colorimetric determination of triglycerides in serum using Cayman colorimetric assay kit (**Cole et al., 1997**)
3. Serum LDL cholesterol was calculated from the values of total cholesterol (TC), HDL and triglycerides using Friedewald equation: LDL (mg/dl) = TC - HDL – $(TG/5.0)^2$ **(Ahmadi SA et al., 2008)**.
4. Serum malondialdehyde (MDA) has been identified as the result of lipid peroxidation reaction with thiobarbituric acid to give red species absorbing at 535 nm. It was measured by using free-SH groups estimation method **(Janero, 1990)**.
5. Serum reduced glutathione (GSH) was measured by using glutathione peroxidase assay kit (**Cayman Chemical, Ann Arbor, MI, USA**) according to the company's instruction. Briefly, 8 ml of phosphate buffer, 3 ml of precipitating solution, and 1 ml of DTNB were added to the blood hemolysate filtrate. The optical density was measured spectrophotometrically at a wave length 410 nm **(Ceballos-Picot et al., 1992)**.
6. Serum tumor necrosis factor alpha (TNF-α) level was measured by commercial ELISA kits (RayBio® Rat, RayBiotech, Norcross, GA, USA) according to company's protocol. The kit's sensitivity level was lower than 25 pg TNF-α/ml (**Engelmann et al., 1990**).
7. Serum interleukin-6 (IL-6) level was measured by commercial ELISA kits (RayBio® Rat, RayBiotech, Norcross, GA, USA) according to company's protocol. The kit's sensitivity level was lower than 30 pg IL-6/ml **(Ferrari et al., 2003).**
8. Vascular cell adhesion molecule 1(**VCAM1**) was measured by commercially available ELISA and standards (**R&D System Europe Ltd**). The kit's sensitivity level was lower than 3.9 ng/ml (**Pigott et al., 1992**).

STATISTICAL ANALYSIS:

Data were presented as Mean ± Standard Error of Mean [SEM]. Statistical analysis was conducted using one-way analysis of variance [ANOVA] followed by Bonferroni post hoc multiple comparison test using the program Statistical Package for the Social Sciences (SPSS), IBM SPSS Statistics (version 18). The values of $P < 0.05$ were considered as statistically significant.

RESULTS:

Effect of flaxseed oil on body weight of hyperlipidemic rats:

HFD feeding for eight weeks significantly augmented the body weight when compared to normal control rats (Table 1). When flaxseed oil was given orally for 4 weeks before inducing hyperlipidemia, as prophylactic, the body weight growth of rats significantly decreased vs hyperlipidemic rats ($P < 0.001$). Notably, there was no significant difference in body weight among flaxseed prophylactic group and control group. When flaxseed oil was given after inducing hyperlipidemia, the body weight significantly decreased vs hyperlipidemic rats, but it was still significantly higher than control rats (Table 1).

Effect of flaxseed oil on lipid profile of hyperlipidemic rats.

a. Serum concentration of total cholesterol and triglycerides:

HFD for eight weeks significantly augmented the fasting serum total cholesterol level in hyperlipidemic vs control rats (Table 1). In comparison to hyperlipidemic group, flaxseed groups (prophylactic and treatment) showed significantly lower total cholesterol and triglycerides ($P<0.0001$) but insignificant difference was found between prophylactic flaxseed group and control rats on total cholesterol and triglycerides (Table 1), while its use as a treatment showed 20 - 25% higher total cholesterol and triglycerides ($P<0.0001$) vs control rats.

b. Serum concentration of LDL and HDL:

Similar to the above results, HFD supplementation increased the serum LDL in hyperlipidemic rats compared to control rats and flaxseed both prophylactic and treatment significantly lowered LDL level ($P<0.0001$) as shown in Table 1. On the other hand, a 58% reduction in serum HDL was observed on the 8^{th} week of high fat diet supplementation when compared to control rats. Flaxseed oil-pretreated and treated groups significantly elevated HDL level when compared to hyperlipidemic rats and completely returned to the normal level in case of the pretreatment but not in the flaxseed treated hyperlipidemic group (Table 1).

Antioxidant effects of flaxseed oil on hyperlipidemic rats.

a. **Serum concentration of MDA:**

Serum MDA level as a marker of lipid peroxidation was extremely high with HFD supplementation for eight weeks compared to normal rats ($P<0.0001$). A significant decline of serum MDA concentration was observed in flaxseed oil-pretreated and treated groups when compared to hyperlipidemic group (Table 2). The increased MDA indicated enhanced lipid peroxidation in the hyperlipidemic group. When flaxseed oil was given as prophylactic, it prevented the lipid peroxidation to occur, and the MDA level was not significantly different from control rats. Yet its use as a treatment showed significant increase in MDA vs control

rats.

b. Serum concentration of Glutathione (GSH):

Daily feeding of HFD for 8 weeks to rats significantly decreased the level of serum GSH **(P<0.001**) compared to control rats. In comparison to hyperlipidemic rats, GSH level showed significant elevation with flaxseed oil pretreatment and treatment (**Table 2**). The GSH level was not significantly different from control when rats were pretreated with flaxseed before inducing hyperlipidemia. Although it was significantly lower in flaxseed treated group when compared to control group (**Table 2**).

Anti-inflammatory effects of flaxseed oil on hyperlipidemic rats.

a. Serum TNF-α and IL-6:

HFD supplementation for eight weeks up-regulated both TNF-α and IL-6 by ~3 and ~4-fold, respectively, compared to control rats (**Figure 1, A and B**). A significant down-regulation of serum TNF-α and IL-6 concentrations was observed in flaxseed oil-pretreated and treated groups when compared to hyperlipidemic group. The flaxseed oil was better at decreasing both TNF-α and IL-6 concentrations when used as a pretreatment before inducing hyperlipidemia. It showed no significant difference from the control rats (**Figure 1**), while its use as a treatment increased TNF-α and IL-6 concentrations vs control rats.

b. Serum VCAM1:

Assessment of VACM1 concentration could be useful to identify the risk for atherosclerotic lesions in hyperlipidemic rats and to estimate the effect of flaxseed supplementation. HFD supplementation for eight weeks to normal rats induced significant elevation in serum VCAM1 compared to control rats. The VACM1 level decreased when the hyperlipidemic rats received flaxseed oil before and after the HFD supplementation (**Figure 1 C**). When flaxseed oil was used before the HFD supplementation, there was a complete reduction of VACM1 level compared to control. Moreover, flaxseed treated group showed significantly elevated VACM1 level in comparison to control rats.

DISCUSSION :

Dietary saturated fatty acids are coupled with metabolic and cardiovascular diseases. Potentially interesting strategies to diminish disease risk is rather modification of the quality of fat consumed or using medicinal plants which are rich sources of unsaturated fatty acids, antioxidants and fibers (**Morrison et al., 2015**). Until now, few effective, safe and convenient approaches for cardiovascular diseases are clinically available. In this study, we offer a possible natural cardio-protective agent which can effectively ameliorate HFD- induced hyperlipidemia.

In the present study, we demonstrated that HFD for 8 weeks significantly increased the body weight, serum total cholesterol, triglycerides, LDL, MDA, and inflammatory markers (**TNF- α, IL-6, and VACM1**) of male rats. However, HDL and reduced glutathione levels were decreased by HFD (**Table 1 and 2**). We also demonstrated that, giving flaxseed oil (**high bioavailability**) at a dose of 1.8 mg/kg to hyperlipidemic rats can reduce concentrations of serum total

cholesterol, triglycerides, and LDL (**Table 1**). Conversely, it can increase the concentration of serum HDL and reduced glutathione (**Table 1 and 2**). In association with modifying the lipid profile, we also demonstrated that, consumption of flaxseed oil significantly reduced the lipid peroxidation marker, malondialdehyde. Furthermore, flaxseed oil suppressed the increase in serum IL-6, TNF-α and VCAM1 levels in hyperlipidemic rats (**Figure 1**). Taken together, the protective effect of flaxseed oil during hyperlipidemia is dependent on lowering the risk of atherosclerosis by reducing VACM1 serum level.

HFD containing ~30% of total energy from fats lead to obesity in many animals like mice, rats, dogs and primates due to bigger energy intake and efficient energy storage **(Hill et al., 2000).** The significant increase of body weight with hyperlipidemic diet for eight weeks in the present study is in consistent with previous studies who attributed this increase to the high energy density of fat, since it supplies ~9 kcal per gram in comparison to the carbohydrates and protein which provides only 4 kcal. Therefor, increased fat intake can promote high energy utilization, increased energy density and palatability **(Hill et al., 2000; Stubbs et al., 1995**). The increase in body weight may occur because hyperphagia and consequently high energy intake induced by adipocyte-derived leptin hormone secretion (**Dodd et al., 2015**).

Many studies reported that flaxseed may have protective effect against diseases like CVD. Flaxseed oil administration before and after induction of hyperlipidemia significantly decreases the body weight. These results are in keeping with previous studies of Vijaimohan et al., who proposed that the hypolipidemic and antioxidant effects of flaxseed oil are responsible for its beneficial action on body weight gain **(Vijaimohan et al., 2006)**. Moreover, Baranowski et al., found that the effect of dietary flaxseed oil on the body weight is attributed to its content of ALA which reduces the adipocyte hypertrophy, protein levels inflammatory markers, monocyte chemoattractant protein-1 (MCP-1), TNF-α and T-cell infiltration in adipose tissue **(Baranowski et al., 2012).** In contrary, a previous study showed that supplementation of flaxseed oil to hyperlipidemic rats insignificantly affects the body weight gain **(Deng et al., 2012).**

As noted above, our result showed a marked increase in the lipid profile (**total cholesterol, triglycerides and LDL**) in the high fat diet group, while serum HDL showed significant decrease. Administration of flaxseed oil prior to and after induction of hyperlipidemia markedly improved these parameters. These results are consistent with the studies by others **(Amin et al., 2013; Li et al., 2015**). The cholesterol level in plasma and liver of hyperlipidemic rats increased due to the higher uptake of exogenous cholesterol and its subsequent accumulation in addition to the lower cholesterol catabolism as proven by a reduction in bile acid formation and turnover of bile acids **(Barakat and Mahmoud, 2011**). In the present study, Flaxseed oil administration improved the lipid profile to normal levels. This was supported by a previous study who reported that flaxseed oil supplementation to rats fed high cholesterol diet decreases the serum lipid profile **(Hussein et al., 2014**). They attributed this beneficial effect to ALA which results in a greater cholesterol secretion into bile leading to depletion of the intrahepatic pool of cholesterol, and therefore to an augmentation in cholesterol synthesis and metabolism. Moreover, diet rich in ALA reduces hepatic lipid deposition both by stimulating β-oxidation and by inhibiting fatty acid synthesis **(Murase et al., 2005**). The triglycerides reducing effect of flaxseed oil is made

through regulation of peroxisome proliferative-activated receptor-γ (PPARγ) and sterol regulatory element-binding protein-1 (**SREBP-1**), which control hepatic fatty acid catabolism and synthesis respectively **(Han et al., 2015)**. Conversely, **(Deng et al., 2012**) and (**Xu et al., 2012**) reported that flaxseed oil does not markedly affect plasma HDL-C level.

We assessed a major compound included in the downregulation of substances formed during oxidative stress, GSH. Our study showed significant changes in oxidative stress markers (**MDA and GSH**) (**Table 2**). We also measured pro-inflammatory cytokines like IL-6 and TNF-α in order to investigate the effect of flaxseed oil on hyperlipidemia-induced inflammation in rats. We showed that flaxseed oil downregulated the hyperlipidemic-induced increase in IL-6, TNF-α, and VCAM1 **(Figure 1**). The results with oxidative stress, inflammatory markers and endothelial dysfunctional markers were consistent with the studies of (**Peairs et al., 2011**) and (**Herieka and Erridge 2014**) who reported that HFD stimulates oxidative stress, impairs endothelial function and causes a rise in circulating inflammatory factors as soluble intercellular adhesion molecule-1 (ICAM-1), TNF-α and C-reactive protein **(CRP**). Also (**Shi et al., 2005**) found that the high TNF-α level, which are mostly produced by macrophages and monocytes, were due to the acute responses to the high fat diet as these cytokines most likely return to baseline concentration once the acute phase response is diminished. Finally, We have showed that flaxseed oil administration before and after high fat diet treatment decreased the levels of oxidative stress and inflammatory markers significantly which was supported by the studies of **(Xu et al., 2014; Xu et al., 2012)** who reported that flaxseed oil elicit anti-inflammatory effects by reducing inflammatory cytokine production such as IL-1, IL-6, CRP and TNF-α via an inhibition of NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells)-induced gene expression and/or an activation of PPAR-γ (Peroxisome proliferator-activated receptor gamma). Another mechanism was reported by **(Tripathi et al., 2013)** who attributed this effect to the presence of omega-3 fatty acid which regulates the adhesion proteins expression like vascular cell adhesion molecule-1, which contribute in leukocyte-endothelium interactions.

Interestingly, the present study showed that the use of flaxseed oil as a prophylactic agent against hyperlipidemia and vascular changes exerted more significant effect on body weight with marked improvement of oxidative stress, inflammatory and vascular adhesion molecule parameters in comparison to its use after induction of hyperlipidemia. Presumably, the use of flaxseed as a treatment after induction of hyperlipidemia, in this study, was insufficient and they may need a longer study period to return all parameters to the normal level completely. Alternatively, HFD may induce atherosclerotic lesions which were not completely healed by administration of flaxseed oil alone and may need a combination therapy. A recent study demonstrated that flaxseed oil + ALA-ester of plant sterol were synergistically ameliorating atherosclerosis and optimizing overall lipid profile, inhibiting inflammation and reducing oxidative stress (**Han et al., 2015**). Further studies are needed to evaluate the potential of this oil for the management of hyperlipidemia, the most effective duration of flaxseed oil treatment to return all parameters to normal levels, and the adverse effects of them for being used as anti-atherogenic drugs in human beings.

In conclusion, flaxseed oil possessed significant anti-hyperlipidemic and anti-inflammatory properties. Flaxseed oil may contribute to reduce the risk factors of cardiovascular disease by

enhancing plasma antioxidant defenses and lipids profiles. Flaxseed oil pretreatment was more effective than its use as a treatment. Thus, flaxseed oil may be a novel therapeutic strategy for atherosclerosis prevention or treatment.

Abbreviations:

ALA, α–linolenic acid; CRP, C-reactive protein; GSH, reduced glutathione; HDL, high density lipoprotein; IL-6, Interleukin 6; ICAM-1, intercellular adhesion molecule-1; LDL, low density lipoprotein; MDA, malondialdehyde; NF-κB, Nuclear factor kappa-light-chain-enhancer of activated B cells; PPARγ, peroxisome proliferative-activated receptor-γ; SREBP-1, sterol regulatory element-binding protein-1; TNF-α, tumor necrosis factor alpha; VCAM1, vascular cell adhesion molecule.

Conflict of interest:

The authors declare that they have no conflicts of interest with the contents of this article.

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Table 1

Effect of flaxseed oil on body weight and lipid profile of hyperlipidemic rats:

	Control	**Hyperlipidemic**	**Flaxseed-prophylactic**	**Flaxseed-treated**	**P- value**
Body weight (g)	215.9 ± 1.5	305.5^{a} ± 2.9	218.7^{b} ± 2.0	$246.9^{a,b}$ ± 1.6	0.001
Serum cholesterol (mg/ml)	100.1 ± 0.8	186.0^{a} ± 2.0	100.9^{b} ± 0.5	$124.8^{a,b}$ ± 2.4	0.001
Serum triglycerides (mg/ml)	81.1± 0.9	150.5^{a} ± 3.4	84.8^{b} ± 1.4	$98.1^{a,b}$ ± 1.2	0.001
Serum LDL (mg/ml)	51.5 ± 0.6	117.0^{a} ± 1.6	52.7^{b} ± 0.4	$69.5^{a,b}$ ± 0.9	0.001
Serum HDL (mg/ml)	59.8 ± 1.0	25.1^{a} ± 1.2	57.4^{b} ± 1.1	$69.5^{a,b}$ ± 0.9	0.001

Values are presented as means ± SEM

a: Significant values compared to control.

b: Significant values compared to hyperlipidemic group.

Table (2)

Effect of flaxseed oil (pretreatment and treatment) on Serum Malondialdehyde (MDA)

and Glutathione (GSH):

	Control	Hyperlipidemic	Flaxseed-prophylactic	Flaxseed-treated	P- value
Serum MDA (nmol/ml)	1.9 ± 0.1	6.7^{a} ± 0.2	2.3^{b} ± 0.2	$3.1^{a,b}$ ±0.2	0.001
Serum GSH (nmol/ml)	61.6 ± 1.2	32.3^{a} ± 0.9	59.6^{b} ± 1.4	$49.2^{a,b}$ ± 1.3	0.001

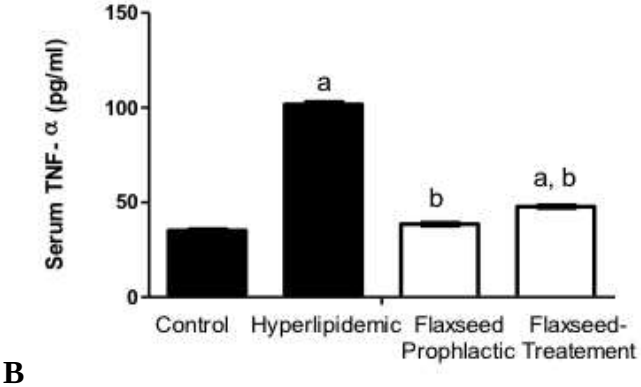
Values are presented as means ± SEM

a: Significant values compared to control.

b: Significant values compared to hyperlipidemic group.

Figure (1)

A



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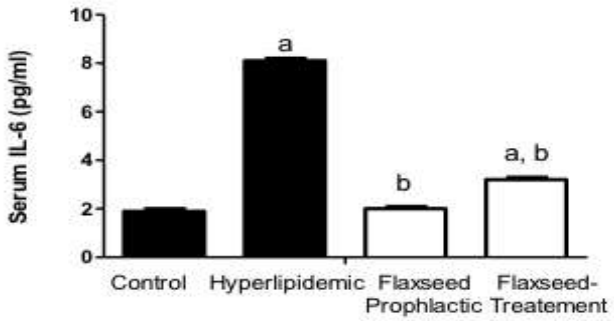

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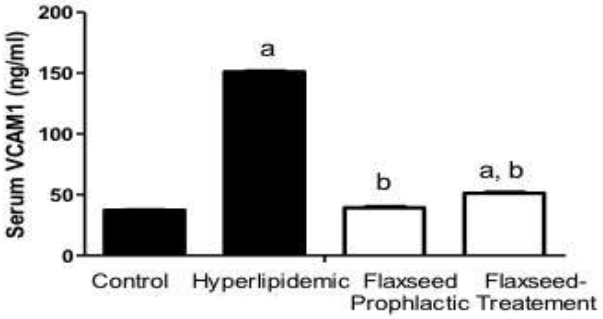

FIGURE TITLE AND LEGEND

Effect of flaxseed oil (pretreatment and treatment) on Serum Tumor Necrosis Factor Alpha (TNF-α; panel A), Interlukin-6 (IL-6; panel B) and Vascular Cell Adhesion Molecule

1 (VCAM1; panel C):

HFD administration increased the the serum levels of TNF-α, IL-6 and VCAM1 in rats in comparison to control group (panels A, B and C). Flaxseed oil prophylactic administration decreased the TNF-α, IL-6 and VCAM1 levels in rats in comparison to hyperlipidemic group ($P<0.0001$). Flaxseed oil treatment decreased the TNF-α, IL-6 and VCAM1 levels in rats in comparison to hyperlipidemic group, but not to the basal control level.

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