Journal of Applied Pharmacy
ISSN: 1920-4159

Elimam and Ramadan, J Appl Pharm 2018, 10:1
DOI: 10.4172/1920-4159.1000257

Research Article Open Access

Comparative Study of the Possible Prophylactic and Curative Effects of Flaxseed Oil on the Lipid Profile and Antioxidant Status of Hyperlipidaemic Rats

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Abstract

Background: Globally, the prevalence of overweight and obesity is increasing, predisposing people of 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 hyperlipidaemia.

Material and methods: Forty rats were equally distributed into four groups: Group I (control group), Group II (hyperlipidaemic group), Group III (flaxseed oil-pretreated group), and Group IV (flaxseed oil-treated group). At the end of the experiment, the body weight, serum lipid profiles, and serum levels of malondialdehyde (MDA), reduced glutathione (GSH), interleukin 6 (IL-6), tumour necrosis factor alpha (TNF-α), and vascular cell adhesion molecule 1 (VCAM1) were determined in all groups.

Results: Flaxseed oil pretreatment and treatment significantly decreased body weight by 28% and 19%, respectively, relative to HFD feeding alone. In addition, flaxseed oil supplementation significantly decreased serum total cholesterol, triglyceride and low-density lipoprotein (LDL) cholesterol levels while significantly increasing the HDL cholesterol levels compared to HFD feeding alone. Furthermore, flaxseed oil significantly suppressed the increases in the serum levels of MDA, IL-6, TNF-α, and VCAM1 while upregulating the serum GSH levels.

Conclusion: Flaxseed oil possesses anti-hyperlipidaemic and anti-inflammatory activities and may reduce the risk factors of cardiovascular disease. Flaxseed oil pretreatment was more effective against hyperlipidaemia than flaxseed oil treatment. Thus, flaxseed oil supplementation may be a novel therapeutic strategy for the prevention of atherosclerosis.

Keywords: Flaxseed oil; Hyperlipidaemia; Atherosclerosis; Cardiovascular diseases

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 Proliferator-Activated Receptor γ; SREBP-1: Sterol Regulatory Element-Binding Protein-1; TNF-α: Tumour Necrosis Factor-alpha; VCAM1: Vascular Cell Adhesion Molecule 1.

Introduction

Cardiovascular diseases are a leading cause of death and a major economic challenge in the health care system [1]. Cardiovascular diseases have become one of the most serious threats to global public health. Of the various causes of cardiovascular diseases, hypertension and atherosclerosis is the most common [2]. Physiologically, lipids play an essential role in the appropriate functioning of the cardiovascular system. Hyperlipidaemia is an extremely predisposing factor for arteriosclerosis and cardiovascular diseases [3]. Scientific evidence indicates that a diet with high intake of saturated fat, trans fat, and salt and low intake of vegetables, fruits and fish has been associated with cardiovascular risk factors [4,5]. Both saturated fats and trans fats tend to block low-density lipoprotein (LDL) receptors, thus preventing the uptake of LDL from the bloodstream. When LDL becomes oxidized, it induces endothelial cell injury as well as foam cell formation, causing atherosclerosis development. 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 [6].

Medicinal plants have been used as possible sources of novel therapeutic compounds and as traditional treatments for many human diseases for thousands of years in many parts of the world. In rural regions of developing countries, people continue to use plants as the primary, and sometimes only, source of medicine. It is estimated that up to four billion people living in the developing world rely on medicinal plants [7]. Pharmacologically and biologically active compounds have been extracted from medicinal plants. Many of these compounds have been the basis for the development of potentially therapeutic drugs to target a specific disease [8,9]. Flax, pumpkin and purslane seeds are medicinal plant products that are productive sources of unsaturated fatty acids, antioxidants and fibre.

Flax *(Linum usitatissimum)* is a blue flowering crop that produces small flat seeds (flaxseed or linseed). These seeds range in colour from golden yellow to reddish brown. Flaxseed was recognized as a substitute plant source of ω-3 fatty acids. However, the ω-3 fatty

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Received: January 02, 2018; **Accepted:** January 08, 2018; **Published**: January 16, 2018

Citation: Elimam H, Ramadan BK (2018) Comparative Study of the Possible Prophylactic and Curative Effects of Flaxseed Oil on the Lipid Profile and Antioxidant Status of Hyperlipidaemic Rats. J Appl Pharm 10: 257. doi: 10.4172/1920-4159.1000257

acids (α–linolenic acid, ALA) found in flaxseed is different from those found in fish. Flax seeds are usually consumed in one of three ways: whole seed, ground seed or flaxseed oil. ALA has higher bioavailability in flaxseed oil than in ground or whole flax seed [10]. Flaxseed has gathered attention due to its health benefits related to three important components: ALA, lignans and fibre. ALA from flaxseed was suggested to be the principal constituent that provided its antiarrhythmic effect [11]. Additionally, flaxseed supplementation in hypercholesterolaemic rabbits prevented atherosclerosis, suggesting that flaxseed has significant anti-atherogenic effects [12]. Lignans are one of the main groups of phytoestrogens that have antioxidant and antitumourigenic properties [13]. The consumption of flaxseed has been beneficial to health in different respects, such as prevention of cardiovascular disease and reduction of plasma total cholesterol (TC) and triglyceride levels [14]. Additionally, it has been reported that flaxseed consumption is beneficial in minimizing menopause symptoms. This effect may represent tissue-specific responses to the lignans contained in flaxseed [15]. Furthermore, certain types of cancer that develop under the influence of hormones, breast and uterine cancer, were prevented by the consumption of flaxseed. The inhibitory effect of flaxseed on the growth and metastasis of breast cancer xenografts is attributed to its lignans and oils [16].

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

Material and Methods

Animals

Forty male albino rats of a local strain (7-8 weeks old, weighing 130-150 g) were purchased from Nile Pharmaceuticals Company (Cairo, Egypt). They were housed in standard cages (5 rats/25 X 30 X 30 cm cage) under specific pathogen-free conditions in facilities maintained at a controlled room temperature with 40-60% relative humidity and a natural light-dark cycle. All animals had free access to rat chow diet (see below) and water ad libitum and were allowed to adapt to the 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" and specific national laws were followed, where applicable.

Materials

A 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). A high-fat diet (HFD), consisting of 88% standard pellet animal diet, 10% lard and 2% cholesterol, was prepared and used to induce hyperlipidaemia. The major components of the diets used in this study were previously characterized [17,18]. The HFD was composed of the following energy sources: 52% carbohydrates, 30% fat and 18% protein (total calories: 4.8 kcal/g) [17]. Natural cold-pressed flaxseed oil was purchased from Imtenan Health Shop (Cairo, Egypt).

Experimental design

After a one-week acclimatization period, the rats were equally distributed into 4 groups.

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

Group II: The rats assigned to the HFD group were given normal balanced chow for the first 4 weeks; then, the normal diet was replaced with the HFD for the next 8 weeks [17,18]. These rats were also supplemented with saline via a gastric gavage tube.

Group III: The rats assigned to the HFD-flaxseed prophylactic group were given flaxseed oil orally at a daily dose of 1.8 mg/kg for 4 weeks with normal balanced chow [19] then; the rats received the HFD for the following 8 weeks.

Group IV: The rats assigned to the HFD-flaxseed treatment group were given the HFD for 8 weeks; then, the rats were given the normal balanced diet and supplemented with flaxseed oil orally at a daily dose of 1.8 ml/kg for the next 4 weeks.

The body weight of each rat in all groups was measured and recorded weekly. Hyperlipidaemia was confirmed by measuring serum lipid and lipoprotein levels in the HFD-treated groups.

At the end of the experiment, after overnight fasting, the rats were anaesthetized in the morning, and blood samples were collected from the retro-orbital venous plexus under light ether anaesthesia. A 0.2 ml sample of the blood was haemolysed via the addition of 1.8 ml of H_2O, and the haemolysate was used for the assessment of GSH levels. The remainder of the blood was then centrifuged at 3000 rpm for 15 minutes for serum collection. Serum was separated into aliquots in Eppendorf tubes and kept frozen at -80°C until analysis. The separated serum was analysed to estimate the lipid profiles and levels of oxidative stress markers, inflammation markers and cell adhesion molecules.

Biochemical analysis

- Serum TC and HDL levels were measured by quantitative enzymatic colorimetric determination of TC and HDL cholesterol in serum using Biomed Diagnostic assay kits [20].
- Serum triglyceride levels were measured by quantitative enzymatic colorimetric determination of triglycerides in serum using a Cayman colorimetric assay kit [21].
- Serum LDL cholesterol levels were calculated from the levels of TC, HDL cholesterol and triglycerides using the Friedewald equation: LDL cholesterol (mg/dl) = TC - HDL cholesterol (TG/5.0) [22].
- The serum malondialdehyde (MDA) level was determined based on lipid peroxidation reactions with thiobarbituric acid to generate red species with absorption at 535 nm using the free-SH group estimation method [23].
- The serum level of reduced glutathione (GSH) was measured using a glutathione peroxidase assay kit (Cayman Chemical, Ann Arbor, MI, USA) according to the manufacturer's instructions. Briefly, 8 ml of phosphate buffer, 3 ml of precipitating solution, and 1 ml of DTNB were added to the blood haemolysate filtrate. The optical density was measured spectrophotometrically at a wavelength of 410 nm [24].
- Serum tumour necrosis factor alpha (TNF-α) levels were measured using a commercial ELISA kit (RayBio® Rat, RayBiotech, Norcross, GA, USA) according to the manufacturer's protocol. The sensitivity level of this assay was less than 25 pg TNF-α/ml [25].

- The serum interleukin-6 (IL-6) levels were measured using a commercial ELISA kit (RayBio® Rat, RayBiotech, Norcross, GA, USA) according to the manufacturer's protocol. The sensitivity level of this assay was less than 30 pg IL-6/ml [26].
- The level of vascular cell adhesion molecule 1 (VCAM1) was measured with a commercially available ELISA kit and standards (R&D System Europe Ltd). The sensitivity level of this assay was less than 3.9 ng/ml [27].

Statistical analysis

The data are presented as the means ± standard error of the mean (SEM). Statistical analysis was conducted using one-way analysis of variance (ANOVA) followed by Bonferroni's post hoc multiple comparison test using Statistical Package for the Social Sciences (SPSS) software (version 18, IBM). $P<0.05$ was considered statistically significant.

Results

Effect of flaxseed oil on the body weight of hyperlipidaemic rats

HFD feeding for eight weeks significantly augmented body weight compared to normal diet feeding (Table 1). When flaxseed oil was given as a prophylactic orally for 4 weeks before inducing hyperlipidaemia, the body weight growth of the HFD-treated rats was significantly decreased ($P<0.001$). Notably, there was no significant difference in body weight among the HFD-flaxseed prophylactic group and the control group. When flaxseed oil was given after inducing hyperlipidaemia, body weight was significantly decreased compared with hyperlipidaemia induction alone, but the body weight of the rats in the HFD-flaxseed treatment group was still significantly higher than that of the control rats (Table1).

Effect of flaxseed oil on the lipid profiles of hyperlipidaemic rats

Serum concentrations of TC and triglycerides: HFD feeding for eight weeks significantly augmented the fasting serum TC level compared with normal diet feeding (Table 1). In comparison to the hyperlipidaemic group, the flaxseed prophylactic and treatment groups showed significantly lower levels of TC and triglycerides ($P<0.0001$). No significant difference in TC or triglyceride levels was found between the flaxseed prophylactic group and the control group (Table 1), while the flaxseed treatment group showed 20-25% higher levels of TC and triglycerides ($P<0.0001$) than the control group.

Serum concentrations of LDL and HDL: Similar to the above results, HFD supplementation increased the serum LDL level in hyperlipidaemic rats compared to control rats, and flaxseed prophylaxis and treatment significantly lowered the LDL levels ($P<0.0001$), as shown in Table 1. In contrast, a 58% reduction in the serum HDL level was observed on the 8th week of HFD feeding compared to normal diet feeding. The HFD-flaxseed prophylactic and treatment groups showed significantly elevated HDL levels than the HFD group, and the HDL concentration returned to the normal level in the HFD-flaxseed prophylactic group but not in the HFD-flaxseed treatment group (Table 1).

Anti-inflammatory effects of flaxseed oil on hyperlipidaemic rats

Serum TNF-α and IL-6 levels:

HFD supplementation for eight weeks up-regulated both TNF-α and IL-6 by ~3-fold and ~4-fold, respectively, compared to control feeding (Figure 1A and B). Significant reductions in serum TNF-α and IL-6 concentrations were observed in the HFD-flaxseed prophylaxis and treatment groups compared to the HFD group. Flaxseed oil was more effective at decreasing both the TNF-α and IL-6 concentrations when used as a pretreatment before induction of hyperlipidaemia than when used as a treatment. There was no significant difference in the TNF-α and IL-6 concentrations between the flaxseed oil-pretreated rats and the control rats (Figure 1), while the HFD-flaxseed treatment group displayed higher TNF-α and IL-6 concentrations than the control group.

Serum VCAM1 level: Assessment of the VACM1 concentration could be useful in identifying the risk for atherosclerotic lesions in hyperlipidaemic rats and in estimating the effect of flaxseed supplementation. HFD administration to normal rats for eight weeks induced a significant elevation in serum VCAM1 compared to control treatment. The VACM1 level was decreased when the rats received flaxseed oil before or after HFD supplementation (Figure 1C). When flaxseed oil was given before HFD supplementation, the VACM1 concentration remained at the control level. However, the HFD-flaxseed treatment group showed significantly elevated VACM1 levels compared to the control group.

	Control	Hyperlipidaemic	Flaxseed prophylactic	Flaxseed treatment	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 difference compared to the control group.
[b]:Significant difference compared to the hyperlipidaemic group.

Table 1: Effect of flaxseed oil on body weight and lipid profiles of hyperlipidaemic rats.

	Control	Hyperlipidaemic	Flaxseed prophylactic	Flaxseed treatment	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 difference compared to the control group.
[b]:Significant difference compared to the hyperlipidaemic group.

Table 2: Effect of flaxseed oil (pretreatment and treatment) on serum malondialdehyde (MDA) and glutathione (GSH) levels.

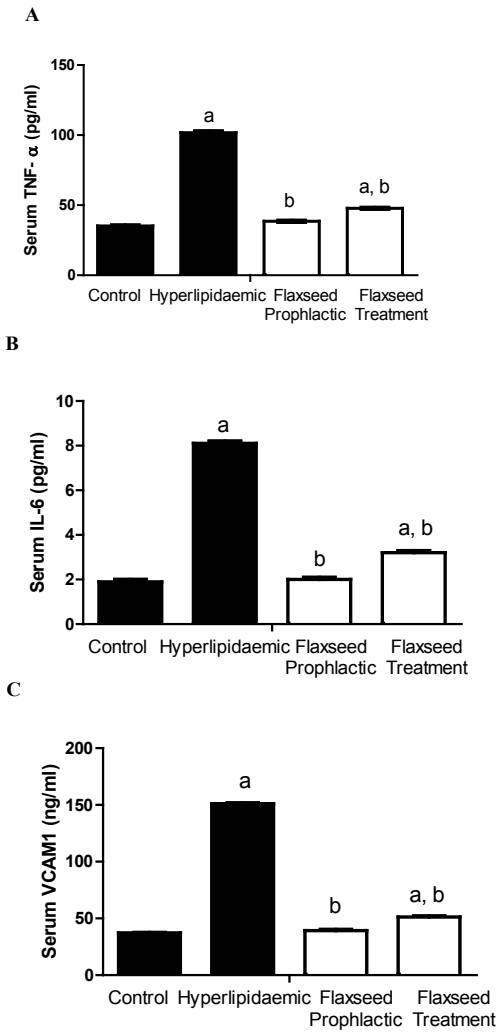

Figure 1: Effect of Flaxseed oil (pretreatment and treatment) on the serum levels of tumour necrosis factor alpha (TNF-α; panel A), interlukin-6 (IL-6; panel B) and vascular cell adhesion molecule 1 (VCAM1; panel C). HFD administration to rats increased the serum levels of TNF-α, IL-6 and VCAM1 compared to the control treatment (panels A, B and C). Prophylactic administration of Flaxseed oil decreased the TNF-α, IL-6 and VCAM1 levels in rats compared to HFD feeding alone ($P<0.0001$). Flaxseed oil treatment decreased the TNF-α, IL-6 and VCAM1 levels in rats compared to HFD feeding alone, although not to the control levels.

Discussion

Dietary saturated fatty acids are associated with metabolic and cardiovascular diseases. Potentially promising strategies to diminish the risk of these diseases include partial modification of the quality of fat consumed or use of medicinal plants, which are rich sources of unsaturated fatty acids, antioxidants and fibre [28]. To date, few effective, safe and convenient approaches for the treatment of cardiovascular diseases have been clinically available. In this study, we identify a possible natural cardioprotective agent that can effectively ameliorate HFD-induced hyperlipidaemia.

In the present study, we demonstrated that HFD for 8 weeks significantly increased body weight and the serum levels of TC, triglycerides, LDL, MDA, and inflammation markers (TNF-α, IL-6, and VACM1) in male rats. However, the HDL and GSH levels were decreased by HFD feeding (Tables 1 and 2). We also demonstrated that flaxseed oil (high bioavailability) administration at a dose of 1.8 mg/kg to hyperlipidaemic rats reduced the serum concentrations of TC, triglycerides, and LDL (Table 1). Conversely, this treatment increased the serum concentrations of HDL and GSH (Tables 1 and 2). In association with modifying the lipid profile, consumption of flaxseed oil significantly reduced the level of the lipid peroxidation marker MDA. Furthermore, flaxseed oil suppressed the increases in serum IL-6, TNF-α and VCAM1 levels in hyperlipidaemic rats (Figure 1). Taken together, the protective effect of flaxseed oil during hyperlipidaemia is dependent on its reduction of the risk of atherosclerosis by decreasing the serum VACM1 levels.

Provision of a HFD with ~30% of all energy derived from fats leads to obesity in many animals, such as mice, rats, dogs and primates, due to increased energy intake and efficient energy storage [29]. The significant increase in body weight with provision of the HFD for eight weeks in the present study is consistent with the results of previous studies, which attributed this increase to the high energy density of fat: fat supplies ~9 kcal/g, whereas carbohydrates and protein provide only 4 kcal/g. Therefore, increased fat intake can promote high energy utilization, increased energy density and increased palatability [29,30]. Such an increase in body weight may occur because hyperphagia and, consequently, high energy intake are induced by secretion of the adipocyte-derived hormone leptin [31].

Many studies have reported that flaxseed may have protective effects against diseases such as cardiovascular disease. Flaxseed oil administration before or after induction of hyperlipidaemia significantly decrease body weight. These results are consistent with those of previous studies by Vijaimohan et al., who proposed that the hypolipidaemic and antioxidant effects of flaxseed oil are responsible for its beneficial effect on body weight gain [32]. Moreover, Baranowski et al. found that the effect of dietary flaxseed oil on body weight is attributable to its content of ALA, which reduces adipocyte hypertrophy, protein levels, the concentrations of inflammation markers, monocyte chemoattractant protein-1 (MCP-1), and TNF-α, and T cell infiltration in adipose tissue [33]. In contrast, a previous study showed that supplementation of flaxseed oil to hyperlipidaemic rats did not significantly alter weight gain [34].

As noted above, our results showed a marked increase in the lipid profile (TC, triglycerides and LDL) in the HFD group, while serum HDL levels showed a significant decrease. Administration of flaxseed oil prior to or after induction of hyperlipidaemia markedly improved these parameters. These results are consistent with the findings from studies by others [35,36]. The cholesterol level in the plasma and liver of hyperlipidaemic rats increased due to elevated uptake of exogenous cholesterol and its subsequent accumulation in addition to decreased cholesterol catabolism, as demonstrated by a reduction in bile acid formation and turnover of bile acids [37]. In the present study, flaxseed oil administration improved the lipid profile to normal levels. This finding was supported by a previous study reporting that flaxseed oil supplementation to rats fed a high-cholesterol diet decreased the serum lipid profile [38]. They attributed this beneficial effect to ALA, which promotes cholesterol secretion into bile, leading to depletion of the intrahepatic pool of cholesterol and, therefore, increased cholesterol synthesis and metabolism. Moreover, a diet rich in ALA reduces hepatic lipid deposition both by stimulating β-oxidation and by inhibiting fatty acid synthesis [39]. The triglyceride-reducing the effect of flaxseed oil is mediated by its regulation of peroxisome proliferator-activated receptor-γ (PPARγ)

and sterol regulatory element-binding protein-1 (SREBP-1), which control hepatic fatty acid catabolism and synthesis, respectively [40]. Conversely, Deng et al. and Xu et al. reported that flaxseed oil does not markedly affect the plasma HDL cholesterol level [41].

We assessed a major compound implicated in the downregulation of substances formed during oxidative stress, GSH. Our study showed that flaxseed oil supplementation induced significant changes in the levels of oxidative stress markers (MDA and GSH) (Table 2). We also measured the levels of pro-inflammatory cytokines, such as IL-6 and TNF-α, to investigate the effect of flaxseed oil on hyperlipidaemia-induced inflammation in rats. We found that flaxseed oil ameliorated the hyperlipidaemia-induced increases in IL-6, TNF-α, and VCAM1 levels (Figure 1). The results for oxidative stress, inflammation markers and endothelial dysfunctional markers were consistent with those of studies by Peairs et al. and Herieka and Erridge, who reported that HFD treatment stimulates oxidative stress, impairs endothelial function and increases the circulating levels of inflammatory factors such as soluble intercellular adhesion molecule-1 (ICAM-1), TNF-α and C-reactive protein (CRP) [42]. Additionally, Shi et al. found that the high levels of TNF-α, which is predominantly produced by macrophages and monocytes, were due to acute responses to the HFD, as the concentrations of these cytokines typically return to baseline levels once the acute phase response is diminished [43]. Finally, we have shown that flaxseed oil administration before or after HFD treatment significantly decreased oxidative stress and the levels of inflammation markers, which was supported by previous studies [44] reporting that flaxseed oil elicits anti-inflammatory effects by reducing the production of inflammatory cytokines such as IL-1, IL-6, CRP and TNF-α via inhibition of NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) and/or activation of PPAR-γ. Another mechanism for the effects of flaxseed oil was reported by [45] who attributed its effects to the presence of ω-3 fatty acids, which regulate the expression of adhesion molecules such as VCAM-1, which contributes to leukocyte-endothelium interactions.

Interestingly, the present study showed that the use of flaxseed oil as a prophylactic agent against hyperlipidaemia and vascular changes exerted a more significant effect on body weight along with marked improvements in oxidative stress, inflammation and vascular adhesion molecule parameters in comparison to its use after induction of hyperlipidaemia. Presumably, the use of flaxseed as a treatment after induction of hyperlipidaemia in this study was insufficient, and a longer treatment period may be required for all parameters to return to their normal levels. Alternatively, HFD feeding may induce atherosclerotic lesions that are not completely repaired by administration of flaxseed oil alone and that may require combination therapy. A recent study demonstrated that combined treatment with flaxseed oil and ALA ester of plant sterol synergistically ameliorated atherosclerosis, optimized the overall lipid profile, inhibited inflammation and reduced oxidative stress [40]. Further studies are needed to evaluate the potential of flaxseed oil for the management of hyperlipidaemia, the most effective duration of flaxseed oil treatment for returning all metabolic and cardiovascular disease risk factors to normal levels, and the adverse effects of these agents for use as anti-atherogenic treatments in humans.

In conclusion, flaxseed oil possessed significant anti-hyperlipidaemic and anti-inflammatory properties. Flaxseed oil may reduce the risk factors of cardiovascular disease by enhancing plasma antioxidant defences and improving lipid 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.

Conflict of Interest

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

Acknowledgements

The authors are particularly grateful to the Laboratory Animal Center, Faculty of Medicine, Al Azhar University, Egypt, for assistance in laboratory tests and for providing laboratory animals.

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