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Evaluation of the Protective Role of Flaxseed Oil on Inflammatory Mediators, Antioxidant Defense System and Oxidative Stress of Liver Tissue in Hypercholesterolemic Rats

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ABSTRACT

Hypercholesterolemia is one of the major risk factors that precipitate coronary heart disease, atherosclerosis, peripheral vascular and ischemic cerebrovascular diseases. Protective effects of flaxseed oil on hepatic oxidative stress and antioxidant status, liver and cardiac marker enzymes in addition to pro-inflammatory cytokines in hypercholesterolemia-induced in rats were evaluated. Forty male rats were divided into four equal groups. Group I :(Control) rats were fed on normal diet. Group Π: hypercholesterolemic diet (HCD) rats were fed [4% cholesterol (w/w) and 1% cholic acid]. Group III: rats were fed HCD and received flaxseed oil (270 mg/kg b.wt/day, orally). Group IV: rats were fed normal diet and administer flaxseed oil (270 mg/kg b.wt/day, orally). Blood and liver tissue samples were collected at 2, 4 and 6 weeks from the onset of treatment with flaxseed oil. The obtained results showed marked increase in serum tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), LDH, CK-MB, ALT, AST and GGT activities in addition to liver nitric oxide (NO) and L-MDA levels in hypercholesterolemic rats. Meanwhile, liver CAT, SOD and GPx activities were significantly decreased. Treatment with flaxseed oil in hypercholesterolemic rats lowered serum TNF-α, IL-6, liver marker enzymes, NO and L-MDA and ameliorate antioxidant enzymatic status in liver tissue. These results suggest that, flaxseed oil may be effective in controlling cholesterolemic status and has the potential in reducing cardiovascular complications due to hypercholesterolemia. Also, administration of flaxseed oil enhanced the antioxidant defense system in liver tissues and have a meliorating effect in hypercholesterolemia induced hepatic oxidative stress.

Keywords: Flaxseed oil; Hypercholesterolemia; oxidative stress; pro-inflammatory cytokines; lipid peroxidation; Cardiac marker enzymes.

1. INTRODUCTION

Hypercholesterolemia is the main risk factor of cardiovascular diseases such as atherosclerosis, myocardial infarction, stroke and cerebrovascular diseases. These diseases are diagnosed through natural increasing of lipids (triglycerides and cholesterol) and lipoprotein in blood [1]. Hypercholesterolemia has been associated with enhanced oxidative stress related to increased lipid peroxidation. Increased generation of oxidized LDL is a major factor in the vascular damage associated with high cholesterol levels. Hence, the inhibition of oxidative stress under hypercholesterolemic conditions is considered to be an important therapeutic approach and efforts have been made to identify the antioxidative functions of various medicinal plants [2].

Hypercholesterolemia and Hyperlipidemia has been ranked as one of the greatest risk factors contributing to the prevalence and severity of coronary heart diseases. In developing countries, the incidence of cardiovascular disease is increasing alarmingly especially; India is on the verge of a cardiovascular epidemic [3]. Feeding animals with cholesterol has

often been used to elevate serum or tissue cholesterol levels to study the etiology of hypercholesterolemia-related metabolic disturbances [4]. Exogenous hypercholesterolemia causes fat deposition in the liver and depletion of the hepatocyte population; it can also cause malfunctioning of the liver, which apparently follows micro vesicular stenosis due to the intracellular accumulation of lipids [5]. Furthermore, free-radical-mediated peroxidative modification of polyunsaturated fatty acids of LDL and very-low-density lipoprotein (VLDL) is thought to contribute to the progression of atherosclerotic lesions. Oxidative stress is an early event in the evolution of hyperlipidemia, and it has been suggested that appropriate support for enhancing antioxidant supply in subjects with abnormally elevated lipid levels can attenuate the course of the disease [6]. It has been reported that high levels of fat increase fat-mediated oxidative stress and decrease antioxidative enzyme activity. Hence, HFD-induced oxidative damage may result in many chronic health problems, such as impairment of liver function [7]. Reactive oxygen species (ROS) have detrimental effects on hepatocytes, by damaging DNA, lipids and proteins, leading to a disruption in cellular homeostasis and aggravation in metabolic syndrome features [8]. Recently, hypercholesterolemia has been associated with enhanced oxidative stress related to increased lipid peroxidation. Increased generation of oxidized LDL is a major factor in the vascular damage associated with high cholesterol levels. Hence, the inhibition of oxidative stress under hypercholesterolemic conditions is considered to be an important therapeutic approach and efforts have been made to identify the antioxidative functions of various medicinal plants [2]. The potential therapeutic and preventive benefits of plant-based medications have been the subject of extensive studies, and many natural constituents have been uncovered with significant pharmacologic activity including agents with antiglycemic, hypolipidemic and antihypertensive properties [9].

Flaxseed (Linum usitatissimum) is the richest dietary source of omega-3 fatty acids among plant sources. Flaxseed is widely used for its edible oil in many parts of the world. A number of investigations have demonstrated that diet supplemented with flaxseed oil has profound beneficial health effects in various pathologies. Flaxseed is also the richest source of lignans, which have been reported to have antioxidant and hypolipidemic effects [10]. Moreover, Vijaimohan *et al.,* (2006) [11] demonstrate that, flaxseed oil present in flaxseeds may be developed as a useful therapy for hyperlipidemia through reducing hepatic lipids, thereby proving its hypolipidemic activity. Addition, of flaxseed in the diet in animal studies has shown inhibit atherogenesis [12] and protect during hypercholesterolemic conditions [13]. Accordingly, the purpose of the present study was to investigate the effect of flaxseed oil against high cholesterol diet induced hypercholesterolemia in rats. Also, to determine whether flaxseed when administered to hypercholesterolemic induced-rats beneficial for prevention and treatment of hypercholesterolemia complications.

2. MATERIALS AND METHODS

2.1 Experimental animals

Sixty male albino rats, 12-16 weeks old and average body weight 180-220 g were used in the experimental investigation of this study. Rats were obtained from Laboratory Animals Research Center, Faculty of Veterinary Medicine, Moshtohor, Benha University. Animals were housed in separate metal cages, fresh and clean drinking water was supplied ad-libitum. Rats were kept at constant environmental and nutritional conditions throughout the period of experiment. The animals were left 14 days for acclimatization before the beginning of the experiment.

2. 2 Flaxseed oil

Flaxseed oil manufactured by (South Egypt Drug Industries Co. (SEDICO), 6 October City-Egypt) and it had a light yellow color. The concentration of flaxseed oil 1000mg and present in the soft gelatine capsulated form. Flaxseed oil was dissolved in propylene glycol and was administered orally in a daily dose of 270 mg/kg body weight using stomach tube.

2. 3 Induction of Hypercholesterolemia

Hypercholesterolemia was induced in rat by feeding high cholesterol diet [4% cholesterol (w/w) and 1% cholic acid (w/w)] for 8-weeks [14].

2. 4 Experimental design

Rats were randomly divided into four main equal groups, 15 rats each, placed in individual cages and classified as follows:

Group 1: Control Normal group: Rats fed an ordinary diet only.

Group 2: High cholesterol diet (HCD) group: Rats fed with hypercholesterolemic diet (HCD) [4% cholesterol (w/w) and 1% cholic acid] and received no drug all over the period of the experiment.

Group 3: High cholesterol diet (HCD) + flaxseed oil treated group: Rats fed with HCD and received flaxseed oil (270 mg/kg. body weight/day, orally) after two weeks (from the onset of induction of hypercholesterolemia).

Group 4: Normal flaxseed oil group: Rats fed with normal diet and administered with flaxseed oil (270 mg/kg. body weight/day, orally).

2.5 Sampling

Random blood sample and liver tissue specimens were collected from all animals groups (control and experimental groups) three times along the duration of experiment after 2, 4 and 6 weeks from the onset of treatment with flaxseed oil.

2.5.1 Blood samples

Blood samples were collected by ocular vein puncture in dry, clean, and screw capped tubes and serum was separated by centrifugation at 2500 r.p.m for 15 minutes. The clear serum was aspirated by automatic

pipette and received in dry sterile samples tube, processed directly for determination of cardiac and hepatic marker enzymes {alanine aminotransaminase (ALT), aspartate aminotransaminase (AST), lactate dehydrogenase (LDH), creatine phosphokinase-MB (CPK-MB) and gamma glutamyl transferase (GGT) } activities, then kept in a deep freeze at -20°C until used for determination of tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) concentrations.

2.5.2. Liver tissue specimens:
Rats killed by decapitation. The livers specimens were quickly removed, rinsed in ice-cold 0.9% sodium chloride solution, quick frozen in a deep freeze at -20°C for subsequent biochemical analyses.

Preparation of liver tissues
Briefly, liver tissues was minced into small pieces, homogenized with ice cold 0.05 M potassium phosphate buffer (pH 7.4) to make 10% homogenates. The homogenates were be centrifuged at 6000 r.p.m for 15 minute at 4°C until used for subsequent biochemical analysis. All liver samples were analyzed for the determination of L-malondialdehyde (L-MDA), Nitric oxide (NO), antioxidant enzymes {(Catalase (CAT), superoxide dismutase(SOD), Glutathione peroxidase(GPX)} and reduced Glutathione (GSH).

2. 6. Biochemical analysis:
Serum ALT and AST, GGT, LDH, CPK-MB, TNF-α and IL-6 levels and liver tissue L-MDA, NO, GSH, CAT, SOD, GPx activities were analyzed according to the methods described by Murray, (1984)[15]; Beleta and Gella, (1990) [16]; Dito, (1979) [17]; Rat Creatine Kinase MB Isoenzyme (CKMB) ELISA (Kamiya Biomedical Company, Cat. No. KT-12247); Beyaert and Fiers, (1998)[18]; Chan and Perlstein, (1987)[19]; Mesbah *et al.,* (2004) [20]; Montgomey and Dymock, (1961)[21]; Moron *et al*., (1979) [22]; Luck, (1974) [23]; Kakkar *et al*., (1984) [24]; Gross *et al*., (1967) [25].

2.7 Statistical analysis
The obtained data were statistically analyzed by one-way analysis of variance (ANOVA) followed by the Duncan multiple test. All analyses were performed using the statistical package for social science (SPSS, 13.0 software, 2009). Values of $P<0.05$ were considered to be significant.

Table 1: Effect of flaxseed oil on serum TNF-α and IL-6 concentrations in normal and hypercholesterolemic rats.

Parameters / Exp.Groups	TNF-α (pg/ml)			IL-6 (pg/ml)		
	2Weeks	4 Weeks	6 Weeks	2Weeks	4 Weeks	6 Weeks
Normal control	16.46±3.44^{a}	14.49±2.61^{c}	13.97±1.43^{b}	14.97±1.58^{a}	10.29±1.24^{c}	14.30±1.27^{b}
High cholesterol diet	23.18±1.30^{a}	29.97±4.96^{a}	31.95±6.78^{a}	17.18±1.44^{a}	25.52±1.16^{a}	37.33±1.01^{a}
Flaxseed Oil treated (270 mg/kg b.wt/day)	12.95±2.88^{b}	24.65±1.66^{a,b}	21.36±0.61^{a,b}	13.52±0.37^{a}	19.40±0.55^{b}	18.95±4.24^{b}
Flaxseed Oil Normal (270 mg/kg b.wt/day)	17.41±0.65^{a,b}	11.92±2.74^{c}	20.35±3.91^{a,b}	14.44±3.24^{a}	9.97±0.77 ^{c}	14.59±2.40^{b}

Data are presented as (Mean ± S.E).S.E = Standard error. Mean values with different superscript letters in the same column are significantly different at (*P≤0.05*).

Table 2: Effect of flaxseed oil on serum AST, ALT and GGT activities in normal and hypercholesterolemic rats.

Parameters / Exp.Groups	ALT (U/L)			AST (U/L)			GGT (U/L)		
	2Weeks	4 Weeks	6 Weeks	2Weeks	4 Weeks	6 Weeks	2Weeks	4 Weeks	6 Weeks
Normal control	76.41 ±3.93^{a,b}	89.37 ±7.68^{a,b}	65.54 ±4.66^{b,c}	120.63 ±9.60^{b}	165.59 ±12.07^{b}	143.78 ±11.03^{b,c}	56.37 ±4.21^{c}	72.52 ±2.84^{a,b}	55.64 ±2.04^{b,c}
High cholesterol diet	79.98 ±2.77^{a,b}	107.93 ±9.42^{a}	98.16 ±3.90^{a}	220.79 ±5.09^{a}	219.18 ±12.19^{a}	222.86 ±21.27^{a}	87.90 ±4.75^{a}	84.75 ±8.25^{a}	95.26 ±10.90^{a}
Flaxseed Oil treated (270 mg/kg b.wt/day)	65.69 ±3.89^{b}	84.40 ±3.52^{b}	82.90 ±2.06^{a,b}	151.05 ±29.21^{b}	165.17 ±7.06^{b}	136.88 ±16.94^{b,c}	72.55 ±3.00^{b}	68.92 ±5.62^{a,b}	75.06 ±5.59^{b}
Flaxseed Oil Normal (270 mg/kg b.wt/day)	92.90 ±2.40^{a}	91.28 ±3.31^{a,b}	71.24 ±8.79^{b,c}	168.57 ±8.80^{b}	142.58 ±13.99^{b,c}	97.98 ±4.98^{c}	60.75 ±5.28^{b,c}	59.76 ±3.14^{b,c}	49.55 ±5.52^{c}

Data are presented as (Mean ± S.E).S.E = Standard error. Mean values with different superscript letters in the same column are significantly different at (*P≤0.05*)

3. RESULTS AND DISCUSSION

3.1. Effect of flaxseed oil on serum TNF-α and IL-6 concentrations in normal and hypercholesterolemic rats
A significant increase in serum TNF-α and IL-6 concentrations were observed in cholesterol fed rats after four and six weeks of the experiment as compared with rats fed normal control diet. Flaxseed oil treatment in rats fed high cholesterol diet resulted in a significant decrease in serum TNF-α level after two weeks of the experiments. However, serum IL-6 level was significantly decreased after two and six weeks as compared to untreated high cholesterol -fed rats (Table 1).

3.2. Effect of flaxseed oil on serum AST, ALT and GGT activities in normal and hypercholesterolemic rats
The obtained results presented in table (2) showed a significant increase in serum ALT activity in cholesterol fed rats after six weeks. Also, serum AST activity was

significantly increased all over the periods of the experiment associated with significant increase in serum GGT activity after two and six weeks as compared with rats fed normal control diet. Flaxseed oil treatment in rats fed high cholesterol diet resulted in a significant decrease in serum ALT activity after four with associated with significant decrease in serum AST and GGT activities all over the periods of the experiments as compared to untreated cholesterol -fed rats.

3.3. Effect of flaxseed oil on serum LDH and CK-MB activities in normal and hypercholesterolemic rats

The obtained data demonstrated in table (3) revealed that, a significant increase in serum LDH and CK-MB activities were observed in cholesterol fed rats all over the periods of the experiment when compared with rats fed normal control diet. Flaxseed oil treatment in rats fed high cholesterol diet resulted in a significant decrease in serum LDH activity after two and sex weeks, associated with a significant decrease in serum CK-MB activity after four and sex weeks as compared to untreated cholesterol -fed rats.

Table 3: Effect of flaxseed oil on serum LDH and CK-MB activities in normal and hypercholesterolemic rats.

Parameters / Exp.Groups	LDH (nmol/ml)			CK-MB (nmol/ml)		
	2Weeks	4 Weeks	6 Weeks	2Weeks	4 Weeks	6 Weeks
Normal control	333.59 ±26.85^{c}	381.44±87.46^{b}	700.60±68.99^{b}	201.01±17.70^{b,c}	205.63±11.13^{c}	259.76±23.99^{b}
High cholesterol diet	1120.47±92.39^{a}	1096.99±113.23^{a}	1050.73±132.62^{a}	441.27±58.47^{a}	467.70±33.41^{a}	443.38±57.28^{a}
Flaxseed Oil treated (270 mg/kg b.wt/day)	606.46±8.07^{b}	922.59±67.73^{a}	354.70±21.92^{c}	378.43±24.88^{a}	341.24±41.13^{b}	108.57±29.47^{c}
Flaxseed Oil Normal (270 mg/kg b.wt/day)	481.03±98.41^{b,c}	545.65±114.07^{b}	403.54±19.58^{c}	129..36±13.69 ^{c}	250.48±13.91^{c}	173.30±16.00^{b,c}

Data are presented as (Mean ± S.E).S.E = Standard error. Mean values with different superscript letters in the same column are significantly different at (*P≤0.05*)

3.4. Effect of flaxseed oil on liver tissue NO, L-MDA and GSH concentrations in normal and hypercholesterolemic rats

A significant increase in liver tissue NO concentration was observed in cholesterol fed rats after two and four weeks of the experiment, associated with a significant increase in liver L-MDA concentration all over the periods of the experiment. However, liver tissue GSH concentration was non significantly decreased when compared with rats fed normal control diet. Flaxseed oil treatment in rats fed high cholesterol diet resulted in a significant decrease in liver NO concentration after two and six weeks and in liver L-MDA concentration after two and four weeks of the experiments. Also, a significant decrease in liver tissue GSH concentration was observed after two weeks of the experiment when compared to untreated cholesterol -fed rats (Table 4).

Table 4: Effect of flaxseed oil administration on liver tissue NO, L-MDA and GSH concentrations in normal and hypercholesterolemic rats.

Parameters / Exp.Groups	NO (ng/g tissue)			L-MDA (mmol/g tissue)			GSH (ng/g tissue)		
	2Weeks	4 Weeks	6 Weeks	2Weeks	4 Weeks	6 Weeks	2Weeks	4 Weeks	6 Weeks
Normal control	110.68 ±3.94 ^{b}	90.84 ±7.78 ^{b,c}	104.10 ±6.56 ^{a,b}	19.71 ±2.43^{c}	15.98 ±1.61^{c}	17.04 ±0.99^{b}	11.60 ±2.11 ^{a}	11.76 ±3.77 ^{a}	8.66 ±1.13 ^{a}
High cholesterol diet	134.98 ±5.68 ^{a}	126.05 ±5.69 ^{a}	126.91 ±1.64 ^{a}	48.04 ±4.08^{a}	65.33 ±6.48^{a}	40.86 ± 5.77^{a}	10.59 ±1.42^{a}	7.65 ±0.66 ^{a,b}	7.26 ±1.22 ^{a}
Flaxseed Oil treated (270 mg/kg b.wt/day)	98.12 ±4.90 ^{b}	116.90 ±4.14 ^{a,b}	90.14 ±14.20 ^{b,c}	33.74 ±2.91^{b}	29.44 ±9.26^{b,c}	31.13 ±5.71^{a,b}	2.11 ±0.67 ^{c}	2.10 ±0.21 ^{b,c}	5.75 ±0.55 ^{a}
Flaxseed Oil Normal (270 mg/kg b.wt/day)	97.24 ±10.47 ^{b}	96.24 ±6.75 ^{b,c}	84.62 ±8.26 ^{b,c}	19.23 ±1.97 ^{c}	20.87 ±1.79^{c}	21.97 ±2.43 ^{b}	6.63 ±0.33 ^{b}	6.27 ±1.29 ^{a,b,c}	5.79 ±1.15 ^{a}

Data are presented as (Mean ± S.E).S.E = Standard error. Mean values with different superscript letters in the same column are significantly different at (*P≤0.05*)

Table 5: Effect of flaxseed oil administration on liver tissue CAT, SOD and GPX activities in normal and hypercholesterolemic rats.

Parameters / Exp.Groups	CAT(U/g tissue)			SOD (U/g tissue)			GPx (ng/g tissue)		
	2Weeks	4 Weeks	6 Weeks	2Weeks	4 Weeks	6 Weeks	2Weeks	4 Weeks	6 Weeks
Normal control	90.79 ±5.87 ^{a}	69.49 ±4.28^{a,b}	59.01 ±4.93^{a,b}	44.06 ±5.26^{a}	43.38 ±7.18^{a}	37.81 ±3.77^{a}	32.99 ±3.72^{a}	30.71 ±2.55^{a}	33.59 ±5.67^{a}
High cholesterol diet	69.60 ±5.49^{b}	56.51 ±2.73^{b}	46.27 ±6.02^{b}	41.32 ±3.49^{a}	26.05 ±3.06^{b}	21.83 ±2.09^{c}	21.52 ±1.70^{c}	22.44 ±2.55^{a}	9.22 ±1.56^{b}
Flaxseed Oil treated (270 mg/kg b.wt/day)	69.87 ±3.33^{b}	81.22 ±1.12^{a}	63.57 ±3.81^{a}	19.95 ±2.29^{c}	28.35 ±1.77^{b}	33.06 ±4.35^{a,b}	24.37 ±2.28^{b,c}	25.94 ±4.53^{a}	24.22 ±2.64^{a}
Flaxseed Oil treated (270 mg/kg b.wt/day)	64.17 ±4.41^{b}	64.87 ±6.27^{b}	63.31 ±5.75^{a}	33.66 ±4.80^{a,b,c}	34.90 ±3.86^{a,b}	39.86 ±3.65^{a}	26.39 ±1.53^{a,b,c}	29.34 ±2.18^{a}	26.30 ±1.72^{a}

Data are presented as (Mean ± S.E).S.E = Standard error. Mean values with different superscript letters in the same column are significantly different at (*P≤0.05*)

3.5. Effect of flaxseed oil on liver tissue CAT, SOD and GPX activities in normal and hypercholesterolemic rats.

The obtained results in table (5) revealed that, a significant decrease in liver CAT activity was observed in cholesterol fed rats after two weeks of the experiment. A significant decrease in liver SOD activity was observed in cholesterol fed rats after four and six weeks. However, liver GPX activity was significantly decreased after two and six weeks when compared with rats fed normal control diet. Flaxseed oil treatment in rats fed high cholesterol diet resulted in a significant increase in liver CAT activity after four and six weeks. Also, a significant increase in liver tissue SOD and GPX activities were observed after six weeks. Meanwhile, liver SOD activity was significantly decreased after two weeks of the experiment as compared to untreated cholesterol-fed rats.

Hypercholesterolemia is a lipoprotein metabolic disorder characterized by high serum levels of low density lipoprotein (LDL-C) and blood cholesterol. Alteration in cholesterol and triglycerides metabolism as a result of hypercholesterolemia has been shown to negatively affect oxidative stress biomarkers and promotes production of reactive oxygen species (ROS) by various mechanisms that lead to increased lipid peroxidation [26]. A significant increase in serum TNF-α and IL-6 concentrations were observed in high cholesterol fed rats when compared with rats fed normal control diet. These results are nearly similar to those recorded by Howard and Culley, (2006)[27] and Rajamannan *et al.,* (2005)[28] who reported that, a significant increase inflammatory markers (CRP, TNFα) level was establish in rabbits fed cholesterol enriched diet as compared with the normal control group. Elevated serum cholesterol concentrations may trigger inflammatory responses in the blood circulation and locally in vascular walls [29]. The present results also showed that, high cholesterol diet results in gross systemic inflammatory responses, as indicated by markedly elevated cytokine concentrations. Because of the in vivo nature of current experimental model, the elevated serum cytokines concentrations could be derived either from circulating white blood cells or from any tissue source, such as endothelium [30]. The mechanisms underlying the different responses over time of different cytokines are not clear. It is possible that the high IL-6 and TNF-α response, which are mainly produced by macrophages and monocytes [31]. Prolonged increases in the concentrations of these cytokines are most likely associated with either severe damage of tissues after trauma or invasion of the body by pathogenic organisms [32].

Previous studies have found that dietary high cholesterol intake can increase the productions of atherogenic inflammatory cytokines such as IL-6 and TNF-α [33]. In addition, excessive intake of cholesterol may cause vascular inflammation, and pro-inflammatory cytokines such as TNF-α and IL-6 may stimulate the expression of adhesion molecules and chemokines such as Vascular Cell Adhesion Molecule-1 (VCAM-1), Intracellular Adhesion Molecule-1 (ICAM-1), and fibronectin in aorta tissue [34,35]. VCAM-1 and ICAM-1 are thought to play an important role in the process of atherosclerosis by recruiting inflammatory cells, and they are both are up-regulated by pro-atherogenic factors [35]. A single cell layer lining the vascular wall, called the vascular endothelium, plays an important role in maintaining the structure and function of vessels. Besides, being the vascular endothelium a mechanical barrier between blood and vessel wall, it is also the origin of production for different bioactive factors that regulate vascular tone, coagulation, cell proliferation, cell death, and inflammation [36]. The cytokines can be represented in two shapes including the anti-inflammatory markers such as IL-1β, IL-6 and TNF-α, the second shape is the adhesion molecules such as ICAM-1 and VCAM-1 [37]. Reactive oxygen species are well documented to function as signaling molecules, stimulating cellular activities ranging from cytokine secretion to cell proliferation, and at higher concentrations, they can induce cell injury and death by oxidative modification of proteins and carbohydrates, lipid peroxidation, and DNA[38]. This is combined with, oxidants lead to the activation of endothelial cells, the action that may results in a wide range of functional changes such as the increase in expression of VCAM-1, ICAM-1, and E-selectin, and the production of chemokines, such as monocyte chemo attractant peptide-1 (AP-1) [39]. In a parallel results, Ceriello *et al.,* (1998)[40] reported that, ICAM-1 is one of the most important intercellular adhesion molecules involved in atherogenesis and noticed increased circulating ICAM-1 plasma levels in NIDDM patients that may be resulted from the acute increase of plasma glucose which leads to produce an oxidative stress and induce cellular expression of ICAM-1. In addition, Ozawa *et al.,* (2003)[41] illustrated the induction of ICAM-1 through pro-inflammatory cytokines (IL-1β, TNF-α, IFN-γ and IL-2) and also, VCAM-1 can be induced by IL-1β [42]. These cytokines have a probable anti-inflammatory action on the artery wall by producing an improvement in endothelial dysfunction in addition to their lipid-controlling action [43].

Flaxseed oil treatment in rats fed high cholesterol diet significantly decreased serum TNF-α and IL-6 concentrations as compared to untreated high cholesterol -fed rats. The pro-inflammatory cytokines IL-6, IL-1 and TNF-α are central mediators of chronic inflammation associated with atherogenesis [44]. Several studies have shown that a diet supplemented with flaxseed oil (high in alpha-linolenic acid (ALA)) significantly decreased serum IL-1β and TNF-α concentrations and other inflammatory mediators such as prostaglandin E2 and leukotriene B4 [45,46]. Also, Zhao *et al.,* (2007)[47] showed that, a diet high in ALA inhibits the peripheral blood mononuclear cells (PBMCs) production of IL-6, IL-1β, and TNF-α and decreases serum TNF-α concentration. The mechanisms by which dietary fatty acids inhibit

cytokine production are not clear. Induction of cytokine gene expression is regulated by a common pathway, i e, activation of nuclear factor-κB (NF-κB) signaling [48,49]. The presence of NF-κB binding sites has been found in the promoter regions of the IL-6, IL-1, and TNF-α genes [50]. Studies have shown that oleic acid inhibit inflammatory responses within the atherosclerotic lesions by inhibiting NF-κB activity in vascular smooth muscle cells and mononuclear cells [51].

Serum ALT, AST and GGT activities were significantly increased in cholesterol fed rats when compared with rats fed normal control diet. These results are nearly similar to those recorded by Suanarunsawat *et al.,* (2011)[52] and Elmhdwi *et al.,* (2014)[53] who reported that, plasma ALT and AST activities were increased in rats fed high fat diet as compared with normal diet group. High cholesterol diet was reported in several studies to cause hepatotoxicity and fatty liver [54,55]. Also, Alkhamees, (2013)[56], indicated that, high cholesterol diet significantly induced elevation in plasma liver enzymes ALT and AST activities. These parameters are known to be markers for hepatotoxicity. Who added that, histopathological findings revealed several impairments in liver sections from high cholesterol diet supplemented rats by showing moderate degree of fat accumulation, degeneration, fibrosis and inflammatory infiltrates. In the present study, the increase of ALT, AST and GGT activities in blood serum indicate that tissue impairment caused by dyslipidemia may be led to adverse effect by increasing lipid peroxidation which in turn produce damage to liver tissue so outflow of these enzymes from the liver cytosol to the blood stream which indicate that inability of liver to metabolize the ALT and AST [57].

Flaxseed oil treatment in rats fed high cholesterol diet resulted in a significant decrease in serum ALT, AST and GGT activities as compared to untreated cholesterol -fed rats. Liver is the most important organ, which plays a vital role in regulating various physiological processes in the body. It is involved in several vital functions, such as metabolism, secretion and storage. It has great capacity to detoxicate toxic substances and synthesizes useful principles [58]. The parameters such as AST and ALT in blood have been found to be of great importance in the assessment of liver damage [59]. A study on omega-3 suggests that, omega-3 polyunsaturated fatty acid (one component of flaxseed oil) supplementation may decrease liver fat [60]. Possible mechanisms that may be responsible for the protection liver damage by omega-3 fatty acids includes membrane stabilizing action on the hepatocytes [59]. Also, Hatzitolios *et al.,* (2004)[61] reported that, the administration of ώ-3 fatty acids (ώ-3 FA) to the patients with hyperlipidemia reduced the values of AST and ALT activities and prevented the occurrence of fatty liver in 35% of the affected patients. Moreover, Alwayn *et al.,* (2005)[62] recorded that, in a nonalcoholic fatty-liver model, ώ-3 FA administration in murine, receiving high carbohydrate intake, led to reduce AST and ALT activities compared with the group without ώ-3 FA treatment. Similarly, Atakisi *et al.,* (2013)[63] demonstrated that, the activity of ALT was decreased in diethylnitrosamine induced toxicity in rats treated with ώ-3 FA compared with diethylnitrosamine non -treated group.

The current study showed significant increase in serum LDH and CK-MB activities in cholesterol fed rats when compared with rats fed normal control diet. These results are nearly similar to those recorded by Suanarunsawat *et al.,* (2011)[52] who reported that, high cholesterol diet markedly suppressed hepatic and cardiac functions as expressed by an augmentation of serum levels of AST, ALT, LDH and CK-MB activities. Increased activities of these enzymes in serum are indicative of cellular damage, loss of functional integrity, and/ or permeability of cell membrane. Flaxseed oil treatment in rats fed high cholesterol diet resulted in a significant decrease in serum LDH activity after two and sex weeks and in serum CK-MB activity after four and sex weeks of experiment as compared to untreated cholesterol -fed rats. Similarly Yu *et al.,* (2013)[64] reported that, α-Linoleic acid pretreatment significantly inhibited doxorubicin-induced increases of CK-MB and LDH activities, thereby further supporting that ALA has cardioprotective properties. Also, Xie *et al.,* (2011)[65] established that, ALA intake confers cardioprotection in myocardial ischemia/reperfusion by exerting anti-inflammatory and anti-oxidative stress effects in diabetic rats. In addition to these anti-oxidative cardioprotective effects, ALA also has antiapoptotic properties. For instance, pretreatment with ALA has been reported to protect against myocardial cell apoptosis by inhibition of reactive oxygen species (ROS) generation, thereby contributing to reducing myocardial infarction size [66].

A significant increase in liver tissue NO concentration was observed in cholesterol fed rats when compared with rats fed normal control diet. Similar results were previously reported in hypercholesterolemic rats[67], rabbits [68] and might be regarded as a defense mechanism to compensate for continuous inactivation of NO by oxygen-derived free radicals in hypercholesterolemia [69,70]. Another possible explanation for the obtained results is increased inducible nitric oxide synthase (iNOS) activity with cholesterol feeding [71,72]. Flaxseed oil treatment in rats fed high cholesterol diet resulted in a significant decrease in liver NO concentration after two and six weeks of the experiments. In the present study, flaxseed oil significantly reduced NO, and it can be suggested that the active flaxseed peptide fractions may have altered the pathway for NO synthesis in the macrophages. Polyunsaturated fatty acid and α-linolenic acid has shown that the activity of potential therapeutic agents of flaxseed oil is responsible for the inhibition of NO production and subsequent inhibition of iNOS mRNA and protein expressions in macrophage [73,74].

Liver tissue L-MDA concentration was markedly increased in cholesterol fed rats all over the periods of the experiment. In the current work, hypercholesterolemia, a major cause for atherosclerosis was associated with increases in the levels of the lipid peroxidation product L-MDA, and decrease in the level of GSH in Liver tissue suggesting an increase in the levels or activity of oxygen radicals. MDA and GSH have been considered as specific indicators of oxidative stress [75]. L-MDA level can be used as a marker of lipid peroxidation and its measurement gives a direct evidence for LDL oxidation and is leading in predicting free radical-induced injury. Therefore, the observed elevation in tissue L-MDA may be attributed to hyperlipidemia that enhances the processes of lipid peroxidation. Hypercholesterolemia could increase the levels of ROS through stimulation of polymorphonuclear leukocytes (PMNLs) and dysfunction of endothelial cells [12,76]. Flaxseed oil treatment in rats fed high cholesterol diet resulted in a significant decrease in liver L-MDA concentration after two and four weeks of the experiments. Similarly, Prasad, (2000) [77] reported that, oral administration of flaxseed oil reduced lipid peroxidation in rats. The protective activity of flaxseed oil could be attributed to its antioxidant effect. The antioxidant activity of flaxseed oil could be attributed to its high content of omega-3 polyunsaturated fatty acid (PUFA) as reported by Khan *et al.,* (2012) [78] who concluded that, the vegetarians who cannot consume fish oil can have similar health benefits from flaxseed oil which contains omega-3 PUFA. However, Lee and Prasad, (2003) [79] concluded that, flaxseed oil (Omega Nutrition) suppresses oxygen radical production by white blood cells and improves cardiovascular health due to its powerful antioxidant activity.

A non-significant decrease in liver tissue GSH concentration was observed in cholesterol fed rats all over the periods of the experiment when compared with rats fed normal control diet. Intracellular reduction oxidation (redox) state is regulated in part by the presence of GSH[80]. The GSH/GSSG ratio may be sensitive indicator of oxidative stress. Significant decrease in the level of total glutathione observed on HCD feeding might be due to impaired GSH biosynthesis and constant on slaught of ONOO- formed by reactions of O2- and NO, both of which increased in hypercholesterolemia. GSH plays critical role in the detoxification process against reactive nitrogen species e.g. NO, NO2 and ONOO- [81]. Oxidized glutathione (GSSG) is formed by the linking of two tripeptides by disulfide bridge. The generation of GSSG takes place during the oxidation of GSH by glutathione peroxides in the following reaction to maintain the sufficient level of GSH. The increased oxidized glutathione levels in hypercholesterolemic rats can be attributed to spontaneous non-enzymatic GSH oxidation [82]. Flaxseed oil treatment in rats fed high cholesterol diet resulted in a significant decrease in liver tissue GSH concentration after two weeks of the experiment. Glutathione and glutathione-related enzymes play a key role in protecting the cells against the damaging effects of reactive oxygen species. Intracellular GSH can act as a reductant, reducing hydrogen peroxide and lipid hydroperoxides directly to H_2O, a reaction catalyzed by GSH-Px. Depletion of intracellular GSH, under conditions of continuous intracellular oxidative stress, leads to oxidation and damage of lipids, proteins and DNA by the reactive oxygen species[83]. In the present study, the observed decrease in GSH concentration effect flaxseed oil administrations may be due to an increase in the free radical associated with hypercholesterolemia. Hypercholesterolemia induces not only atherosclerosis but also produces a lot of free radicals in blood and tissues [84].

Regarding antioxidants enzymatic activities a significant decrease in liver CAT, SOD and GPX were observed in high cholesterol fed rats when compared with rats fed normal control diet. These results are nearly similar to those recorded by Ahmed *et al.,* (2014) [85] who suggested that, feeding of animal with high cholesterol diet associated with a significant decrease in SOD and GPx activities compared with normal fed diet rabbits. Also, Elmhdwi *et al.,* (2014) [53] reported that, the activities of SOD, GPX and Catalase were significantly decreased in hypercholesterolemic mice (3% cholesterol/ 1% cholic acid) group when compared to normal control one. Oxidative stress is an imbalance between the free radicals production especially reactive oxygen species (ROS) and antioxidants systems and has been implicated in accelerated atherosclerosis [86]. The accumulation of cholesterol in erythrocytes, leukocytes, platelets and endothelial cells can lead to an increase in the concentration of reactive species [87,88], and a reduction in the antioxidant defense systems, such as CAT, GPx and SOD enzyme activities [89]. The decrease in the activities of these enzymes could be attributed to the excessive utilization of these enzymes in inactivating the free radicals generated due to the high cholesterol diet [90]. This condition favors a disruption of the redox balance, contributing to the establishment of oxidative stress, which is involved in several metabolic disorders [91,92]. In the present study, flaxseed oil treatment in rats fed high cholesterol diet associated with increase in liver tissue antioxidants enzymes (CAT, SOD and GPx) activities in most of the experimental durations. Similarly, Makni *et al.,* (2008) [93] reported that, administration of flaxseed oil in obese aged rats elevated the levels of vitamin C and the antioxidant enzymes, indicating the antioxidant potential of the flaxseed oil. Also, Jangale *et al.,* (2013) [94] demonstrated that, flaxseed oil administration increased both hepatic-SOD and CAT activities in addition to hepatic SOD and GPx gene expression in diabetic rats as compared with diabetic non treated group. Superoxide dismutase is the first antioxidant enzyme to deal with oxy-radicals by accelerating the dismutation of superoxide to hydrogen peroxide, while CAT is a peroxisomal heme protein that catalyses the removal of hydrogen peroxide formed during the

reaction catalyzed by SOD. Thus, SOD and CAT acts mutually supportive anti-oxidative enzymes, which provide protective defense against reactive oxygen species [95]. It is commonly accepted that SOD protects against the free radical injury by converting O^{2-} and prevent the formation of OH radicals, and the H_2O_2 can be removed by catalase. The main physiological benefits of flaxseeds are attributed primarily to the high linoleic acid content which contributes to their antioxidant properties [96]. Flaxseed oil might be ameliorating the damage caused by high cholesterol diet in two possible ways: First, flaxseed oil supplementation increases the levels of SOD, catalase and GPx in the liver tissue resulting in enhanced defense against ROS. Second, the constituent omega-3 PUFA of dietary flaxseed oil may have replaced the polyunsaturated fatty acid components of the cell membrane that had been attacked by oxygen free radicals [97,98]. Also, flaxseed oil a major source of omega-3 fatty acids by altering membrane fatty acid composition appeared to affect membrane organization and functions. Flaxseed oil appears to accelerate repair and/or regeneration of injured organelles e.g., mitochondria and lysosomes and plasma membrane by activating endogenous antioxidant defense mechanism [99]. Thus, flaxseed oil provided protection from high cholesterol diet-induced free radical attack. These results suggest that, flaxseed oil may be effective in controlling cholesterolemic status and improving dyslipidemia and has the potential in reducing cardiovascular complications due to hypercholesterolemia.

4. CONCLUSION

This study suggest that flaxseed oil possess antioxidant activity and produces potent antiatherogenic and an effective treatment against hypercholesterolemia. Administration of flaxseed oil significantly reduced lipid peroxidation and have a modulating role in hypercholesterolemia induced oxidative stress as well as it improved the antioxidant defense system in hepatic tissues. Hence, dietary consumption of flaxseed oil may provide benefits for patients with hypercholesterolemia-induced oxidative stress. We recommended that, administration of diet rich in the natural antioxidant is very important for protection of different body tissue, against oxidative stress or hypercholesterolemia and cardiovascular disease and may be beneficial for patients who suffer from hyperlipidemia, hypercholesterolemia and/or arteriosclerosis.

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