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Research Article

THE BENEFECIAL EFFECTS OF FISH OIL SUPPLEMENTATION ON HYPERLIPIDEMIC AND HYPOTHYROID ALBINO RATS

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ABSTRACT

Background: Fish oils (FO) contain the omega-3 fatty acids eicosapentaenoic acid, and docosahexaenoic acid, precursors of certain eicosanoids that offering multiple health benefits.

Objectives: The current study performed to evaluate the effect of FO administration on hyperlipidemic and hypothyroid albino rats.

Methods: Hyperlipidemia was inducted by adding cholesterol powder, cholic acid and animal lard to the standard diet, while hypothyroidism was induced by administration of carbimazole. Hyperlipidemic and hypothyroid rats received FO to rats through gastric intubation. Rats divided into five groups each group contains six rats, and all treatments were performed orally and daily for 6 weeks.

Results: The current results revealed that FO administration was increased significantly thyroid hormones concentrations in the serum of hyperlipidemic and hypothyroid rats as compared to control rats. However, leptin hormone concentration recorded a significant decrease by in both groups. Moreover, FO treatment ameliorated significantly the elevated level of lipid parameters in hyperlipidemic rats while treated hypothyroid rats recorded decreased levels of total lipids and atherogenic index only. Moreover, FO treatment ameliorated the non-enzymatic antioxidant, liver malondialdehyde and glutathione (GSH) concentration and the enzymatic antioxidant, liver GSH-S-transferase and catalase activities, and also the cardiac enzymes lactate dehydrogenase and creatine kinase activities in the treated groups.

Conclusion: FO administration showed a beneficial therapeutic effect on hyperlipidemic and hypothyroid albino rats due to the presence of omega-3 fatty acids, which showed hypolipidemic, antioxidant and hyperthyroid action.

Keywords: Omega 3 fatty acids, Fish oil, Antioxidants, Hyperlipidemia and hypothyroidism.

INTRODUCTION

Fish oil (FO) is oil derived from the tissues of oily fish (e.g. tuna) or from the livers of lean fish (e.g. cod liver) [1]. FO contain omega-3 fatty acids eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), precursors of certain eicosanoids that are known to reduce inflammation throughout the body [2]. Two long-chain n-3 polyunsaturated fatty acids (PUFAs) (EPA and DHA) from FO have been widely recognized due to their beneficial effects on health, and are considered as essential supplements in human food [3]. Extensive research has established that EPA and DHA play a vital role in the prevention of Alzheimer's disease, atherosclerosis, heart attack, angina, stroke, congestive heart failure, depression, and cancer [4].

Furthermore, hyperlipidemia; hypercholesterolemia (HC) and hypertriglyceridemia, remains a formative burden on the health care systems of North America [5]. Hyperlipidemia is a risk factor for vascular diseases such as atherosclerosis and coronary artery diseases [6]. Furthermore, the beneficial effects of omega-3 fatty acids are possibly secondary to their anti-inflammatory, antithrombotic, hypolipidemic and vasodilatory properties [7]. The American Heart Association has acknowledged that EPA and DHA may decrease sudden death, decrease the rate of atherosclerosis and has recommended FO supplementation as a therapeutic strategy to reduce cardiovascular disease (CVD) [8].

Hypothyroidism is associated with cardiovascular risk factors, subclinical CVD, and overt CVD, all of which predispose to atrial fibrillation [9]. N-3 PUFAs present in FO potently decrease serum lipids, which is also has the effect of thyroid hormones (TH). Both PUFAs and TH affect hepatic lipid metabolism. Therefore, long-term diet rich in n-3 PUFAs would enhance TH action in the liver [10]. In addition, n-3 fatty acids are known to exert multiple beneficial effects including anti-inflammatory actions that may diminish oxidative stress [11]. According to the several beneficial effects of omega 3 fatty acids, the main objective of the current study was to evaluate the effect of FO on various biochemical parameters in hyperlipidemia and hypothyroid rats.

METHODS

Experimental animals

White male albino rats (*Rattusnorvegicus*) weighing between 100 g and 120 g were used as experimental animals in the present investigation. They were obtained from the animal house of National Research Institute, El-Giza, Egypt. They were kept under observation for about 15 days before the onset of the experiment to exclude any intercurrent infection. The chosen animals were housed in metal (stainless steel) separate bottom cages at normal atmospheric temperature (25±5°C) as well as under good ventilation and received water and standard balanced diet. All the procedures were performed in accordance with the Institutional Animal Ethics Committee in Beni-Suef University recommendations.

Hyperlipidemic and hypothyroidism agents

Cholesterol used as a hyper-cholesterolemic agent, was purchased from Oxford Laboratory (India), cholic acid sodium salt was purchased from Fluka - Biochemical, (Switzerland) and animal lard was purchased from a market in El-Giza. Otherwise, carbimazole used as hypothyroidismic agent, was purchased from Chemical Industries Development (Egypt).

FO dosage

FO capsules contain 1000 mg FO (13% EPA and 9% DHA) manufactured by SEDICO for pharmaceuticals (Egypt). Hyperlipidemic and

hypothyroid rats received FO in a dose of 0.5 ml/kg b.wt./day [12] to rats through gastric intubation for 6 weeks.

Induction of hyperlipidemia and hypothyroidism

Hyperlipidemia was induced by addition of cholesterol powder, cholic acid and animal lard to the standard diet in percentage of 1%, 0.5% and 5% respectively [13], to rats for about 45 days. On the other hand, hypothyroidism was induced by injection of 30 mg of carbimazole/kg b.wt./day [14] to rats through gastric intubation for 6 weeks.

Animal grouping

There are five groups each group contains six rats:

- Group 1 was regarded as control group and given distilled water by gastric intubation for 6 weeks
- Group 2 was regarded as hyperlipidemic group and given hyperlipidemic agents for 6 weeks
- Group 3 was regarded as hyperlipidemic group treated with FO by gastric intubation for 6 weeks
- Group 4 was regarded as hypothyroid group and given hypothyroid induced agent for 6 weeks
- Group 5 was regarded as hypothyroid group treated with FO by gastric intubation for 6 weeks.

All treatments were performed orally and daily between 8.00 a.m. and 10.00 a.m.

Biochemical studies

Serum TH determination

The quantitative determination of free triiodothyronine and tetraiodothyronine was estimated according to the procedure of [15] using kits purchased from Siemens Healthcare Diagnostics.

Serum leptin hormone concentration

Leptin hormone concentration was determined according to the procedure of enzyme-linked immunosorbent assay kit [16] using kits purchased from RayBiotech, Inc. (USA).

Serum lipid profile determination

Lipid profile parameters were estimated according to; serum total lipids [17], total cholesterol (Tc) [18], triglycerides (TG) [19] and high-density lipoprotein (HDL)-cholesterol [20] by kits purchased from bio-diagnostic, Egypt. In addition, low-density lipoprotein (LDL)-cholesterol was calculated according to the formula [21]. Furthermore, serum very LDL (VLDL) was determined according to formula [22]. While, the atherogenic index was calculated according to formula [23].

Serum cardiac enzymes determination

Lactate dehydrogenase (LDH) activity was determined according to the procedure [24]. While, creatine kinase-NAC (CK) activity was estimated according to the procedure [25] using reagent kits purchased from Centronic GmbH, Germany.

Oxidative stress on liver tissue

Liver lipid peroxidation malondialdehyde (MAD) content was determined according to the procedure [26] and liver glutathione concentration (GSH) was estimated according to the procedure [27]. Moreover, liver GSH-S-transferase activity (GST) was determined according to the procedure [28] while, liver catalase activity (CAT) was determined according to the procedure [29], using reagent kits purchased from bio-diagnostic, Egypt.

Statistical analysis of the results

The Statistical Package for the Social Sciences (IBM SPSS for WINDOWS 7, version 20; SPSS Inc, Chicago) was used for the statistical analysis. Comparative analysis was conducted by using the general linear models procedure (IBM SPSS). $p>0.05$ were considered statistically non-significant, while $p<0.05$ were considered statistically significant.

RESULTS

Serum TH

The present study elucidated a significant decrease in TH (free T3 and free T4) concentrations in serum of hyperlipidemic and hypothyroid rats as compared to control rats. However, the treatment with FO induced significant improvement in both groups as shown in Figs. 1 and 2.

Serum leptin hormone concentration

The serum leptin concentration was significantly increased in the hyperlipidemic, and hypothyroid rats compared to control rats while it improved significantly after FO administration as shown in Fig. 3.

Serum lipid profile

Our data showed that Tc, LDL-cholesterol, TG, VLDL-cholesterol, total lipids and atherogenic index were significantly increased as

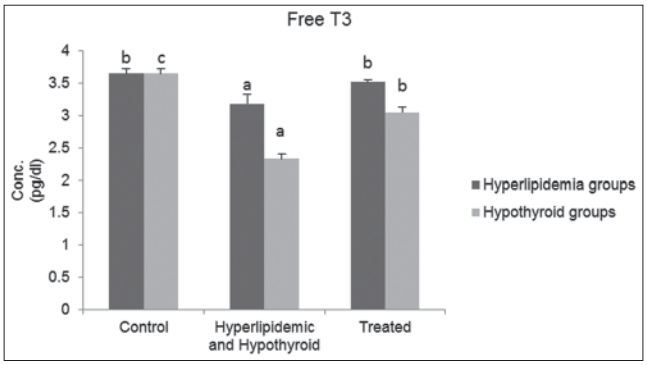

Fig. 1: The effect of fish oil administration on serum free T3 concentration in hyperlipidemic and hypothyroid rats

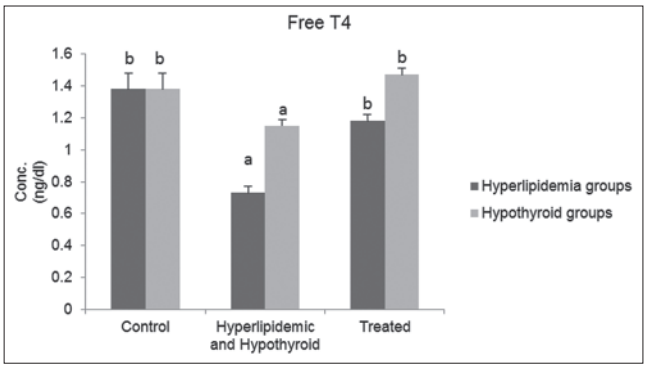

Fig. 2: The effect of fish oil administration on serum Free T4 concentration in hyperlipidemic and hypothyroid rats

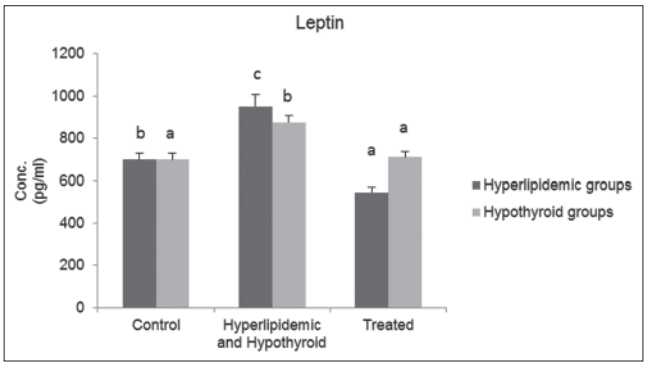

Fig. 3: The effect of fish oil administration on serum leptin concentration in hyperlipidemic and hypothyroid rats

compared to control rats. However, HDL-cholesterol was significantly decreased in hyperlipidemic rats as compared with control rats. On the other hand, the hypothyroid rats exhibited a significant increase in Tc, LDL-cholesterol, total lipids and atherogenic index but HDL-cholesterol was significantly decrease and non- significant change in TG and VLDL-cholesterol were showed as compared to control rats. After FO administration, the hyperlipidemic rats showed a significant improvement in Tc, LDL-cholesterol, VLDL-cholesterol, total lipids and atherogenic index as compared with control rats. Otherwise, the hypothyroid rats recorded, HDL and the atherogenic index only as shown in Tables 1 and 2.

Serum cardiac enzymes

The cardiac enzymes (LDH and CK) activities were significantly increased in the serum of hyperlipidemia and hypothyroid rats as compared to control rats, however after FO supplementation the activities of these enzymes were improved significantly as compared to control rats as shown in Figs. 4 and 5.

Hepatic oxidative stress

Non-enzymatic antioxidant (MAD and GSH) were significantly increased in their contents while, the enzymatic antioxidants (GST and CAT) were decreased its activities in the hepatic tissue of hyperlipidemic rats as compared to control rats. On the other hand, the hypothyroid rats showed a significant decrease in GSH content and GST activity and non-significant change in MDA and CAT in hepatic tissue were observed as compared to control. The FO administration caused significant amelioration in the enzymatic and non enzymatic antioxidant in the both groups as shown in Tables 3 and 4.

Table 1: The serum concentration of lipid profiles of control, hyperlipidemic and hyperlipidemic treated rats with FO

Group	Control	Hyperlipidemic	Treated	LSD
Tc (mg/dl)	63.08±2.95^a	124.83±8.49^b	76±4^a	61.75
HDL (mg/dl)	21.39±2.18^c	8.20±0.29^a	16.57±0.07^b	4.80
LDL (mg/dl)	40.45±1.50^a	111.53±3.36^b	46.13±3.46^a	71.08
TG (mg/dl)	48.33±0.88^a	95.5±4.32^c	65.33±1.80^b	17.00
VLDL (mg/dl)	9.67±0.18^a	19.45±0.79^c	16.4±1.36^b	3.48
Total lipids (mg/dl)	465.67±10.81^a	961±17.58^c	797.67±23.26^b	332
Atherogenic index	2.79±0.68^a	14.41±1.36^c	6.28±0.89^b	3.4

Values significantly different to control at (p≤0.05), Data are expressed as mean±SE, Values which share the same superscript symbol are not significantly different, F-Probability: p<0.05, SE: Standard error, LSD: Least significant difference, HDL: High density lipoprotein, LDL: Low density lipoprotein, VLDL: Very low density lipoprotein, Tc: Total cholesterol, TG: Triglycerides, FO: Fish oil

Table 2: The serum concentration of lipids profiles of control, hypothyroid and hypothyroid treated rats with FO

Group	Control	Hypothyroid	Treated	LSD
Tc (mg/dl)	63.08±2.95^a	83.17±3.76^b	74.33±3.77^b	11.25
HDL (mg/dl)	21.39±2.18^b	14.69±0.32^a	25.45±2.01^b	6.70
LDL (mg/dl)	40.45±1.50^a	62.33±2.37^b	54.37±4.68^b	13.91
TG (mg/dl)	48.33±0.88^a	54.33±2.19^a	51.63±2.42^a	6.00
VLDL (mg/dl)	9.67±0.18^a	10.87±0.44^a	10.33±0.48^a	1.2
Total lipids (mg/dl)	465.67±10.81^a	595.33±20.34^b	449.67±21.37^a	129.67
Atherogenic index	2.79±0.68^a	4.69±0.34^b	2.03±0.30^a	1.90

Values significantly different to control at (p≤0.05), Data are expressed as mean±SE, Values which share the same superscript symbol are not significantly different, F-Probability: p<0.05, SE: Standard error, LSD: Least significant difference, HDL: High density lipoprotein, LDL: Low density lipoprotein, VLDL: Very low density lipoprotein, TC: Total cholesterol, TG: Triglycerides, FO: Fish oil

DISCUSSION

Marine omega-3 PUFAs are essential fatty acids offering multiple health benefits. FO and flaxseed oil are among few dietary sources of these fatty acids [30]. Also, a number of studies showed the efficacy of omega-3 fatty acids in the metabolic syndrome-related conditions [31].

This study shows a significant decrease in free T3 and free T4 hormones concentrations in the serum of hyperlipidemic rats as compared to control rats. The decrement in TH concentrations may be due to a significant reverse relationship between TH and fat level and increase in fat and its residue in liver results in the dysfunction of liver [32]. The hyperlipidemic rats treated with FO in the current study showed significant improvement in TH (free T3 and free T4) level as compared to control rats, which was agree with that of [33].

Moreover, the hypothyroid group, in the current study, showed a significant decrease of free T_3 and free T_4 concentrations as compared to control rats. Our data revealed that the hypothyroid rats treated with FO showed a significant improvement in free T_3 and free T_4 hormones. The recorded result was supported by the finding [34] who reported that thyroid peroxidase activity might, therefore, be stimulated by the consumption of polyunsaturated n-3 USFAs. In addition, stimulating effects of n-3 USFAs have also been observed for other elements of hypothalamic-pituitary-thyroidal axis activity [35]. These data indicated that FO administration could improve the TH action and concentrations of both hyperlipidemic and hypothyroid rats.

Leptin hormone identified and cloned in 1994, is synthesized and secreted specifically from white adipose cells [36]. Leptin has a variety of important central and peripheral actions to regulate energy balance and metabolism, fertility, and bone metabolism that are mediated by specific cell surface leptin receptors [37]. The current study showed a significant elevation in leptin hormone level in the serum of

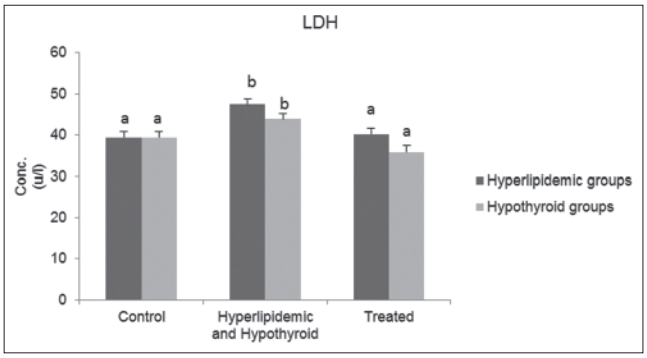

Fig. 4: The effect of fish oil administration on serum lactate dehydrogenase activity in hyperlipidemic and hypothyroid rats

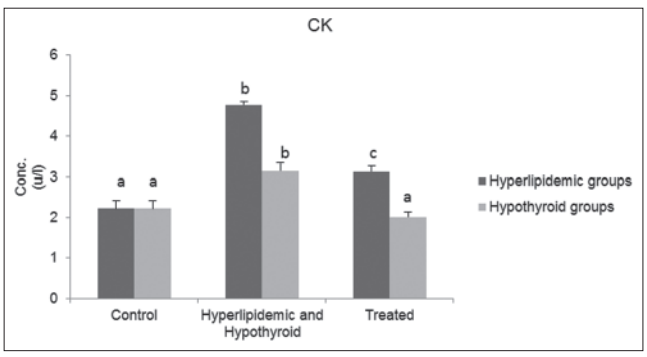

Fig. 5: The effect of fish oil administration on serum creatine kinase activity in hyperlipidemic and hypothyroid rats

Table 3: Hepatic lipid peroxidation (MAD) , GSH and GST concentrations and CAT activity of control, hyperlipidemic and hyperlipidemic treated rats with fish oil.

Group	Control	Hperlipidemic	Treated	LSD
MAD (nmol/g. tissue)	25.60±3.50[a]	42.02±1.08[b]	28.88±1.76[a]	16.47
GSH (mg/g.tissue)	5.08±0.18[a]	9.25±0.38[c]	7.22±0.34[b]	2.13
GST (u/g.tissue)	8.91±0.06[c]	3.74±0.36[a]	4.85±0.18[b]	4.05
CAT (u/g.tissue)	1.98±0.01[b]	1.91±0.02[a]	1.96±0.01[b]	0.07

Values significantly different to control at (p≤0.05), Data are expressed as mean±SE, Values which share the same superscript symbol are not significantly different, F-probability: p<0.05, SD: Standard deviation, LSD: Least significant difference, MAD: Malondialdehyde, GSH: Glutathione, GST: Glutathione-s-transferase, CAT: Catalase

Table 4: Hepatic lipid peroxidation (MAD) ,GSH and GST concentrations and CAT activity of control, hypothyroid and hypothyroid treated rats with fish oil.

Group	Control	Hypothyroid	Treated	LSD
MAD (nmol/g.tissue)	25.60±3.50[a]	21.51±2.08[a]	19.85±1.94[a]	-
GSH (mg/g.tissue)	5.08±0.18[b]	3.53±0.24[a]	5.02±0.28[b]	1.5
GST (u/g.tissue)	8.91±0.06[b]	8.59±0.09[a]	8.83±0.07[b]	0.32
Catalase (u/g.tissue)	1.98±0.01[a]	1.96±0.02[a]	1.95±0.02[a]	-

Values significantly different to control at (p≤0.05), Data are expressed as mean±SE, Values which share the same superscript symbol are not significantly different, F-probability: p<0.05, SD: Standard deviation, LSD: Least significant difference, MAD: Malondialdehyde, GSH: Glutathione, GST: Glutathione-s-transferase, CAT: Catalase

hyperlipidemic rats as compared with control rats and this result was an agreement with the data recorded [38]. Moreover, the hypothyroid rats were recorded significant elevation in leptin level as compared with control rats which in parallel with that of [32]. Furthermore, leptin levels correlate with body fat content [39] and the increase of fat cells in number and in size is coupled with an increase in leptin secretion [40] and this may be the reason of leptin level elevation. In addition, there is a direct relationship between the amount of lipids and leptin hormone concentration [41]. The hyperlipidemic and hypothyroid treated rats, in the present investigation, were shown improvement of leptin levels as result of FO administration, which contain n-3 PUFAS. These data are supported with the finding of [42]. These results may be due to that omega-3 PUFA can down regulate leptin in association with reduced adiposity or up regulate its level in association with increased adiposity [43]. The decreasing level of leptin in hypothyriod treated rats, which treated by FO may be due to there is a direct negative relationship between the level of fat and leptin, and also there is a significant negative relationship between T3 and leptin [32] and the good relationship between fat, leptin and TH [44]. Therefore, it may be speculated that stimulation of peroxisome proliferator-activated receptor (PPARγ) is one of the mechanisms by which omega-3 PUFA mediate their insulin sensitizing, lipid lowering, and antiinflammatory properties [42].

High blood cholesterol (hypercholesterolaemia) is a risk factor for both fatal and non-fatal CVD events in people with and without a past CVD, and lowering cholesterol, in particular LDL cholesterol, is an important target for pharmacotherapy [45]. The present results elucidated a significant increase in Tc, total lipids, TG, LDL-cholesterol, VLDL-cholesterol concentrations and atherogenic index in the serum of hyperlipidemic rats as compared to control rats. This result runs parallel with the data of [46]. However, HDL-cholesterol concentration showed a significant decrease in the serum of hyperlipidemic rats compared to control rats which are in accordance with that of [47]. However, after FO administration the lipid profile in hyperlipidemic treated group showed significant improvement of Tc, LDL-cholesterol, TG, VLDL-cholesterol and total lipids concentrations which are in agreement with the study of [30]. In addition, FO showed a hypolipidemic effect and act predominantly through the modulation of the transcription of hepatic metabolism genes [48]. The hypolipidemic effect of FO has been explained via PPAR-α [49] and other hepatic transcriptional factors such as sterol regulatory element-binding protein [50] and retinoic X receptor [51].

On the other hand, the results of lipid profile in hypothyroid group exhibited a significant increase of Tc, total lipids, LDL-cholesterol levels and atherogenic index in the serum of hypothyroid rats as compared to control rats. These results are agreed with the investigation of [52]. This may be due to the decreased LDL-receptors activity, resulting in decreased catabolism of LDL [53]. Moreover, TH are potent modulators of lipid metabolism [54], and hypothyroidism is associated with higher serum lipids [55]. On the other hand, treatment of hypothyroid rats with FO showed an amelioration of total lipids concentrations and HDL-cholesterol and atherogenic index, but non-significant effect on Tc, LDL-cholesterol, triglycerides and VLDL-cholesterol concentrations was recorded. Furthermore, [56] reported that most of the hypolipidemic effects of TH are due to their action in the liver via the TH receptor (TR). Moreover, the interaction of triiodothyronine (T3) with TR promotes the recruitment of cofactors, resulting in regulation of the transcriptional activity of genes encoding key enzymes, and other factors, involved in lipid metabolism. Otherwise, many studies have shown a clear link between hypothyroidism and HC mediated through decreased production of the LDL cholesterol receptor [57].

Cardiac marker enzymes are measured to evaluate the heart function. The diagnosis of acute myocardial infarction can be achieved by electrocardiogram changes and elevation of cardiac marker enzymes like CK, LDH [58]. The current study showed that a significant elevation in serum LDH and CK activities of the hyperlipidemic rats as compared with control rats, which are agree with that of [59]. The activities of these enzymes after FO administration were improved significantly, which are an agreement with that of [60]. The author attributed the effects of FO treatment to the hypolipidemic action. Furthermore, the cardiac marker enzymes (LDH and CK) in the hypothyroid group were increased significantly as compared with control rats, which are an agreement with that [61]. These enzymes (LDH and CK) showed a negative correlation with T3 and T4 levels [62]. Hence, our results in the hypothyroid treated rats with FO observed that serum LDH and CK activities were decreased by FO administration and this result may be due to the improvement of thyroid function and the hypolipidemic effect of FO.

Antioxidants are substances that either directly or indirectly protects cells against adverse effects of xenobiotics, drugs, carcinogens and toxic radical reactions [63]. Oxidative stress contributes to the development of atherosclerosis in the vascular wall through the formation of reactive oxygen species (ROS) [64]. This study in liver tissue of hyperlipidemic rats recorded a significant increase in GSH reduced content GSH and lipid peroxidation (MDA) level, and a significant decrease in GST and CAT activities, which were agreement with the finding of [65]. Concerning the present study, the hepatic GSH and CAT activities and hepatic GST and lipid peroxidation contents were ameliorated significantly after FO administration in the hyperlipidemic treated rats. The improvement in hepatic oxidative stress after FO administration may be due the hepatoprotective effect of omega 3 fatty acids, which found in FO [30]. In addition, [66] reported that Omega-3 fatty acid supplementation leads to a significantly lower level of MDA compared to the control group, which had good clinically relevant aspects. The authors suggested that mechanisms for the decrease in MDA may relate to the assembly of omega-3 fatty acids in membrane lipids and lipoproteins making the double bonds less available for free radical attack, inhibition of the pro-oxidant enzyme phospholipase A2 and stimulation of anti-oxidant enzymes. In this regard, [67] reported that omega-3 fatty acids up regulate gene expressions of antioxidant enzymes and down regulate genes associated with production of ROS.

On the other hand, the hypothyroid rats in the current study showed a significant decrease of hepatic GSH activity and GST concentration

while, non-significant change of hepatic CAT activity and lipid peroxidation content were recorded as compared to control rats. These data are in parallel with that [68]. Furthermore, there is no statistically significant difference found between hypothyroid and control groups in the lipid peroxidation indicator MDA [69]. The present study showed that omega 3 fatty acids in FO induced hepatoprotective effect against oxidative stress, which may be due to its hypolipidemic, hyperthyroid and anti-oxidative effects.

CONCLUSION

The above results confirmed the benefit therapeutic effect of FO administration on hyperlipidemic and hypothyroid albino rats due to the presence of omega-3 fatty acids, which showed hypolipidemic, antioxidant and hyperthyroid effects. Additionally, the results elucidated a positive relationship between the hypothyroidism and hyperlipidemia.

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