Hypolipidemic Efficacy of Omega-3 Fatty Acids in Comparison with Rosuvastatin in Induced Hyperlipidemic Albino Rats

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ABSTRACT

There is evidence that omega-3 fatty acids, i.e. eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), found in oil of fish, can help prevent and treat atherosclerosis by averting the development of plaque and blood clotting. This study was conducted to evaluate the effect of omega-3 fatty acids and rosuvastatin on lipid profile, liver function enzymes, renal function tests, hematological parameters, blood glucose, malondialdehyde (MDA), and superoxide dismutase (SOD) in hyperlipidemic rats. In the current study, 32 male albino rats, aging between (8-10) weeks and weighing (200–250 g) were randomly selected. The rats were housed under highly standard laboratory conditions exposed to a photoperiod of 12:12h light: dark and maintained at 22±2 ℃. The rats were divided into 4 groups, each of which contained eight individuals. The first group received a standard diet throughout the experimental period. The second group represented the model, as the laboratory rats were given chow (5% cholesterol+0.5% cholic acid) for six consecutive weeks. The third group received omega-3 oil at a concentration of 35 mg/kg daily. The fourth group received a daily dose of rosuvastatin (10 mg/kg). The results revealed that after six weeks of therapy, omega-3 fatty acids significantly decreased serum total cholesterol (TC), serum low-density lipoprotein cholesterol (LDL-C), and triglyceride (TG) when compared to hyperlipidemic rats. In addition, daily administration of rosuvastatin (10 mg/kg) for six weeks significantly attenuated serum TG, LDL-C, and TC levels when compared to hyperlipidemic rats.

Key Words: Hyperlipidemia, omega-3, Rosuvastatin, lipid profile.

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INTRODUCTION

Hyperlipidemia is the abnormal level of lipids in the body, which may have familial or genetic origins. It can be due to endocrine, hepatic, or renal illnesses. Primary hyperlipidemia consists of familial or polygenic hypercholesterolemia, familial combined hyperlipidemia, dysbetalipoproteinemia, and familial hypertriglyceridemia [1]. Certain clinical disorders are associated with abnormal lipid metabolisms such as cardiac disease, diabetes, obesity, and associated disorders like atherosclerosis [2].

Atherosclerosis is a disease in which the artery wall thickens due to the build-up of fatty materials such as triglyceride and cholesterol. Atherosclerosis is started by an inflammatory process in the endothelial cells of the vessel wall. It is done because of their action to retained low-density lipoprotein (LDL) molecules. It is mainly caused by the buildup of white blood cells and macrophages and raised by LDL, the plasma proteins that carry cholesterol and triglyceride) while there is no sufficient removal of cholesterol and fats from the macrophage by functional high-density lipoprotein (HDL) [3]. There are some natural products that are used as a treatment for hyperlipidemia such as flavonoids and alkaloids. These products can also be used for both primary and secondary preventions of cardiovascular diseases [4].

Omega-3 fatty acids from the end of the carbon chain have a double bond (C=C) in the third carbon atom and are polyunsaturated fatty acids. They are important for normal metabolism [5]. The three types of omega-3 fatty acids presented in the human body are ALA (α-linolenic acid, 18 carbons, and 3 double bonds), which are also found in plant oils such as EPA (eicosapentaenoic acid, 5 double

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bonds, and 20 carbons), and DHA (docosahexaenoic acid, 6 double bonds, and 22 carbons), both of which are also found in marine fish oils. Mammals have a limited ability in the synthesis of omega-3 fats when the diet includes shorter-chain omega-3 fatty acids such as ALA to form EPA, as the more essential long-chain omega-3 fatty acids, the most important, DHA, which has a greater inefficiency [6].

Omega-3 helps prevent heart disease, leads to lower blood pressure, and reduces the level of triglycerides (fats) in the blood. People with heart diseases or those who need lower triglycerides may need to take fish oil supplements and are characteristic of subjects who exhibit a lipid phenotype typical of combined hyperlipidemia. The evidence associating the consumption of fish with the risk of cancer is poor [7]. There is good evidence that omega-3 fatty acids (namely DHA and EPA) found in fish oil can help to treat and prevent atherosclerosis. Omega-3 fatty acids stop the growth of blood clots and plaques. In addition, they can help prevent heart disease, lower blood pressure, and reduce the level of TG (fats) in the blood. People with heart disease or those who need to lower triglycerides may need to take fish oil supplements. They are representative of subjects who show a lipid phenotype typical of combined hyperlipidemia [8].

The effect of omega-3 fatty acids in reducing cholesterol and triglyceride levels has been proven. Also, omega-3 fatty acids have shown a significant reduction in the levels of TG, LDL-C, and TC in treated hyperlipidemic rats [9]. There is some evidence that shows that people with certain circulatory problems, such as varicose veins, may benefit from the intake of DHA and EPA. This may stimulate blood circulation, increase the fibrin breakdown, a compound involved in scar and clot formation, and also, may decrease the pressure of the blood in the circulatory system [10]. Omega-3 fatty acids reduce triglyceride levels in the blood [9] and the regular intake may decrease the risk of the primary and secondary heart attacks. Studies have proven that individuals who consume large amounts of omega-3 fatty acids tend to have increased HDL-C levels, and decreased negative triglyceride material that circulates in their bloodstream. Research shows that omega-3 fatty acids reduce LDL-C and TG levels [11-17].

MATERIALS AND METHODS

Animal and housing

32 male albino rats were used in this study. All rats were 8-10 weeks of age and weighing about 200-250 grams. The experiment was carried out from 25 July 2014 to 2 February 2015. Animals were housed and bred in the animal house of the Department of Biology, College of Education, Salahaddin University-Erbil. The animals were housed under laboratory conditions at 22±2 ºC and the light schedule for them was 12 hours of light and 12 hours of dark [17]. At the beginning of the experiment, the animals received standard rat tap water and pellets *ad libitum*. The standard pellet had the following ingredients: 66.6% wheat, 25.6% soy, 4.4% sunflower oil, 1.5% limestone, 0.63% salt, 0.15% methionine, 0.06% choline chloride, and 0.05% trace elements.

Induction of Hypercholesterolemia

Hypercholesterolemia was induced by adding 5% cholesterol and 0.5% cholic acid diet to the standard rat pellets [18]. The time required for the induction of hypercholesterolemia in rats was determined by evaluating their serum cholesterol level while they were fed a cholesterol-enriched diet and tap water *ad libitum* for 30-60 days. Rats were starved for 24h, before cholesterol measurement and then serum cholesterol levels were enzymatically determined with diagnostic kits. Cholic acid 0.5% was included since it increases micelle formation and helps intestinal absorption of cholesterol [19].

Experimental Design

In this study, the experiments were designed as the following:

Group 1 (Non-hypercholesterolemic rats, control group): This group included 7 rats that received a standard diet during the experiment.

Group 2 (Hypercholesterolemic rats, Model group): The rats of this group represented the model, as the laboratory rats they were given chow (5% cholesterol+0.5% cholic acid) for six weeks.

Group 3 (Hypercholesterolemic rats + Omega-3): This group received daily omega-3 oil at the concentration of 35 mg/kg body weight.

Group 4 (Hypercholesterolemic rats + Rosuvastatin): This group received a daily dose of rosuvastatin (10 mg/kg). The rosuvastatin solution (10 mg/kg) [20] was freshly prepared in normal saline and fed daily to animals by oral gavage.

At the end of treatment time, the animals were exposed to different biochemical and hematological parameters. They were not given any food during one night and the following day blood samples were collected. The procedure was done by anesthetizing rats by a combination of 5 mg/kg xylazine and 35 mg/kg ketamine [21]. It was followed by cardiac puncture using a sterile disposable plastic syringe, which was then put into a specified numerically labeled blood tubes (normal tubes for serum and containing EDTA tubes for hematological tests) and centrifuged at 3000rpm for 15min. During the experimental period, the body weight of rats was individually recorded for each rat before and after treatment. Total cholesterol, triglycerides, high-density lipoprotein, low-density lipoprotein, alkaline phosphatase, aspartate aminotransferase enzyme, alanine aminotransferase enzyme, bilirubin, blood urea, serum creatinine, serum glucose, creatine kinase, lactate dehydrogenase, and α-amylase were determined by Cobas c111 analyzer.

In the presence of acid and heat, a pink-colored end-product is produced with the reaction of MDA and TBA. The color intensity at 532nm corresponds to the peroxidation level of lipids in the sample. The assessment of the lipid peroxidation process has been achieved via the determination of the end product, malondialdehyde. The serum MDA level was spectrophotometrically determined with a thiobarbituric acid solution.

According to the kit, in order to assess the Rat SOD-1 level in the sample, Purified Rat SOD-1 antibody was used to coat microtiter wells, the solid-phase antibody was made, and then SOD-1 was added to each well. SOD-1 was combined with the antibody, which was labeled with HRP, and the antibody-antigen enzyme-antibody complex was made. After completely washing, TMB substrate solution was added; this substrate becomes blue in the presence of HRP enzyme-catalyzed. The reaction was completed by the addition of sulfuric acid solution and the change in color was measured spectrophotometrically at 450 nm. Then, the OD of the samples was compared with the standard curve, thus determining the concentration of Rat SOD-1 in the samples. Red blood cells (RBCs) count, hemoglobin (Hb) concentration, packed cell volume (PCV), white blood cells (WBCs) count, platelets (PLTs) count, mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH), and mean platelet volume (MPV) were determined automatically by automated hematology analyzer (System model: K-1000, Japan).

Statistical Analysis

All data are expressed as ± standard error of the means. Statistical analysis was done using statistically available software (SPSS Version 19). Statistical differences were determined by Duncan test for multiple comparisons after ANOVA. $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Effect of Omega-3 and Rosuvastatin on Serum Lipid Profiles in Hyperlipidemic Rats

- **Total Serum Cholesterol**

Serum total cholesterol was significantly ($p<0.01$) increased (134.86±9.83 mg/dl) in hyperlipidemic rats compared to the control group (61.42±3.18 mg/dl). Also, in the treatment of hyperlipidemic rats, omega-3 fatty acids (55.00±4.22 mg/dl) and rosuvastatin (46.28±1.52 mg/dl) showed improvement in serum total cholesterol levels. These changes were statistically significant ($p<0.01$) in comparison to the hypercholesterolemic group (Table 1).

- **Serum Triglyceride**

As illustrated in Table 1, hyperlipidemic rats were associated with significantly elevated ($p<0.01$) levels of serum triglyceride (164.86±8.37 mg/dl) in comparison to the control group (57.00±2.51 mg/dl). This elevation significantly decreased with the treatment of hyperlipidemic rats with omega-3 fatty acids (50.28±5.72 mg/dl) and rosuvastatin (33.86±3.65 mg/dl).

- **Serum HDL-C**

As shown in Table 1, there was no significant difference between hyperlipidemic rats and treated groups on serum HDL levels.

- **Serum VLDL**

As shown in Table 1, hyperlipidemic rats were associated with significantly elevated ($p<0.01$) levels of serum VLDL (32.97±1.67 mg/dl) compared to the control group (11.40±0.50 mg/dl). This elevation decreased significantly by treatment of hyperlipidemic rats with omega-3 fatty acids (10.06±1.14 mg/dl) and rosuvastatin (6.77±0.73 mg/dl).

- **Serum LDL**

As illustrated in Table 1, hyperlipidemic rats were associated with significantly ($p<0.05$) elevated levels of serum LDL-cholesterol (23.22±1.80 mg/dl) in comparison to the control rats (9.14±0.40 mg/dl). The present results also showed that the treatments of hyperlipidemic rats with omega-3 and rosuvastatin caused a significant ($p<0.05$) decrease in serum LDL 14.71±1.52 mg/dl and 12.57±1.17 mg/dl, respectively when compared with the untreated hyperlipidemic groups (23.22±1.80 mg/dl).

Table 1: Effects of Omega-3 and Rosuvastatin on Serum Lipid Profiles in Hyperlipidemic Rats

Groups	Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	VLDL-C (mg/dl)
G1 (Control)	$61.42±3.18^{bc}$	$57.00±2.51^{c}$	$22.54±2.14^{a}$	$9.14±0.40^{c}$	$11.40±0.50^{c}$
G2 (Hyperlipidemic)	$134.86±9.83^{a}$	$164.86±8.37^{a}$	$25.33±1.81^{a}$	$23.22±1.80^{a}$	$32.97±1.67^{a}$
G3 (Omega-3 fatty acids)	$55.00±4..22^{c}$	$50.28±5.72^{c}$	$27.42±2.37^{a}$	$14.71±1.52^{b}$	$10.06±1.14^{c}$
G4 (Rosuvastatin)	$46.28±1.52^{c}$	$33.86±3.65^{c}$	$23.29±1.41^{a}$	$12.57±1.17^{bc}$	$6.77±0.73^{c}$

The same letters within columns mean no significant differences and the different letters mean significant differences

Effects of Omega-3 and Rosuvastatin on Liver Function in Hyperlipidemic Rats

- **Serum ALT**

The obtained results showed that there were no significant differences in serum ALT between the control group (42.15±3.09 IU/L) and hyperlipidemic rats (57.98±6.01

IU/L). However, omega-3 (39.38±3.36 IU/L) was associated with significantly decreased ($p<0.05$) levels of serum ALT in comparison to the untreated hyperlipidemic rats. But rosuvastatin (52.03±3.41 IU/L) treated group decreased in ALT levels non-significantly when compared with hyperlipidemic rats (Table 2).

- **Serum AST**

The present results showed that serum AST levels in the hyperlipidemic group (154.30±10.45 IU/L) were significantly different when compared to the control group rats (139.75±7.11 IU/L) in serum AST. Furthermore, the treatment effects of hyperlipidemic rats with rosuvastatin (151.08±7.78 IU/L) were not significant in comparison to untreated hyperlipidemic rats. Whereas, omega-3 (134.28±7.13 IU/L) significantly decreased serum levels of AST in hyperlipidemic rats (Table 2).

- **Serum ALP**

The result showed that serum ALP levels of hyperlipidemic rats (605.89±60.67 IU/L) increased significantly when compared to the control group (412.03±43.33 IU/L). However, hyperlipidemic rats treated with omega-3 (401.76±32.65 IU/L) and rosuvastatin (689.00±63.54 IU/L) were associated with a significant decrease in comparison to the untreated hyperlipidemic rats (Table 2).

- **Total Serum Bilirubin**

A significant difference in total serum bilirubin was observed between the hyperlipidemic group (0.22±0.05 mg/dl) and the control group (0.07±0.01 mg/dl). Whereas, treatment of hyperlipidemic rats with Omega-3 (0.09±0.01 mg/dl) and rosuvastatin (0.17±0.09 mg/dl) caused a significant decrease ($p<0.01$) when compared to untreated hyperlipidemic rats (Table 2).

Table 2: Effects of Omega-3 and Rosuvastatin on Liver Function Tests in Hyperlipidemic Rats

Groups	GPT/ALT (IU/L)	GOT/AST (IU/L)	ALP (IU/L)	T.S.B (mg/dl)
G1 (Control)	42.15±3.09^{ab}	139.75±7.11^{b}	412.03±43.33^{b}	0.07±0.01^{b}
G2 (Hyperlipidemic)	57.98±6.01^{a}	154.30±10.45^{a}	605.89±60.67^{a}	0.22±0.05^{a}
G3 (Omega-3 fatty acids)	39.38±3.36^{b}	134.28±7.13^{b}	401.76±32.65^{b}	0.09±0.01^{b}
G4 (Rosuvastatin)	52.03±3.41^{ab}	151.08±7.78^{ab}	689.00±63.54^{b}	0.17±0.04^{b}

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Effects of Omega-3 and Rosuvastatin on Blood Urea, Serum Creatinin, CPK, LDH-P, and GGT Tests in Hyperlipidemic Rats

Table 3 shows that the serum levels of creatinine, LDH-P, and GGT did not change significantly in rats treated with omega-3 and rosuvastatin and hyperlipidemic rats when compared to the control group. However, hyperlipidemic rats (46.77±1.39 mg/dl) showed a significant change in the mean value of blood urea when compared to the control group (28.71±2.28 mg/dl). Whereas, the treatment of hyperlipidemic rats with omega-3 (35.47±0.82 mg/dl) showed a significant change in blood urea when compared to the control group. But the treatment of hyperlipidemic rats with rosuvastatin did not significantly affect blood urea when compared to untreated rats. As shown in Table 3, the hyperlipidemic group resulted in a significant elevation ($p<0.01$) of CPK when compared to the control group. This elevation was significantly lowered ($p<0.01$) with the treatment of hyperlipidemic rats with rosuvastatin (281.57±29.35 U/L). Whereas, omega-3 (361.57±18.42 U/L) did not cause significant difference when compared to untreated hyperlipidemic rats.

Table 3: Effects of Omega-3 and Rosuvastatin on Blood Urea, Serum Creatinine, CPK, LDH-P, and GGT Tests in Hyperlipidemic Rats

Groups	Blood Urea (mg/dl)	S. Creatinine (IU/L)	CPK (U/L)	LDH-P (U/L)	GGT (U/L)
G1 (Control)	28.71±2.28^{c}	0.47±0.02^{b}	249.85±33.17^{b}	358.71±34.54^{a}	0.142±0.01^{a}
G2 (Hyperlipidemic)	46.77±1.39^{a}	0.53±0.02^{ab}	436.00±50.69^{a}	444.57±59.35^{a}	0.571±0.02^{a}
G3 (Omega-3 fatty acids)	35.47±0.82^{bc}	0.43±0.01^{b}	361.57±18.42^{ab}	370.00±39.21^{a}	0.146±0.01^{a}
G4 (Rosuvastatin)	41.41±1.00^{ab}	0.57±0.01^{ab}	281.57±29.35^{b}	293.57±29.56^{a}	0.134±0.01^{a}

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Effects of Omega-3 and Rosuvastatin on Total Protein, Serum Albumin and Serum Globulin Tests in Hyperlipidemic Rats

- **Total Protein**

The present results showed that serum levels of the total protein in the hyperlipidemic group decreased significantly (5.44±0.13 g/dl) when compared to the control group (6.18±0.22 g/dl). Furthermore, the treatment effect of hyperlipidemic rats with omega-3 (5.56±0.14 g/dl) and rosuvastatin (5.75±0.14 g/dl) was not significant when compared to untreated hyperlipidemic rats (Table 4).

- **Serum Albumin**

The obtained results showed that there were no significant differences in serum albumin between the control group

(3.94±0.06 g/dl) and hyperlipidemic rats (3.51±0.09 g/dl). Rosuvastatin (3.33±0.08 g/dl) and omega-3 (3.58±0.15 g/dl) treated groups did not have any significant relationship when compared to hyperlipidemic rats (Table 4).

- **Serum Globulin**

As shown in Table 4.4, there were no significant differences in serum globulin between untreated and treated rats.

Table 4: Effects of Omega-3 and Rosuvastatin on Total Protein, Serum Albumin and Serum Globulin Tests in Hyperlipidemic Rats

Groups	Total Protein (g/dl)	S. Albumin (g/dl)	S. Globulin (g/dl)
G1 (Control)	6.18±0.09^{a}	3.94±0.06^{ab}	2.25±0.07^{ab}
G2 (Hyperlipidemic)	5.44±0.13^{b}	3.51±0.09^{b}	1.92±0.11^{ab}
G3 (Omega-3 fatty acids)	5.56±0.22^{ab}	3.58±0.15^{b}	1.98±0.13^{ab}
G4 (Rosuvastatin)	5.75±0.14^{ab}	3.33±0.08^{b}	2.41±0.10^{a}

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Effects of Omega-3 and Rosuvastatin on Blood Sugar, Amylase and Serum Uric Acid in Hyperlipidemic Rats

- **Serum Blood Glucose**

In the present study, hyperlipidemic rats (192.42±7.99 mg/dl) caused a significant difference in serum blood glucose when compared to the control rats (122.14±3.15 mg/dl). Furthermore, the treatment of hyperlipidemic rats with omega-3 (186.14±13.85 mg/dl) and rosuvastatin (159.15±19.29 mg/dl) for six weeks did not produce any alteration in serum blood glucose when compared to untreated hyperlipidemic rats (Table 5).

- **Serum Uric Acid**

As shown in Table 5, a significant difference was observed between hyperlipidemic rats (4.01±0.19 mg/dl) and the control group (2.14±0.18 mg/dl) in serum uric acid. Also, there were no statistical differences in serum uric acid when hyperlipidemic rats were treated with omega-3 (2.54±0.20 mg/dl) and rosuvastatin (3.17±0.27 mg/dl).

- **Serum Amylase**

In the present study, hyperlipidemic rats (866.94±11.66 su/dl) showed a significant difference in serum amylase when compared to control rats (478.08±71.33 su/dl). Whereas, the treatment of hyperlipidemic rats with omega-3 (794.36±65.76 su/dl) and rosuvastatin (685.80±58.68 su/dl) for six weeks did not produce any alteration in serum amylase when compared to untreated hyperlipidemic rats (Table 5).

Table 5: Effects of Omega-3 and Rosuvastatin on Blood Sugar, Amylase, and Serum Uric Acid in Hyperlipidemic Rats

Groups	Blood Glucose (mg/dl)	S. Uric Acid (mg/dl)	Amylase (su/dl)
G1 (Control)	122.14±3.15^{b}	2.14±0.18^{b}	478.08±71.33^{b}
G2 (Hyperlipidemic)	192.42±7.99^{a}	4.01±0.19^{a}	866.94±11.66^{a}
G3 (Omega-3 fatty acids)	186.14±13.85^{a}	2.54±0.20^{a}	794.36±65.76^{a}
G4 (Rosuvastatin)	159.15±19.29^{ab}	3.17±0.27^{a}	685.80±58.68^{ab}

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Effects of Omega-3 and Rosuvastatin on Serum MDA and Serum SOD in Hyperlipidemic Rats

- **Serum MDA**

The present results showed serum MDA levels in the hyperlipidemic group (6.18±0.09 µmol/L). There was a significant relationship when compared to the control group rats (3.28±0.19 µmol/L). Furthermore, treatment of hyperlipidemic rats with omega-3 (3.40±0.17µmol/L) and rosuvastatin (3.87±0.14µmol/L) produced a significant change when compared to untreated hyperlipidemic rats (Table 6).

- **Serum SOD**

A significant difference was observed in SOD between the hyperlipidemic group (4.01±0.19 U/ml), control group (2.14±0.18 mg/dl), and omega-3 (2.54±0.20 U/ml). Furthermore, the hyperlipidemic group, which was treated with rosuvastatin (3.17±0.27 U/ml) showed no significant changes in SOD when compared to the model group rats (Table 6).

Table 6: Effects of Omega-3 and Rosuvastatin on MDA and SOD in Hyperlipidemic Rats

Groups	MDA (µmol/L)	SOD (U/ml)
G1 (Control)	3.28±0.19^{b}	2.14±0.18^{b}
G2 (Hyperlipidemic)	5.64±0.30^{a}	4.01±0.19^{a}
G3 (Omega-3 fatty acids)	3.40±0.17^{b}	2.54±0.20^{b}
G4 (Rosuvastatin)	3.87±0.14^{b}	3.17±0.27^{a}

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Effects of Omega-3 and Rosuvastatin on RBCs, Hb, PCV, and PLTs in Hyperlipidemic Rats

- **Red Blood Cell Count (RBC)**

Cholesterol and cholic acid diet given to the animals for six weeks insignificantly changed RBC count (6.63±0.17 x106/µL) when compared to the control group (6.87±0.17x106/µL). However, omega-3 (7.49±0.10 x106/µL) treatments produced a significant change. However, rosuvastatin (7.11±0.05 x106/µL) did not lead to a significant change in the mean value of RBC count (Table 7).

- **Hemoglobin concentration (Hb)**

As shown in Table 7, hyperlipidemic rats did not significantly alter hemoglobin concentration (11.85±0.28 g/dl) when compared to the control group (12.80±0.25 g/dl). Additionally, treatments of hyperlipidemic rats with omega-3 (14.01±0.19 g/dl) showed a significant alteration in comparison to untreated hypercholesterolemic rats. Whereas, hyperlipidemic rats, which were treated with rosuvastatin (12.85±0.29 g/dl) did not show any significant change in the mean value of hemoglobin concentration.

- **Packed Cell Volume (PCV)**

Hyperlipidemia in rats (36.40±1.25 %) significantly changed the mean value of PCV when compared to the control group (40.38±0.94 %). However, omega-3 (44.28±0.63 %) and rosuvastatin (40.57±0.76 %) administration to hyperlipidemic rats produced a significant increase ($p<0.05$) in PCV value in comparison to hyperlipidemic rats (Table 7).

- **Platelet Count (PLTs)**

The results showed that PLTs did not have a significant change in hyperlipidemic rats when compared to the control group. In addition, the hyperlipidemic group treated with omega-3 and rosuvastatin did not cause any significant differences in PLTs (Table 7).

Table 7: Effects of Omega-3 and Rosuvastatin on Rbcs, Hb, PCV, and PLTs in Hyperlipidemic Rats

Groups	RBCs (x10^{6}/µL)	Hb (g/dl)	PCV (%)	PLTs (x10^{3}/µL)
G1 (Control)	6.87±0.17^{bc}	12.80±0.25^{b}	40.38±0.94^{b}	455.42±21.57^{a}
G2 (Hyperlipidemic)	6.63±0.17^{c}	11.85±0.28^{b}	36.40±1.25^{c}	469.28±24.42^{a}
G3 (Omega-3 fatty acids)	7.49±0.10^{a}	14.01±0.19^{a}	44.28±0.63^{a}	439.00±14.13^{a}
G4 (Rosuvastatin)	7.11±0.05^{abc}	12.85±0.29^{b}	40.57±0.76^{b}	465.14±27.65^{a}

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Effects of Omega-3 and Rosuvastatin on MCHC, MCH, MCV, and MPV in Hyperlipidemic Rats

The results showed that MCV, MCH, and MPV did not have a significant change in hyperlipidemic rats compared to the control group. Furthermore, the hyperlipidemic group treated with omega-3 and rosuvastatin also did not show any significant change in MCV, MCH, and MPV (Table 8). As shown in Table 8, hyperlipidemia in rats significantly changed MCHC (6.14±0.30 g/dl) when compared to the control group (5.15±0.24 g/dl). However, significant differences were observed in hyperlipidemic rats treated with omega-3 (4.31±0.12 g/dl) and rosuvastatin (4.93±0.12 g/dl) in MCHC in comparison to the untreated hyperlipidemic group.

Table 8: Effects of Omega-3 and Rosuvastatin on MCV, MCH, MCHC, and MPV in Hyperlipidemic Rats

Groups	MCV (fl)	MCH (Pg)	MCHC (g/dl)	MPV (Pg)
G1 (Control)	18.58±0.16^{b}	31.74±0.30^{a}	5.15±0.24^{b}	58.65±0.62^{a}
G2 (Hyperlipidemic)	19.30±0.11^{a}	31.90±0.15^{a}	6.14±0.30^{a}	60.52±0.17^{a}
G3 (Omega-3 fatty acids)	19.01±0.13^{a}	31.35±0.20^{a}	4.31±0.12^{cd}	58.91±0.69^{a}
G4 (Rosuvastatin)	18.40±0.42^{a}	31.61±0.15^{a}	4.93±0.12^{bc}	57.82±1.11^{a}

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DISCUSSION

Effect of Omega-3 Fatty Acids on Hyperlipidemic Rats

Accompanying increased levels of circulating cholesterol-rich LDL and triglyceride-rich VLDL is identified when it is associated with an increased risk of premature coronary artery disease [22] and it belongs typically to subjects who exhibit a lipid phenotype, which is the typical of combined hyperlipidemia [23]. In this investigation, serum TG significantly decreased in hyperlipidemic rats treated with 35 mg/kg of omega-3 fatty acids after 6 weeks of treatment. This result is in agreement with another study by Harris et al. [24] in which they found that in hypertriglyceridemic patients, omega-3 significantly decreased serum TG by 25-35% after 12 weeks of therapy. Similar results have been reported by Negakawa et al. [25], Sanders and Hochland [26], and Zucker et al. [27] who found that fish oil (< 20 g/d) induced a marked decrease in triglyceride concentration in hyperlipidemic patients.

The anti-triglyceridemic effect of omega-3 on hyperlipidemic rats is consistent with Thomas et al. [28] and Simopoulos [29] who reported that omega-3 considerably decreased TG concentration in individuals with hypertriglyceridemia. The mechanism, which is the cause of the TG-lowering effect of omega-3, is not defined sufficiently. In theory, first, it is probably related to a decreased VLDL production, which is supposedly secondary to reduced availability of hepatic free cholesterol for particle assembly. Second, it could be related to the increased clearing of VLDL through the LDL receptor or other lipoprotein receptors. Next, it could be related to the increased decay of VLDL particles via LPL. And it could be related to a combination of the above mechanisms [30].

In this study, the level of total cholesterol was significantly decreased in hyperlipidemic rats treated with omega-3 after 6weeks of treatment. This finding is consistent with Kobatake et al. [31] study in which it was found that in hyperlipidemic subjects, omega-3 significantly decreased serum total cholesterol after 20days of therapy. Whereas Harris [9] found that a large dose of omega-3 (4 g per day) has no significant effect on the level of total cholesterol in hyperlipidemic subjects after 2 weeks of treatment. So this difference might be due to the short term treatment with omega-3.

In the present study, hyperlipidemic rats treated with omega-3 at the doses (35mg/kg) showed no significant increase in HDL-C level after 6 weeks of treatments, which is incompatible with the study by Mori et al. [32] who showed that in hyperlipidemic subjects, HDL-C significantly increased. Furthermore, Harris [9] found that 4 g/day of omega-3 following 4 weeks of treatment increased HDL-C levels by 1-3%. This effect of omega-3 could be because omega-3 significantly lowered total cholesterol in normal and hyperlipidemic rats.

LDL-C was significantly decreased in hyperlipidemic rats treated with omega-3 after 6 weeks of treatment. This is inconsistent with the study of Mori [32] in which it was found that the administration of omega-3 in hyperlipidemic subjects does not usually lead to any significant changes in the concentration of LDL-C. In contrast, LDL levels may increase by 10%, especially with high doses of omega-3 fatty acids in the treatment of hypertriglyceridemia, which is even more pronounced in individuals with severe TG raises at baseline. Sanders and Hochland [25] found modest reductions in LDL-C for the normal individuals who were taken (< 20 g/d) of fish oil after 4 weeks of treatment; similar findings were reported by Negakawa et al. [25] and Zucker et al. [27].

In this investigation, HB and RBC were significantly increased in hyperlipidemic rats treated with 30mg/kg of omega-3 fatty acids. These results are consistent with another study by Abbas et al. [33] who found that the administration of omega-3 fatty acids is associated with an increase in the RBC and HB levels in rats treated with sucrose.

In this study, PCV was significantly increased in hyperlipidemic rats treated with omega-3 fatty acids, which is incompatible with another study [34] in which it was determined that the administration of 1g of fish oil in elderly individuals has no significant effect on the HTC level.

In the present study, hyperlipidemic rats treated with omega-3fatty acids showed a significant change in the level of MDA and SOD levels after 6 weeks of treatment. This result is compatible with another study by Ahmed and Hoda [35] who found that omega-3 fatty acids (20 mg/kg body weight) significantly affect the antioxidant and free radical activity. The results demonstrate that in rats, omega-3 fatty acids neutralize changes in lipid peroxidation and biochemical parameters by activating the antioxidant defense system.

Effect of Rosuvastatin on Hyperlipidemic Rats

According to this study, it was found that rosuvastatin (10 mg/kg) caused a significant decrease in serum TC, TG, LDL-C and VLDL-C of hyperlipidemic rats, this result was in line with Ansari et al. [20] study in which it was proven that the oral administration of rosuvastatin (10 mg/kg/day) for 21days along with a high-fat diet significantly reduced serum TC, TG, and LDL-C when compared to hyperlipidemic rats. This reduction of serum cholesterol of rosuvastatin happens because of the inhibition of HMG-CoA reductase. HMG-CoA reductase catalyzes the conversion of HMG-CoA to mevalonate, which decreases cholesterol synthesis [36, 37].

Serum alkaline phosphate of hyperlipidemic rats was increased by daily administration of rosuvastatin (10 mg/kg), this result was similar to a study reported by Dodiya et al. [38] who found that orally administration of rosuvastatin (40 mg and 80 mg) for 21 days to rats significantly increased AST, ALP, and total bilirubin levels. The serum ALP

elevation might be in response to the direct irritant effect of rosuvastatin on hepatic cells.

CONCLUSION

Omega-3 fatty acids had beneficial effect on must tests of rats and were efficient in reducing serum TC, TG and LDL-C. However it was not effective in significantly altering serum HDL-C in hyperlipidemic rats.Omega-3 fatty acids were effective in increasing the levels of HB, RBC and HTC in hyperlipidemic rats.

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