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Impact of Omega-3 on Hypercholesteremia Induced Male Rabbits

Fadhil A. Rizij[1], Ali M. Janabi[2*], Ayad A. Ameen[3], Najah R. Hadi[4]
[1]Department of Clinical Pharmacy, Faculty of Pharmacy, University of Kufa, Najaf, Iraq
E mail: fadhil.abduljabbar@uokufa.edu.iq
[*2]Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Kufa, Najaf, Iraq
E mail: alim.hashim@uokufa.edu.iq
[3] Department of Clinical Pharmacy, Faculty of Pharmacy, University of Kufa, Najaf, Iraq
E mail: ayada.hussein@uokufa.edu.iq
[4]Department of Pharmacology and Therapeutics, Faculty of Medicine, University of Kufa, Najaf, Iraq
E mail: drnajahiraq@gmail.com

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ABSTRACT
Atherosclerosis is a disorder of vasculature wall resulting mainly from the inflammatory processes. This condition involves vascular endothelial dysfunction, recruitment and activation of phagocytic cells in early stages. Omega-3 polyunsaturated fatty acids comprise part of the cellular membrane function many roles including increased development and functionality of neuronal synapses. This study assesses the significance of omega-3 fatty acids on atherosclerosis events via lowering oxidative and inflammatory insults. In this study, twenty-four domestic rabbits (male) were randomly distributed into three groups. The first one was the negative control group in which, rabbits were received normal diet (Oxide) for twelve week-period. The second group was the hypercholesteremia-induced, untreated rabbits fed with cholesterol (1%) enriched diet. The third group consumed cholesterol (1%) enriched diet supplemented with 5% omega-3 fatty acids. Blood samples were collected after twelve weeks to measure high-density lipoprotein-C (HDL-C), total cholesterol (TC), serum triglycerides (TG), endothelin-1 (ET-1), intracellular adhesion molecule-1 (ICAM-1) and highly sensitive C-reactive protein (hs-CRP) levels. After twelve weeks, aorta of each rabbit was isolated to identify glutathione (GSH), malondialdehyde (MDA) and intimal thickness. Administration of omega-3 was un-capable to modify lipid parameters significantly when compared with high cholesterol-fed rabbits. Administration of omega-3 results in improvement of ICAM-1, hs-CRP, ET-1, aortic MDA and intimal thickness of aorta significantly when compared with untreated high cholesterol-fed rabbits (P<0.05), Aortic GSH level was restored in rabbits receiving omega-3 fatty acids (P<0.05). Omega-3 fatty acid may reduce atherosclerosis progression in high cholesterol-fed rabbits via suppression of inflammatory and oxidative cascades with the lack of impacting of lipid profile.

Keywords: Omega 3 fatty acid, intracellular adhesion molecule-1, endothelin-1, aortic intimal thickness, oxidative stress, atherosclerosis.

Correspondence:
Ali M. Janabi
Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Kufa
Najaf, Iraq
Email: alim.hashim@uokufa.edu.iq

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INTRODUCTION
Atherosclerosis is now considered one of the main leading reasons of mortality and morbidity over the world [1]. While a variety of processes have been involved in atherosclerosis, it is likely resulted from consuming high-fat diet as well as lacking of regular exercise and living in sedentary lifestyle [2]. Elevated blood cholesterol level is a significant contributory factor to the development of cardiovascular disease and atherosclerosis. The latter refers to biological events including structural and functional vascular disorders that initiate molecular and cellular cascades prompted by endothelial dysfunction. These result in inflammation, increased ET-1 production, elevated cyclooxygenase activity and decreased nitric oxide production [3]. Diabetic patients are 3 - 4 times more likely to develop atherosclerosis at comparable plasma total cholesterol levels in comparison with nondiabetic individuals [4]. All stages of atherogenesis have been extensively ascribed to the inflammatory processes encountered the vasculature [5]. The potential anti-atherosclerotic activity of many anti-inflammatory drugs has been investigated since inflammation is a common condition which has considerable impact on pathogenesis of atherosclerosis. There is a growing body of literature that recognizes the importance of some anti-inflammatory agents in improving atherosclerosis events and subsequent risk for cardiovascular problems [6]. Omega-3 has emerged as potential therapeutic agent capable to decrease heart problems in people with an excessive sea food intake [7]. The cardio-protection presented by omega-3 may be attributable to the modulatory roles played on inflammation, lipid metabolism and thrombosis. However, data collected from experimental, observational and randomized studies are conflicting and not consistently supporting the cardioprotective efficacy mediated by omega-3 [8]. Omega-3 is able to diminish triglyceride levels via lowering hepatic output of triglyceride-rich lipoproteins [8]. Those apparent differences of using omega-3 might be due to, for instance, distinct effects of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) on the action of lipoprotein lipase, since DHA has potential downregulatory effect on LDL-C receptor [9-10]. DHA has agonistic activity on the G-protein coupled receptor, GPR120, which is responsible for beneficial metabolic profiles and anti-inflammatory effects of omega-3 in experimental studies [10]. Omega-3 polyunsaturated fatty acids comprise an important element of the cellular membrane function many roles including increased development and functionality of neuronal synapses [11-12].

MATERIALS AND METHODS
Twenty-four domestic rabbits (male) were randomly distributed into three groups. The first group was the negative control group in which, animals received normal diet (Oxide) for twelve week-period. The second group was the hypercholesteremia-induced, untreated rabbits fed with cholesterol (1%) enriched diet. The third group consumed cholesterol (1%) enriched diet supplemented with 5%

omega-3 fatty acids. Blood samples were collected after twelve weeks to measure serum HDL-C, TC, TG, hs-CRP, ET-1 and ICAM-1 levels. After twelve weeks, aorta of each rabbit was isolated to identify GSH, MDA and intimal thickness. ELISA kits of the corresponding markers were used according to the manufacturer's instructions.

STATISTICAL ANALYSES

Statistical comparisons were achieved using one-way ANOVA followed by Bonferroni's test for multiple comparisons using Prism (GraphPad 7 Software Inc., CA, and USA). Statistical significance was considered for P<0.05.

RESULTS

The body weight of rabbits treated with omega-3 fatty acids was not significantly different when compared with other study groups suggesting that food consumption probably was similar in all the groups and cholesterol or omega-3 fatty acid had insignificant consequence on body weight. In comparison with the levels of normal rabbits, levels of TC, TG, HDL, figure (1), hs-CRP, ET-1, ICAM-1, figure (2), aortic MDA and intimal thickness of aorta were increased significantly (*P*<0.05) whereas a decrease in the level of aortic GSH (*P*<0.05) was shown in the animals fed with Atherogenic diet figures (1-3). Treatment with omega-3 fatty acids could not significantly affect lipid profile, figure (1). However, omega-3 fatty acids were able to reduce the elevation in serum hs-CRP, ET-1, ICAM-1, aortic MDA and intimal thickness of aorta in comparison with the untreated animals (*P*<0.05), figures (2-3). Additionally, treatment with omega-3 reestablished the level of GSH in the aortal significantly (*P*<0.05).

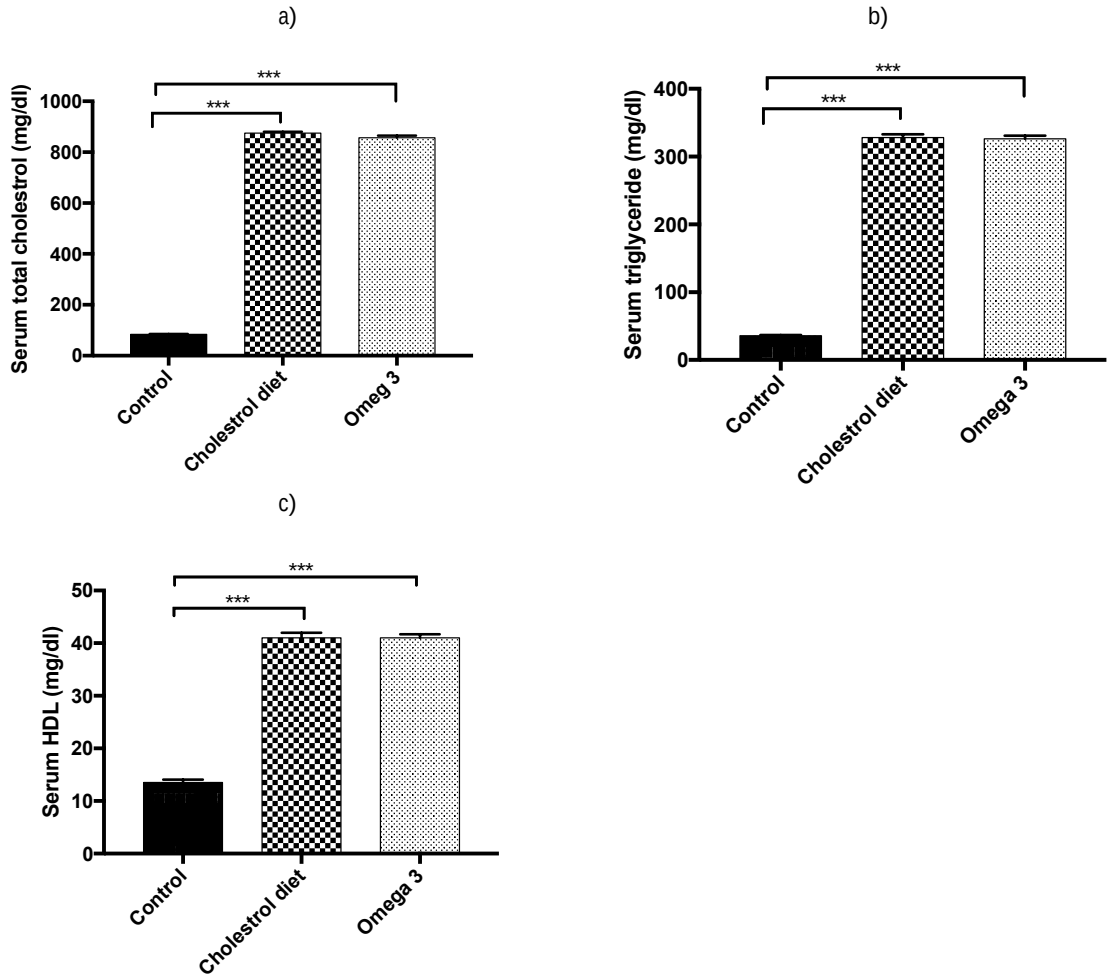

Figure (1): Serum TC (a), TG (b) and HDL (c) of the experimental groups
Data are mean ± SEM, n=8. ***P<0.001 vs control by Bonferroni's multiple comparison test following one-way ANOVA.

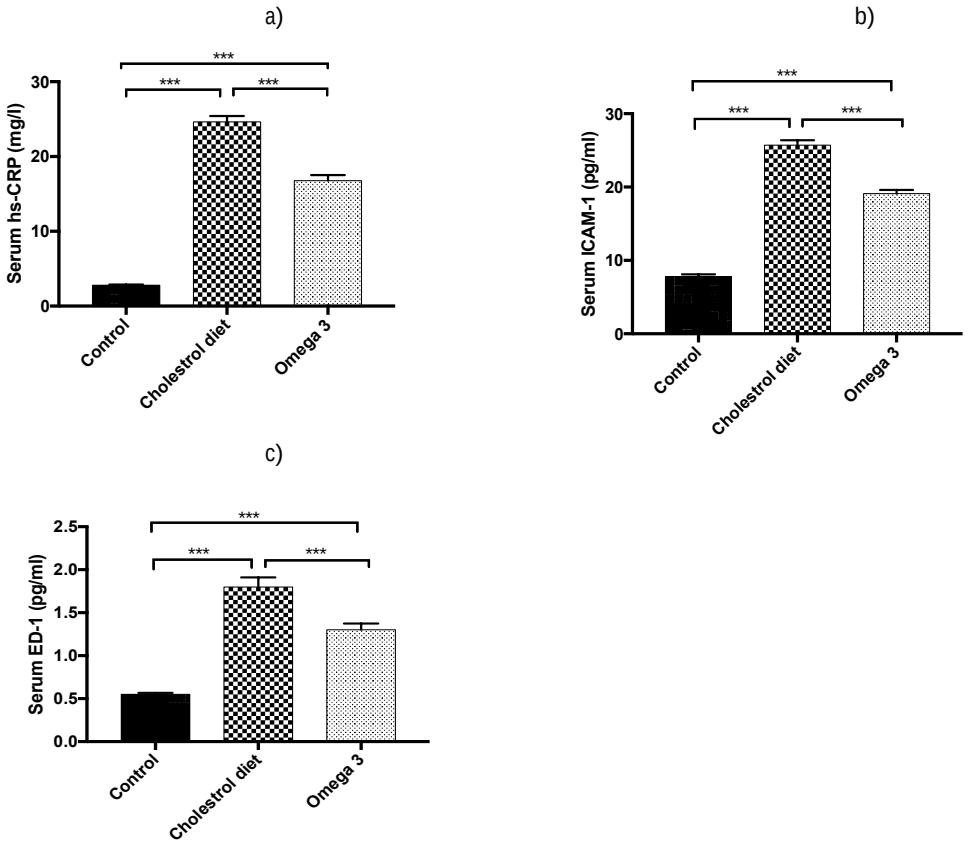

Figure 2: Serum hs-CRP (a), ICAM-1 (b) and ED-1 (c) of the experimental groups
Data are mean ± SEM, n=8. ***P<0.001 vs control by Bonferroni's multiple comparison test following one-way ANOVA.

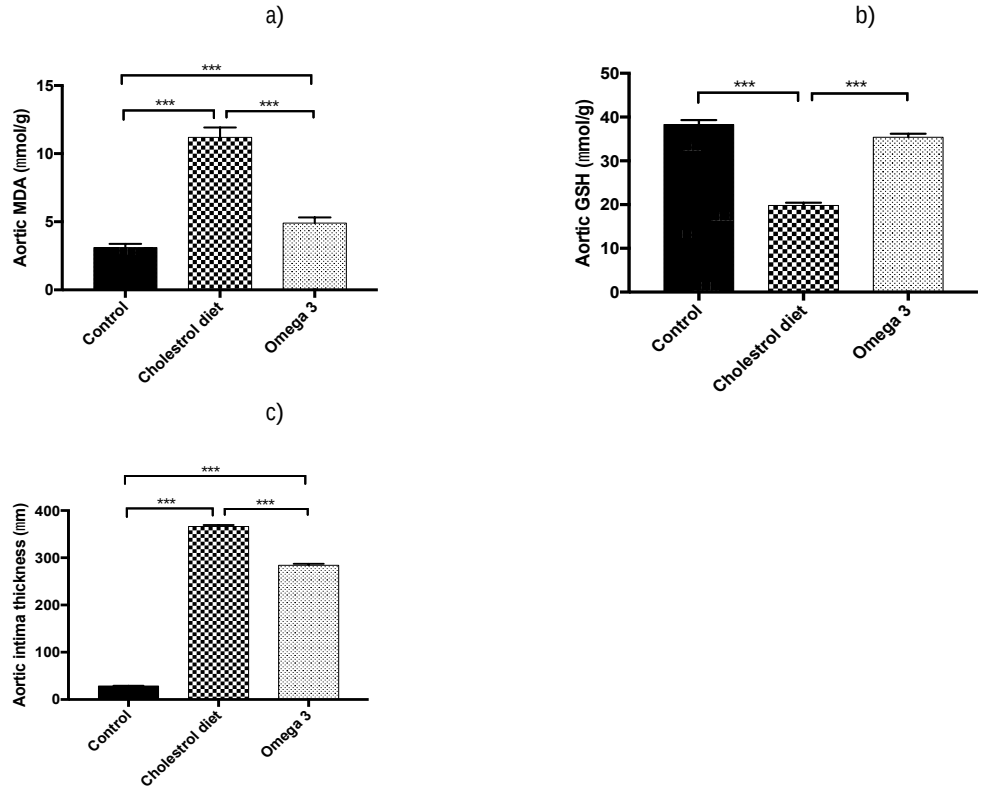

Figure 3: Aortic MDA (a), GSH (b) and intimal thickness (c) of the experimental groups
Data are mean ± SEM, n=8. ***P<0.001 vs control by Bonferroni's multiple comparison test following one-way ANOVA.

DISCUSSION

The present study attempts to explore the effect of omega-3 on atherosclerosis and whether these acids can interfere with inflammatory and oxidative systems or not. Data in this study showed that 12 week-consumption diet enriched with 1% high-cholesterol elevated serum TC and TG and generated atherosclerotic lesions including intimal thickening of aorta and disposition of lipid droplets under arterial endothelial layers. Data from this work also showed that generation of hs-CRP, ET-1 and ICAM-1 were significantly elevated in the atherosclerotic rabbits and, enrichment of food with omega-3 fatty acid could significantly lower them. This study established that treatment with omega-3 fatty acids appeared to have insignificant effect on lipid homeostasis when compared with untreated rabbits. This finding is contrary to a previous study conducted by Harris and coworkers, 2006 which has suggested that omega-3 fatty acids diminish triglycerides via reduction of hepatic output [13]. Another study showed that supplementation with DHA was superior to EPA in lowering TG levels; however DHA in this meta-analysis study augmented LDL-C [14]. These variations seen on the usage of omega-3 could be stemming from the distinct actions mediated either DHA or EPA on lipoprotein lipase activity as well as downregulatory effect of DHA on LDL-C receptor [15]. Omega-3 was capable to reduce the elevated serum MDA significantly in rabbits fed with high cholesterol diet suggesting a decrease in ROS generation and later lipid peroxidation. Additionally, omega-3 increased GSH level significantly in hypercholesterolemic rabbit providing a better antioxidant capacity to protect vasculature from lipid peroxidation. Omega 3 fatty acids have been commonly used in the treatment oxidative stress-linked diseases including peripheral vascular, cardiovascular and cerebrovascular diseases. The beneficial effects of omega-3 are mainly ascribed to its antioxidant properties. The data revealed in this study demonstrated a significant effect of omega-3 on hs-CRP, ET-1 and ICAM-1 in rabbit fed high cholesterol diet suggesting a potential blockage activity of omega-3 on the vascular inflammatory responses induced by high cholesterol intake. The mechanism of this effect is likely attributed to its antioxidative activity. Several reports have shown that antioxidant agents offer potential therapeutic agents encounter the generation of pro-inflammatory and cytotoxic mediators [16]. The most obvious finding to emerge from the analysis is that dietary intake of n-3 PUFAs and the improved ration of omega-6/-3 could control inflammatory response. After 21 days of administration of 3.2gm EPA and 2.2gm DHA, content of EPA was elevated in inflammatory cells (neutrophils and monocytes) [17]. Inhibition of lipoxygenase pathway in these immune cells and function of LTB5 have been shown to underly the anti-inflammatory effects of fish oils. In addition, omega-3 decreases the level of interleukin-1 and interleukin-6 thereby diminishing inflammation [18]. The present study demonstrated that omega-3 decreased the intimal thickness of aorta in rabbits receiving atherogenic diet when compared with rabbits not treated with omega-3. Introduction of diet enriched with omega-3 rich oil in mice with induced atherosclerosis results in augmented incorporation of omega-3 fatty acids in the tissue of the aorta and the heart, whereas the arachidonic acid content is diminished [19]. Despite conflicting data raised from a pile of studies, efficient effect of dietary omega-3 on atherosclerosis in mice is established significantly with a reduction in atherosclerotic lesion size [20].

CONCLUSION

The research has shown that supplementing omega-3 exerts an anti-inflammatory effect, thereby reducing the investigated inflammatory mediators and antioxidant effect by diminishing lipid peroxidation and enhancing glutathione. Thus, data in this study revealed a potential mode of action of how omega-3 lower aortic intima thickness by several pathways including suppression of oxidative reactions and systemic inflammation. Additionally, omega-3 fatty acids prevented lipid peroxidation (via reduction of serum MDA) providing another protective tool against hypercholesterolemic atherosclerosis.

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