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Research article

Synergistic anti-atherosclerotic role of combined treatment of omega-3 and co-enzyme Q10 in hypercholesterolemia-induced obese rats

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ABSTRACT

Hypercholesterolemia is a metabolic disorder associated with atherosclerosis. This study aimed to investigate the effects of omega-3 and/or coenzyme Q10 (CoQ10) on hypercholesterolemia-induced atherosclerosis. Rats were divided into five groups; (1): served as the negative control, (2): served as hypercholesterolemic (HC) control, (3): HC-rats administrated omega-3 orally, (4): HC-rats administrated CoQ10 orally, and (5): HC-rats administered the combination treatment of both omega-3 and CoQ10. Lipid profile was assayed and cardiovascular risk indices were calculated. Serum levels of Adiponectin (APN) and creatine kinase (CK-MB) were determined using ELISA. Besides, oxidative stress markers, malondialdehyde (MDA), nitric oxide (NO) and glutathione (GSH) were assayed in the heart homogenate. Histopathological investigation of the aortae and heart tissues were investigated. The results revealed that atherogenic HC-rats demonstrated a significant elevation in lipid profiles, except for HDL-C, along with decreased levels of APN, but increased CK-MB activities. Hypercholesterolemia increased lipid peroxidation, reduced NO production, and decreased GSH content in the cardiac tissue. Treatment of atherogenic HC-rats with omega-3 and/or CoQ10 improved dyslipidemia and ameliorated most of the HC-induced biochemical and histopathological changes. The histological observations of aortae and cardiac tissues validated our biochemical results. We concluded that the combined treatment of nutraceuticals such as omega-3 and CoQ10 demonstrated the best outcome, demonstrating their anti-hyperlipidemic, cardioprotective, and atheroprotective potentials. Together, this study supports a beneficial role of dietary co-administration of omega-3 and CoQ10 in obese patients who are prone to develop cardiovascular disorders.

1. Introduction

Consumption of high-fat diet is one of the most important factors in the development of many pathological conditions and metabolic disorders [1]. Hyperlipidemia is second, after hypertension, in a list of the 10 most common chronic disorders [2]. Hypercholesterolemia is regarded as a risk factor for atherosclerosis and cardiovascular diseases (CVDs) [3]. Obesity resulted in increased risk for CVDs through the remodeling of both cardiac tissues and adipose tissues by inducing structural alterations and subclinical dysfunction [4, 5]. There is an existing link between the development of CVDs and large amounts of visceral adipose tissue [6]. CVDs is the leading cause of mortality and morbidity worldwide, more than 80% of all CVD-related deaths occur in low- and middle-income countries [7]. The World Health Organization (WHO) expected that nearly 23.6 million people would die from CVDs each year by 2030 [8].

Atherosclerosis is one of the most common causes of CVDs including unstable angina, myocardial infarction, and ischemic heart failure [9]. The incidence of atherosclerosis is strongly associated with the metabolic disorder of blood lipids and endothelial dysfunction [10]. Development of atherosclerosis is orchestrated by endothelial dysfunction, lipid deposition, inflammation, and oxidative stress; all are intimately associated with adipokines [5], the "inside to outside" theory of atherosclerosis development demonstrates that adhesions of inflammatory cells to the disrupted endothelium that triggers cholesterol deposition within the arterial walls [11]. Atherosclerotic plaques are usually found in the aortae; the coronary arteries, cerebral arteries, and peripheral arteries [12]. Advanced atherosclerotic plaques could block blood flow resulting in insufficient blood supply to the coronary arteries and subsequent myocardial ischemia, which are basic pathological processes of coronary heart disease (CHD) [13].

Hypercholesterolemia is supposed to play an essential role in the development of atherosclerosis among other risk factors; it is "a lipoprotein metabolic disorder characterized by high serum levels of low-density lipoprotein (LDL-C) and blood cholesterol" [14]. The elevated

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LDL-C levels can be oxidized into "oxidized-LDL" (ox-LDL), resulting in oxidative stress, inflammation and endothelial dysfunction [15], leading finally to increased lipid peroxidation [16]. Therefore, as a clinical condition of the cholesterol, atherosclerosis is characterized by the narrowing and hardening of the arteries because of fatty deposition [17].

Atherosclerotic plaque formation was suggested to begin with endothelial dysfunction caused by adipose tissues-released factors that promote adhesions of immune cells that launch the development of atherosclerosis [18]. Perivascular adipose tissue (PVAT) is the adipose tissue that surrounds the blood vessels and is involved in both non-pathological vascular homeostasis [19] and pathological atherosclerosis [20]. PVAT acts as a "paracrine regulator of vascular function" through secreting "metabolically active" adipokines, chemokines, and hormone-like factors, such as leptin and adiponectin and vasoactive substances [19]. Interestingly, superoxide released by the vasculature and adiponectin released by 'healthy' PVAT seem to equilibrate each other, indicating "a counter-regulatory protective mechanism" [21]. However, the role of adiponectin (APN) in the modulation of PVAT properties in obesity is still under investigation [22]. For example, hypoadiponectinemia is associated with obesity and is linked to impaired endothelium-dependent vasodilation [23], while hyper-adiponectinemia has been associated with increased cardiovascular mortality [24]. Thus, further research is needed to elucidate if APN can be considered a therapeutic agent.

Therapies designed to reverse or even to prevent; the devastating outcomes of atherosclerosis remain elusive [25]. Current treatments include hypolipidemic agents and anti-hypertensive medications [26], such as the commonly used statins that are capable of reducing serum LDL-C levels and inhibiting the vascular risk, but the continuous and long-term use of statins lead to more side effects such as; rhabdomyolysis, myopathy, liver damage, muscle toxicity, and acute renal failure [27]. Despite the hype surrounding the development of new drugs, global CVDs and hypercholesterolemia are still puzzling.

Dietary omega-3 fatty acids are one of the therapeutic modalities for atherosclerosis; however, their efficacy in secondary prevention remains controversial [28]. Dietary consumption of omega-3 fatty acids (Fish Oil) exhibited a cardioprotective potential and demonstrated a primary preventive role in several metabolic disorders, including atherosclerosis and diabetes [29]. Moreover, Omega-3 demonstrated anti-inflammatory potential, as well as, antioxidant actions, especially DHA [30, 31]. Besides, omega-3 showed numerous functions related to the structure and function of the membrane, tissue metabolism, and gene regulation [32]. Long chain omega-3 fatty acids, including eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n- 3) are predominantly sourced from marine fish [33].

On the other hand, coenzyme Q10 (CoQ10) or ubiquinone is a safe supplement in humans with minimal side effects [34, 35]. CoQ10 is a redox component of the mitochondrial respiratory chain that synthesizes Adenosine triphosphate (ATP); thus, it is especially vital to the heart that has a high demand for oxygen [36]. CoQ10 is derived from mevalonate, a precursor of endogenous cholesterol and other metabolites [37]. The reduced form of ubiquinone "Ubiquinol" is a powerful lipophilic antioxidant that participates in reviving antioxidants such as ascorbate and tocopherol [38]. Ubiquinone (CoQ10) acts as an electron carrier that transfers electrons in the mitochondrial electron transport chain between complexes I and III and complexes II and III, while in its reduced form, it acts as the most abundant and efficient antioxidant [35].

Due to the high prevalence of atherosclerosis and considering that its exact pathophysiology is not clearly understood, besides the absence of fully effective therapy, it is therefore urgent to explore novel therapeutic and preventative approaches to reduce the prevalence of atherosclerosis in high-risk populations. Administration of omega-3 or CoQ10 (either alone or in combination) as therapeutic tools for atherosclerosis is attracting attention; due to their favorable safety profile, being non-invasive, besides being widely available and inexpensive compounds, which contribute to a better acceptance and adherence to the therapeutics.

The working hypothesis was to investigate the cardioprotective and atheroprotective potentials of omega 3 and/or CoQ10 in hypercholesterolemic (HC) rats, by exerting anti-oxidative functions, modulating adiponectin (APN) levels, and decreasing creatine kinase (CK-MB) activities. The main objectives of the hypothesis were: (1) to compare the effects of omega 3 and/or CoQ10 on induced hypercholesterolemia in rats. (2): to demonstrate the "cardioprotective and anti-atherosclerotic" potentials of omega 3 and/or CoQ10; (3) to examine the effect of omega 3 and/or CoQ10 on factors that are responsible for development of atherosclerosis including low-density lipoprotein cholesterol (LDL-C), adiponectin (APN) and creatine kinase (CK-MB).

2. Materials and methods

2.1. Drugs and chemicals

2.1.1. Drugs

Omega-3 oil was purchased in the form of capsules containing 1.0 g omega-3 fish oil EPA and DHA (SEDICO Pharmaceutical Co., Egypt). Omega-3 fatty acids were administered to the rats in pure form without the use of any vehicle. CoQ10 powder was bought from (MEPACO Pharmaceutical Co., Egypt).

2.1.2. Chemicals

Serum Adiponectin (APN) and creatine kinase (CK-MB) levels were determined using commercially available ELISA kits according to manufacturer instruction (Biovision and CUSABIO, USA). While colorimetric Kits of total cholesterol (TC), triacylglycerols (TAG), high-density lipoprotein-cholesterol (HDL-C), malondialdehyde (MDA), nitric oxide (NO), and glutathione reduced (GSH) were purchased from Biodiagnostic Company (Egypt).

2.2. Animals

Forty Male Wistar rats (115–120 g) were obtained from the Animal House of the National Research Center (NRC), Egypt. Rats were housed under controlled room temperature of 23 ± 1 °C and humidity with 12 h light and dark cycles. The animals were fed with standard pellet diet and water ad libitum. Animals received care in compliance with the National Institutes of Health Guide for the Use and Care of Laboratory Animals; in addition, all efforts were made to minimize animal suffering, as well as to reduce the number of animals. The ethics committee of NRC approved this study (Ethical approach number: 19 105).

2.3. Experimental design

After a week of acclimatization, five groups of rats were selected for this study (n = 8 per group). **Group (1):** Negative control rats were fed the standard pellet diet with water *ad libitum*. **Group (2):** Hypercholesterolemic (HC) rats served as atherosclerotic/atherogenic control group. Rats were administrated a daily oral dose of cholesterol (30mg/0.3 ml olive oil/1 kg animal) and were fed a high fat (lard-based) diet according to the method of **Adaramoye *et al.*** [39] for six consecutive weeks. **Group (3):** atherogenic HC-rats administered a daily oral dose of omega-3 (500 mg/kg body weight) for six consecutive weeks [40], after stopping HC-induction. **Group (4):** atherogenic HC-rats administered a daily oral dose of CoQ10 (10 mg/kg body weight) for six consecutive weeks [41], after stopping HC-induction. **Group (5):** atherogenic HC-rats

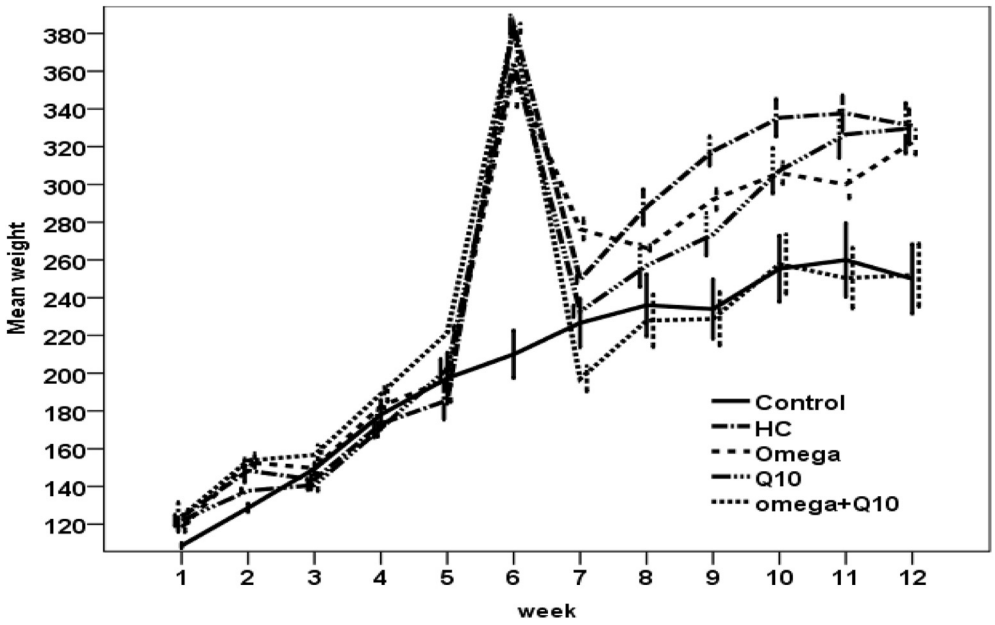

Figure 1. The hypocholesterolemic effect of omega-3, CoQ10, and combination (omega-3+ CoQ10) on the bodyweights of rats of different therapeutic groups. Data are presented as mean ± SE, (n = 8).

administered combination treatment of both omega-3 and CoQ10 orally for six consecutive weeks, after stopping HC-induction.

2.4. Body weight

The body weight was measured every week during the experimental period and after the last treatment administration; to evaluate the effect of the administration of high-fat diet and the effect of treatments on body weight of rats using electronic weighing balance.

2.5. Sample preparations

2.5.1. Blood collection and biochemical analysis

At the end of the experimental period (12 weeks), all rats were fasted for 12 h.; weighted and anesthetized, 4 mL of blood samples were collected from abdominal aorta, the blood samples were left to clot in clean, dry test tubes for 30 min. at room temperature and then centrifuged at 4000 RPM for 10 min. The clear supernatant serum was then frozen and stored at -20 °C for biochemical analysis of total cholesterol (TC), triacylglycerols (TAG), and high-density lipoprotein-cholesterol (HDL-C), using colorimetric kits. Serum Adiponectin (APN) levels and creatine kinase (CK-MB) activities were determined colorimetrically using ELISA kits.

2.5.2. Tissue collection

After blood sampling, rats were immediately euthanized and the heart from each rat was excised immediately and cleaned. The left portion of the heart was homogenized for biochemical assay, while the right portion of the heart and aorta were fixed in 10% neutral formalin for histological examination.

2.5.3. Preparation of the heart homogenates

The heart samples (0.5 g) were homogenized in 4.5 ml of Phosphate Buffer Saline (PBS; pH 7.4) with a homogenizer at 4 °C to obtain cardiac homogenate. The homogenates were centrifuged at 4000 RPM for 10 min. at 4 °C. The supernatants were collected and used for assessment of cardiac malondialdehyde (MDA), nitric oxide (NO), and glutathione reduced (GSH).

2.6. Biochemical analyses

2.6.1. Lipid profile

Enzymatic colorimetric methods were used to value serum total cholesterol (TC), serum triacylglycerols (TAG), and serum HDL-C levels according to **Allain *et al.*** [42]; **Fassati and Prencipe** [43]; **Lopez-Virella *et al.*** [44]. Serum LDL-C concentration was calculated according to **Friedewald's** equation [45]: LDL-C = TC-(HDL-C + VLDL-C). Serum very low-density lipoprotein cholesterol (VLDL-C) was determined according to **Norbert** [46]: VLDL-C = TAG/5.

2.6.2. Atherogenic risk predictor indices (ARPIs) (cardiovascular risk indices)

The Atherogenic index (AI) was calculated according to the method of **Harnafi *et al.*** [47] AI = TC-HDL/HDL. The atherogenic risk predictor indices were calculated as following coronary risk index or Castelli's Risk Index-I (CRI-I) = TC/HDL and Castelli's Risk Index-II (CRI-II) = LDL/HDL as described by **Asare *et al.*** [48].

2.6.3. Oxidative stress markers in the cardiac tissue

Homogenates of the heart were used to estimate lipid peroxidation (LPO) by reaction of thiobarbituric acid (TBA) [49], nitric oxide (NO) was estimated according to the method of **Montgomery and Dymock** [50], and glutathione reduced (GSH) using the method of **Beutler *et al.*** [51].

2.6.4. Determination of serum levels of adiponectin (APN) levels and creatine kinase-MB (CK-MB) activities by ELISA

Serum levels of APN and CK-MB were quantified by ELISA (a sandwich enzyme Immunoassay) by the methods of **Aazmi *et al.*** [52] and **Al-Rasheed *et al.*** [53], respectively, and were calculated with the help of the calibration curve generated by using known amounts of standards.

2.7. Histopathological study

For light microscopy, the isolated hearts and aortae were cleaned, dried, and fixed in a buffer solution of 10% neutral buffered formalin, the

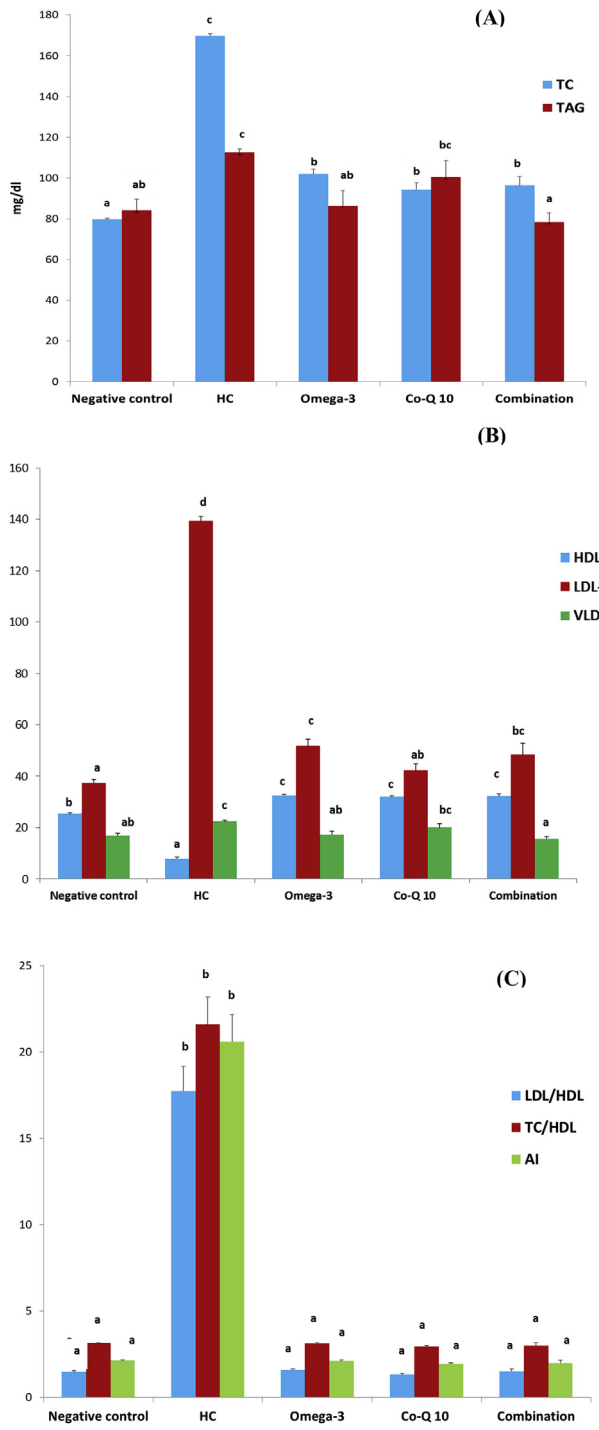

Figure 2. (A, B, and C). The hypocholesterolemic effect of omega-3, CoQ 10, and combination (omega-3+CoQ10) on serum levels of total cholesterol (TC), triacylglycerols (TAG), high-density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C), LDL/HDL, coronary risk index (TC/HDL), atherogenic index (AI = TC-HDL/HDL) in HC-rats. Data are presented as mean ± SE, (n = 8). Mean with different superscripts (a–c) are significant at $p \leq 0.05$.

fixed tissues were processed using analytical grade ethanol and xylene treatment and were embedded in paraffin, sectioned to about 5μm thickness and stained with hematoxylin and eosin (H&E) for general histopathological evaluation [54]. The morphometric measurements were performed on real–time image from the microscope, the area was measured (μm^2).

2.8. Statistical analysis

Data were analyzed using computer software, Statistical Package for the Social Sciences (SPSS) version 16 (SPSS Inc. Released 2007, SPSS for Windows, and Version 16.0. Chicago, SPSS Inc.). Differences among experimental groups were determined by simple one-way analysis of variance (ANOVA) followed by Duncan's multiple range test. All data were expressed as Mean ± SE (Standard Error), for n = 8 rats of each group. A *p*-value of less than 0.05 was considered statistically significant.

3. Results

3.1. Effects of omega-3 and/or CoQ10 on body weight in HC-rats

As compared to negative control rats, HC-rats showed significant increase in body weight. It is apparent that the highest body weight was observed in HC-group. There seems to be a parabolic rise in body weight from 5-6 weeks, followed by a rapid decline at 7 weeks. Treatment of HC-rats with omega-3 and/or CoQ10 for six weeks showed significant reduction in body weight, it is demonstrated that the lowest body weight was observed in the combination treatment (Figure 1).

3.2. Anti-hyperlipidemic activity and anti-atherogenic potential of omega-3 and/or CoQ10 in HC-rats

The serum levels of TC, TAG, LDL-C, VLDL-C in HC-rats were significantly higher than those of control group (113.4, 33.6, 272.8, 33.6%), denoting that high-fat diet feeding for 6-weeks caused hypercholesterolemia in rats. Treatment of HC-rats with omega-3, CoQ10, and a combination of both significantly decreased these changes for TC (40, 44.5, 43.3%), for TAG (23.3, 10.7, 30.3%), for LDL-C (62.8, 69.8, 65.3%) and for VLDL-C (23.3, 10.8, 30.3%). On the other hand, HDL-C level was significantly decreased in HC-rats by 68.6% as compared to control rats, by treatment HDL-C was increased significantly in treated HC-rats by omega-3 (309.9%), CoQ10 (300.1%), and a combination of both (303.6%), as compared to HC-rats (Figure 2 A, B).

The ARIs of atherogenic HC-group were significantly ($p < 0.05$) higher than that of the three treated groups, the ratios of LDL/HDL, TC/HDL and AI were 1108.2, 588.2, and 863.1% as compared to control group. By treatment, the values were significantly decreased by (91, 85.6 and 89.8%) for omega 3, (92.6, 86.3 and 90.5%) for CoQ10, and (91.5, 86.1, and 90.3%) for the combination treatment, as compared to atherogenic HC-rats (Figure 2 C).

3.3. Effects of omega-3 and/or CoQ10 on serum adiponectin (APN) and creatine kinase (CK-MB) levels in HC-rats

In comparison with control rats, HC-rats demonstrated a reduced level of APN that reached to 53.5% and an elevated CK-MB activity that reached to 201.2%. On the other hand, treatment of HC-rats with omega-3, CoQ10, and combination treatment showed a significant elevation in the serum APN with percentages increase reached to 91.4, 92.2, and 79.1%, respectively. On the other side, the cardiac enzyme CK-MB activity was improved by treatment with omega-3 (10.6%), CoQ10 (10.3%), and combination treatment (17.7%) in comparison with HC-rats, but the levels were also significantly higher than control rats (Figures 3 and 4).

3.4. Effects of omega-3 and/or CoQ10 on oxidative stress in the cardiac homogenates of atherogenic HC-rats

Concerning lipid peroxidation, atherogenic HC-rats showed a significant increase in the production of malondialdehyde (MDA) in the heart homogenate by 145 %, as compared to control rats. Treatment of HC-rats showed a significant reduction in MDA by (42.1%) for omega-3, (59.9%)

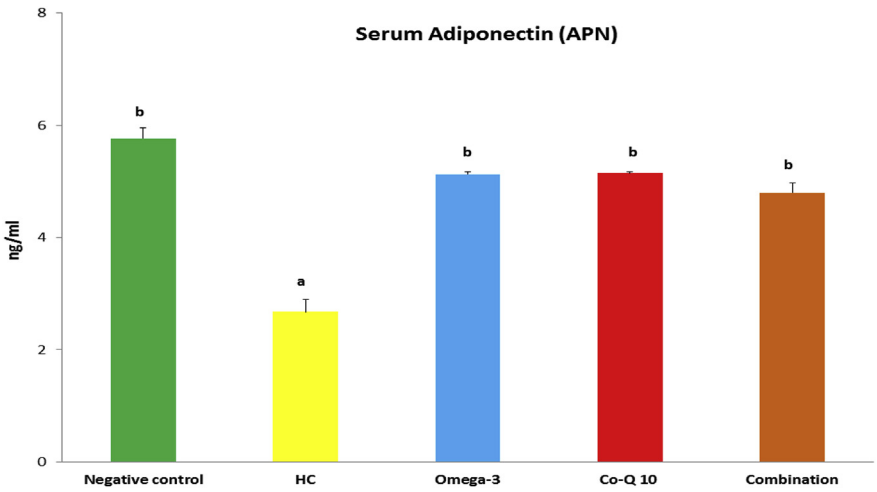

Figure 3. The effect of omega-3, CoQ10, and combination (omega-3+ CoQ10) on serum Adiponectin (APN) levels in HC-rats. Data are presented as mean ± SE, (n = 8). Mean with different superscripts (a, b) are significant at $p \leq 0.05$.

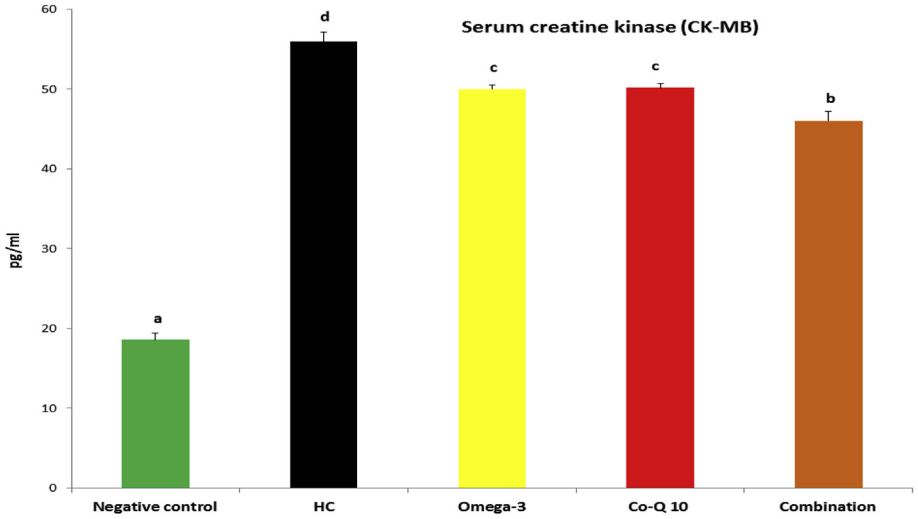

Figure 4. The effect of omega-3, CoQ 10, and combination (omega-3+ CoQ10) on serum creatine kinase (CK-MB) levels in HC-rats. Data are presented as mean ± SE, (n = 8). Mean with different superscripts (a–d) are significant at $p \leq 0.05$.

for CoQ10 and (49.2%) for the combination treatment, as compared to HC-rats (Figure 5 A).

Regarding cardiac NO production, atherogenic HC-rats showed a significant decrease in NO, as a marker of endothelial dysfunction, in the heart homogenate by 40.1%, as compared to control rats. Treatment of HC-rats showed a significant elevation in HC-rats treated with omega-3 (402.7%), CoQ10 (211.3%) and combination treatment (526.2%), as compared to HC-rats (Figure 5 B).

Regarding anti-oxidant defense, HC-rats showed a significant decrease in cardiac GSH production by 47.4%, as compared to control rats. Treatment of HC-rats showed a significant increase in HC-rats treated with omega-3 (79.8%), CoQ10 (34.8%), and combination treatment (77.9%), as compared to HC-rats (Figure 5 C).

3.5. Effects of omega-3 and/or CoQ10 on histopathological study of aortae and hearts of different groups

3.5.1. Morphometric analysis of aorta

The thickness of the aortic arch was examined to evaluate histopathological changes in the aortae in different groups (Table 1). The thickness area of the arterial walls in the atherosclerotic HC-group (596.8 ± 27.6 μm) was about two times thicker than that in the control group (314.4 ± 19.7 μm). By treatment, HC-group demonstrated significant decrements in the thickness area of the arterial walls by (444.8 ± 49.5μm) for omega-3, (376.0 ± 30.7 μm) for CoQ10, and (351.8 ± 33.3 μm) for the combination treatment. On the other hand, the length of the aorta in different groups ranged from 75.9 for normal control to 147.8 μm for atherogenic HC-group.

3.5.2. Histopathological examination of the aortae of different groups

The negative control group (Figure 6 a) exhibited normal architecture and normal endothelial lining represented as intact and regularly arranged endothelial cells with no histopathological changes. The atherogenic HC-group (Figure 6 b) showed the disarranged and proliferated endothelial cells along with thickening of artery tunica, the appearance of a fibrous layer in the intima, and deposition of the foam cells. The aortic wall seemed to be thicker and less smooth. In addition, the elastic fiber plates were disrupted and atherosclerotic plaques were observed, as compared to the control group. Figures 6 c and d of the HC-treated groups with omega-3 or CoQ10 showed the

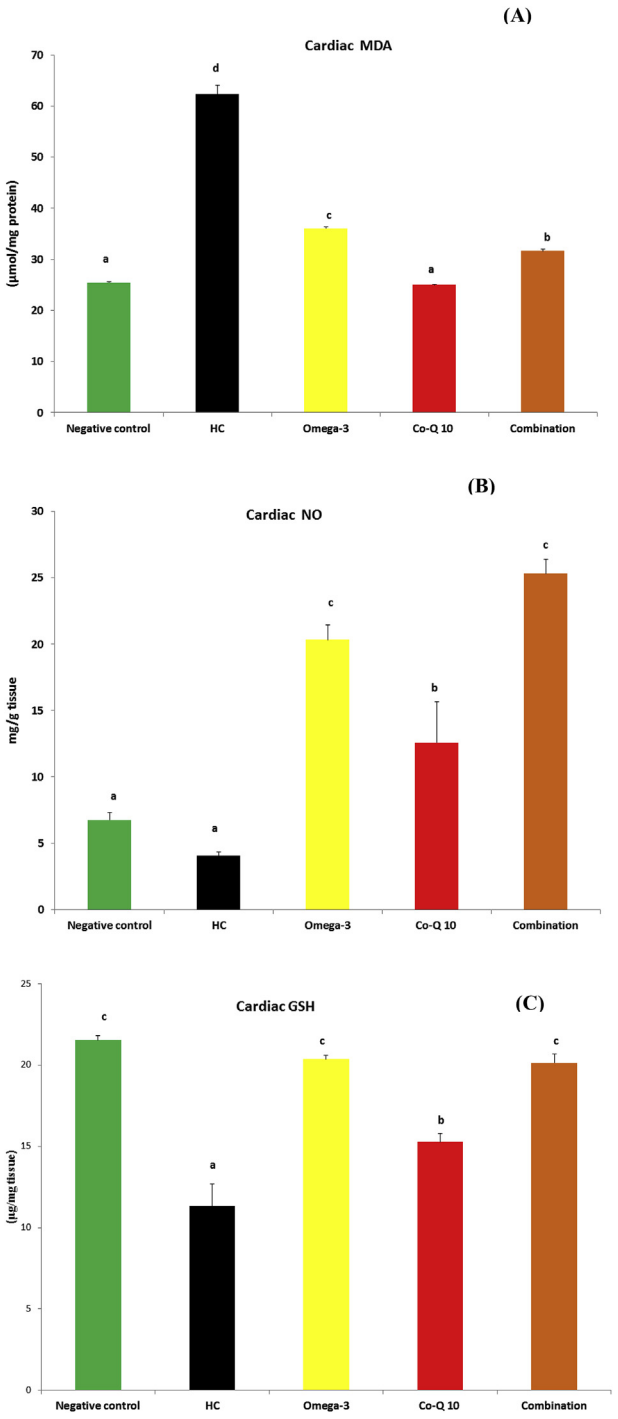

Figure 5. (A, B, and C). The effect of omega-3, CoQ10, and combination (omega-3+ CoQ10) on malondialdehyde (MDA), nitric oxide (NO), and glutathione reduced (GSH) levels in cardiac tissues of HC-rats. Data are presented as mean ± SE, (n = 8). Mean with different superscripts (a–d) are significant at $p \leq 0.05$.

presence of aortic plaques and foam cell-rich lesions, in comparison with HC-rats.

Figure 6 e of the HC-treated groups with the combination treatment of omega-3 and CoQ10 showed the restoration of normal architecture and demonstrated thinner arterial thickness that lacked swelling with no remarkable lesions in aortic tissue, the endothelial cells were basically intact and did not desquamate, in comparison with HC-rats.

3.5.3. Histopathological examination of the cardiac tissues of different groups

The normal control group (Figure 7 a) demonstrated regularly arranged myocardial fibers, muscle bundles, and normal architecture of cardiomyocytes. HC-rats Figure 7 b demonstrated abnormal fatty change, loss of striations, and cytoplasmic vacuolization (black arrow) in the cardiac tissues, as compared to control rats. Figure 7 c, d and e highlighted the treatment of the HC-heart with omega-3 and/or CoQ10 and revealed a normal morphological appearance and the fatty change was completely disappeared, in comparison with HC-group.

4. Discussion

Hypercholesterolemia facilitates the risk of atherosclerosis and subsequent myocardial infarction [55]. There is a positive correlation between serum levels of cholesterol and LDL-C and the development of atherosclerosis [56], furthermore it was reported that decreasing the serum levels of TC, TAG, and LDL-C and increasing that of HDL-C could mitigate the clinical complications of atherosclerosis [57, 58].

This study demonstrated that administration of cholesterol along with a high-fat diet (lard-based) for six weeks resulted in a significant increase in the bodyweight of HC-rats. Figure 1 demonstrated the pattern of the bodyweight of HC-rats and different therapeutic groups during the experimental period. Control rats, fed on the control diet, maintained a consistent weight gain throughout the period, while HC-rats, fed on the HC-diet, gained weight up-till the 7th week, then started a steady decline, with a sudden increase until the end of the experiment. This pattern of bodyweight decrease in HC-rats runs in parallel with a study by **Harb *et al.*** [59]. There are different reasons explain this notable reduction in bodyweight of HC-rats including (1) high-fat diet results in the reduction in the food intake through causing impaired absorption of nutrients, including protein [60, 61, 62]. (2) Anorexia may result in decreased food intake [63]. (3) "ketogenic diet" that is characterized by "high fat" and "low carbohydrate" in the high-fat diet administrated to HC-rats, this "ketogenic diet" results in hepatic insulin resistance, and induces type 2 diabetes and non-alcoholic fatty liver disease [64].

The HC-group demonstrated the highest bodyweight due to the increased energy intake and energy storage [65]. This increase in the bodyweight of HC-rats was associated with a significant decrease in serum levels of adiponectin (APN), which is an adipocyte-specific protein. This runs in coincidence with **You *et al.*** [66]. It was reported that hypoadiponectinemia is associated with obesity [67]. In addition, APN knockout could accentuate high fat diet-induced obesity in mice [68].

Furthermore, this obesity was accompanied with hypercholesterolemia as demonstrated by the significant increase in serum levels of TC, TAG, LDL-C, and VLDL-C along with a significant reduction in circulating HDL-C; these results are in line with several studies [69, 70, 71, 72]. This state of atherogenic dyslipidemia could result in cardiac remodeling and the development of atherosclerosis. The elevation of the serum levels of the lipid profile (TC, TG, and LDL-C) and decrement of HDL-C are indicators for the development of CVDs [73]. Taken together, TAG-rich lipoproteins are precursors for cholesterol-enriched particles that deposit cholesterol in the arterial wall [74], moreover, excessive LDL-C can precipitate in the development of "arterial atherosclerotic lesions" [75]. Besides, reduced serum HDL-C concentration hinders the clearance of cholesterol from the arterial wall and fastens the progression of atherosclerosis [76].

In this study, atherogenic dyslipidemia was developed and HC-rats demonstrated a profound increase in the atherogenic index (AI), TC/HDL ratio, LDL/HDL ratio, and high TAG, as compared to control group, as demonstrated by **Manninen *et al.*** [77]. AI and CRI demonstrated a direct relationship with TC, LDL-C, and TAG; it means that AI and CRI increase with increasing TC, LDL-C, or TAG values [73]. Therefore, they could be strong markers to predict the risk of atherosclerosis and to reflect the abnormality of lipid metabolism [78], regarding the fact that

Table 1. Morphometric analysis of aortae of HC-rats and different therapeutic groups.

	Area (μm)	Mean	Minimum	Maximum	Angle	Length
Negative Control group	314.4 ± 19.7^{a}	199.6 ± 1.3^{c}	164.2 ± 1.4^{b}	238.0 ± 2.5^{b}	43.6 ± 30.1^{b}	75.9 ± 6.2^{a}
HC-control group	596.8 ± 27.6^{c}	178.42 ± 4.9^{a}	138.4 ± 10.6^{a}	219.4 ± 3.1^{a}	-40.7 ± 23.6^{a}	147.8 ± 9.4^{c}
HC + omega 3 group	444.8 ± 49.5^{b}	187.9 ± 4.5^{ab}	158.2 ± 3.6^{ab}	220.0 ± 9.1^{a}	-32.26 ± 20.5^{a}	109.6 ± 11.4^{b}
HC + CoQ10 group	376.0 ± 30.7^{ab}	193.5 ± 2.9^{bc}	158.0 ± 2.9^{ab}	242.2 ± 5.6^{b}	-44.9 ± 11.8^{a}	89.6 ± 8.6^{ab}
HC + omega 3 +CoQ10 group	351.8 ± 33.3^{ab}	211.8 ± 2.4^{d}	178.40 ± 8.9^{b}	252.8 ± 1.5^{b}	3.9 ± 11.1^{ab}	83.1 ± 8.5^{ab}

The morphometric investigations of the aorta in different rat groups, data are presented as mean ± SE (n = 8). Mean with different superscripts (a, b, c, d) are significant at $p \leq 0.05$.

"LDL/HDL ratio > 2.3" is atherogenic and undesirable; "TC/HDL ratio > 3.33" is atherogenic and undesirable [79]. AI is a powerful indicator that is reflective of more atherosclerotic plaque formation [80]. On the other hand, HDL-C levels are inversely correlated with the risk of atherosclerosis [81]. These results were supported by the elevated serum activity of the heart function marker CK-MB in HC-rats.

Treatment of HC-rats with omega-3 resulted in resolution of hyperlipidemia through reversing the increment in TC, TAG and LDL-C and the decrement in HDL-C. These results run in agreement with several studies [82, 83, 84] that demonstrated the hypolipidemic potential of omega-3. On the other hand, administration of CoQ10 to HC-rats resulted in improvement of lipid profile. In addition, CoQ10 improves the hypolipidemic action of omega-3. These results are consistent with **Shakir *et al.*** [85]. This hypolipidemic activity of CoQ10 might be attributed to its ability to affect the negative feedback of hepatic cholesterol [86]. Moreover, HDL-C is involved in the reversed transport of the cholesterol back to the liver for excretion in the bile and metabolism, thereby mitigating the accumulation of LDL-C in the arterial walls [87]. Consequently, improvement of lipid profiles in treated HC-rats was associated with a decrement in AI, LDL/HDL, and TC/HDL ratios, as compared to HC group. Mathematically, relatively low ARPIs are attained by decreasing concentrations of atherogenic LDL-C, TC, and TAG, along with, increasing HDL-C levels. These alterations play a key role in alleviating the atherogenic steps involving cholesterol accumulation; explaining why fewer plaques were found in the aorta of HC-treated group with omega-3 and/or CoQ10. Hence, therapeutic and dietary interventions cause reduction of atherogenic lipoproteins and inhibiting the biosynthesis of cholesterol [85].

On the other hand, rats of different therapeutic groups began to have a linear increase in body weights that is significantly lower in relative to HC-rats. This hypocholesterolemic and anti-obesity potential of omega-3 and/or CoQ10 was reflected in the significant reduction in body weights of different therapeutic groups, this could be linked to the increased serum APN levels to levels approximating those in control rats and highlighting the cardioprotective potential omega 3 and/or CoQ10. The role of APN in reducing food intake and decreasing bodyweight was established through its action on the hypothalamus [88, 89]. Molecules such as adiponectin [90] or omega-3 [91] may target M2-macrophages to endure a non-inflammatory environment, thereby allowing adipocytes to respond efficiently to flexible metabolic demands [92]. Omega-3 increases APN levels by stimulating the production of APN through interaction with specific adipocyte channels [93].

Furthermore, APN upregulates uncoupling protein 1 (UCP1) in brown adipose tissue; thus supporting its thermogenic potential and its mechanistic action to reduce body weight [88] and is capable of inducing AMP-activated protein kinase (AMPK) and the peroxisome proliferator-activated receptor alpha (PPARα) [67]. APN is an anti-inflammatory chemokine [94] that demonstrated anti-atherogenic potential and cardioprotective role [5, 95]; therefore, high levels of APN are linked to a lower risk of coronary heart disease [96, 97]. APN deficiency exacerbates cardiac damage [98]. Therefore, boosting APN generation is a promising therapeutic approach to reduce hypercholesterolemia-induced obesity.

Creatine kinase-Myocardial Band (CK-MB), served as the diagnostic cardiac markers of myocardial tissue damage. CK-MB levels showed a significant increase in the sera of atherogenic rats, indicating structural and functional alterations in the cardiac muscle and demonstrating disruption of cell membrane integrity [99]. CK-MB leaks out from cardiomyocytes into the blood stream because of the disruption and uncontrolled permeability of cell membranes [100], demonstrating increased enzyme activity in HC-group. This runs in parallel with **Choi *et al.*** [101], **Mohamed *et al.*** [99], and **Hassan *et al.*** [102]. The release of CK-MB from the damaged myocardial membranes indicates hypercholesterolemia-induced myocardial necrosis that was confirmed by histopathological investigation of HC-hearts.

On the other hand, administration of omega-3 and/or CoQ10 appeared to attenuate the negative effects of a hypercholesterolemic diet by reducing serum levels of CK-MB. Omega 3 and/or CoQ10 inhibited the increase of CK-MB activity and restricted the release of cardiac CK-MB, but the levels were also significantly higher than control rats. This indicates the ability of omega 3 and/or CoQ10 to maintain, to a certain extent, the cellular membrane integrity. The most remarkable effect resulted from the co-administration of both omega-3 and CoQ10, indicating that cardiac muscle injury in atherogenic rats was attenuated by the combination treatment.

The observed imbalance of lipid metabolism in HC-rats results in lipid peroxidation as demonstrated by **Shen *et al.*** [82]. Our results showed an increment in cardiac MDA level and a decrement in cardiac GSH content because of hypercholesterolemia-triggered oxidative stress. These results are in agreement with several studies [14, 69, 71, 103] that demonstrated that hypercholesterolemia induced oxidative stress through increasing production of free radicals and decreased enzymatic and non-enzymatic antioxidant defense [104]. This might be linked to the accumulation of cholesterol in endothelial cells and cardiomyocytes and subsequent stimulation of oxidative stress [105]. Furthermore, myocardial lipid peroxidation leads to cardiomyocytes damage by inducing alterations in the permeability of cell membranes and the overload of intracellular calcium [106]. Enhanced myocardial lipid peroxidation is associated with depletion of glutathione (GSH) [107], showing its role in inhibiting oxidative stress. Furthermore, atherogenic rats demonstrated a decreased cardiac NO level, in comparison with control rats, indicating endothelial dysfunction and denoting that "NO unavailability" stimulates the pathogenesis of atherosclerosis; this run in accordance with **Lian *et al.*** [9]. Atherogenesis is strongly correlated with endothelial dysfunction that is featured by reduced bioavailability of nitric oxide (NO) [5]. In atherogenic state, cardiac oxidative damage results in release of CK-MB from the necrotic cardiocytes to blood, reflecting the alterations in plasma membrane integrity and/or permeability [108]. Herein, HC-rats showed a significant elevation in serum CK-MB, in comparison with controls, this run in agreement with **Subramani *et al.*** [108] and **Sengupta and Ghosh** [109]. The leakage of cardiac CK-MB could be regarded as a secondary event to the lipid peroxidation of cardiac membranes, with a subsequent elevation in enzyme leakage from cardiomyocytes.

Treatment of HC-rats with of omega-3 or CoQ10 or their combination alleviated the oxidative alterations through decreasing lipid peroxidation

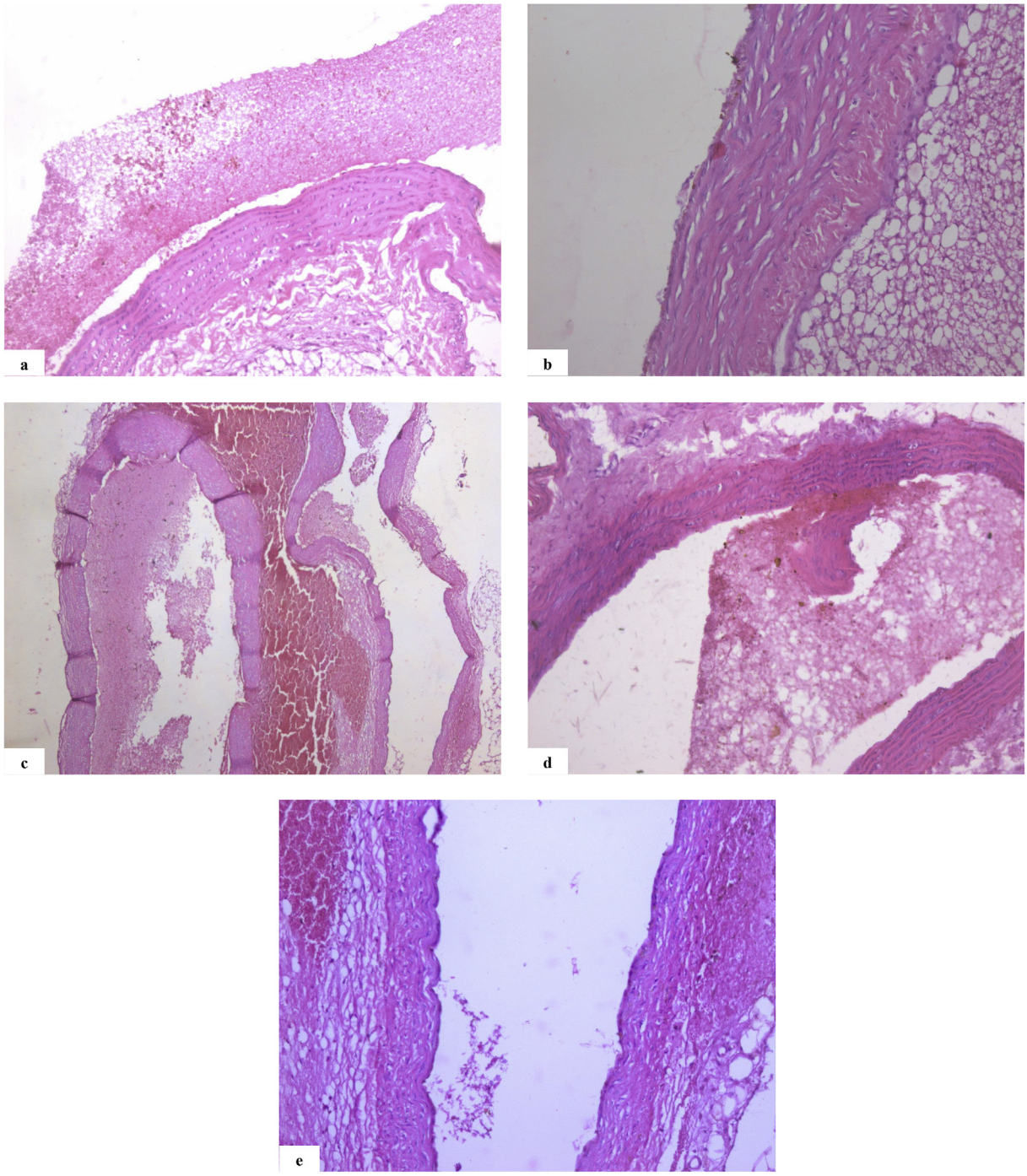

Figure 6. Effects of omega-3 and/or CoQ10 on histopathological study of aortae of different groups (H&EX200): (a): Normal control group demonstrated a normal histological structure. (b): Atherogenic HC-group showed severe fragmentation of elastic and collagen fibers in the irregular tunica media and formation of atherosclerotic plaques. (c): HC + omega-3 group demonstrated almost normal histological structure. (d): HC + CoQ10 group showed almost normal histological structure. (e): HC + Omega-3+CoQ10 group showed almost normal histological structure.

and inducing a significant increment in NO and GSH in the heart tissue. This anti-oxidative potential might be attributed to hypocholesterolemic activity and cardioprotective potential of both omega-3 and CoQ10. Agents that combine hypolipidemic and antioxidant potentials such as omega-3 can prevent the negative impact of cholesterol on the heart and other body tissues [69]. Omega-3 inhibits lipid peroxidation through the aggregation of fatty acids in membrane lipids rendering the double bonds "unexposed" for the attack of free radicals, inhibition of the pro-oxidant phospholipase A2, and stimulation of anti-oxidant enzymes [109]. On the other hand, CoQ10 attenuated cardiac lipid peroxidation, through its ability to terminate pro-oxidant radical and to regenerate the active form of vitamin E [110]. Besides, CoQ10 treatment restored the GSH levels significantly. This favors the dual role of Coenzyme Q10 as a hypocholesterolemic agent, as well as, an antioxidant. This potential may be largely mediated by its ability to maintain mitochondrial function and protect cardiomyocytes against hypercholesterolemia-induced oxidative damage.

In addition, the cardiac NO levels were significantly increased in HC-rats treated with omega-3 and/or CoQ10, this effect was involved in attenuation of atherosclerosis in HC-rats. NO is an endothelium-derived

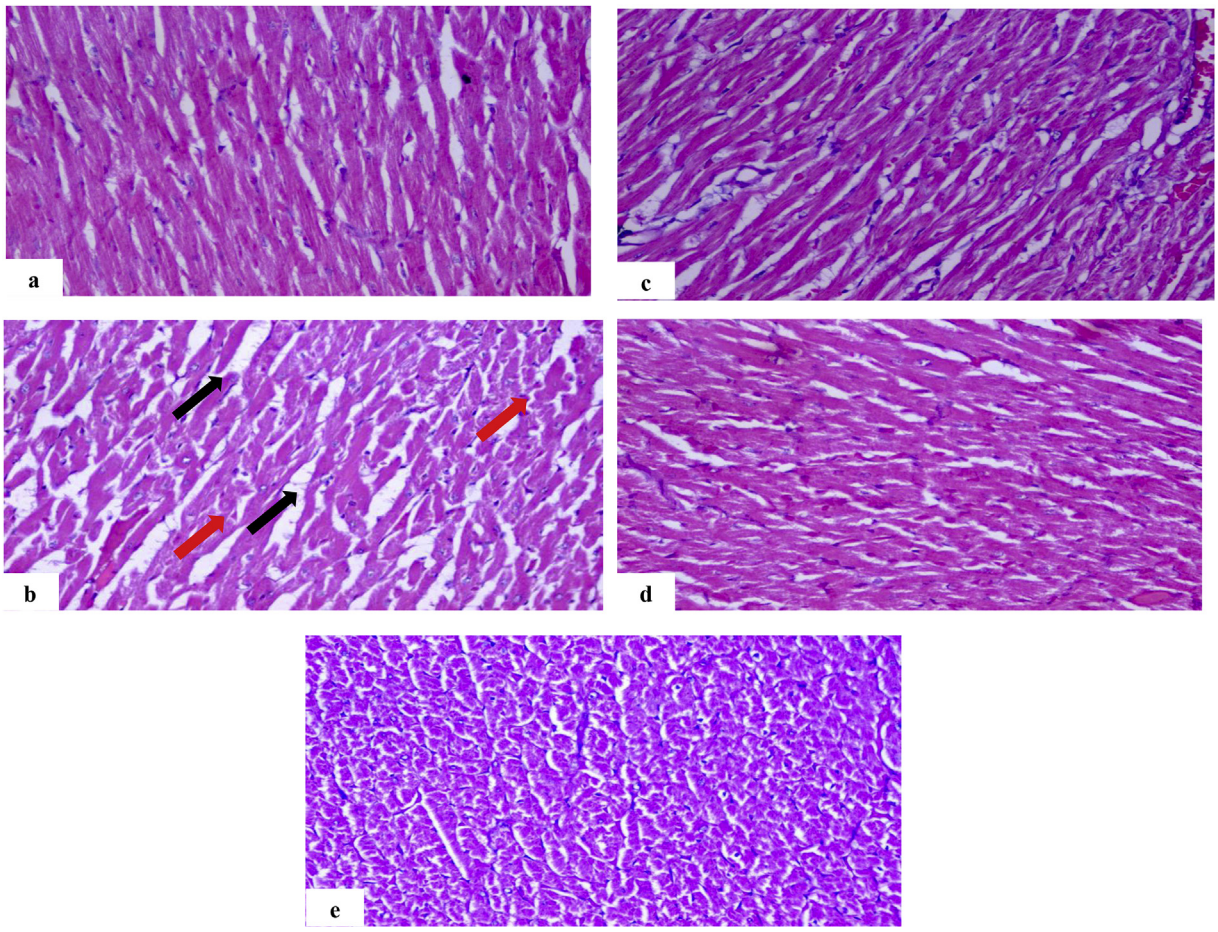

Figure 7. Effects of omega-3 and/or CoQ10 on histopathological study of hearts of different groups (H&E X400): **(a)**: Normal control group showed normal morphological appearance with normal myocardia. **(b)**: HC-group demonstrated fatty changes and loss of striation and discontinuous muscle fibers (black arrow) that resulted in wide-spaced appearance, and extravasation of blood (red arrow). **(c)**: HC + omega-3 group showed almost normal morphological appearance with normal myocardial. **(d)**: HC + CoQ10 group showed almost normal morphological appearance with normal myocardial **(e)**: HC + Omega-3+CoQ10 group showed almost normal morphological appearance with normal myocardial fibers.

vasodilator and a key anti-oxidant molecule that inhibits the progression of atherosclerosis through decreasing monocyte-endothelial cell interaction, inhibiting platelet aggregation, and preventing the proliferation of smooth muscle cells [55]. The improvement of NO production in HC-treated rats might be explained by the contribution of PVAT to vascular NO production [111]; by secreting APN that is capable of activating (by phosphorylation) endothelial NO synthase (eNOS) [112] and normalizing endothelial function [22], demonstrating the anti-oxidative potential of APN [113]. APN is an important vasoactive regulator that exhibits anti-inflammatory and anti-atherogenic potentials through increasing bioavailability of endothelial nitric oxide (NO) [114]. Furthermore, APN can inhibit plaque formation and reduce inflammation denoting the atheroprotective activities of PVAT [115].

In a good connection with the aforementioned findings, we could assume an existing relationship between APN, NO, and CK-MB. Treatment of HC-rats with omega-3 and/or CoQ10 downregulated CK-MB release and significantly improved its serum levels, as compared to HC-rats. This runs in agreement with **Sengupta and Ghosh** [109] who demonstrated the omega-3 ability to decrease CK-MB and with **Ghule *et al.*** [116] and **Ulla *et al.*** [117] who found that CoQ10 inhibited the elevation of CK-MB activity and mitigated oxidative stress. This action exerted by omega-3 and CoQ10 might be attributed to their cardioprotective and atheroprotective properties, to their ability to inhibit membrane lipid peroxidation, and to maintain the integrity of myocardial cell membrane from oxidative attack, thereby restricting the leakage of CK-MB. This improvement concerning CK-MB was further supported by the histopathological investigation that demonstrated amelioration of cardiac tissue damage and disappearance of lipid changes in the cardiomyocytes by treatment. This amelioration of CK-MB is strongly correlated to the improved lipid profile. This hypolipidemic activity is associated with the cardioprotective potential of omega-3 and CoQ10.

Histopathological findings showed that hypercholesterolemia causes structural abnormalities in the arterial walls demonstrated by thickened media-intima layer as compared with the control group. This effect might be due to fatty accumulation in the tunica media. These results are consistent with **Diao *et al.*** [15] that demonstrated that thickness of the aorta could be an indicator of atherosclerosis. This was evidenced by the morphometric parameters of atherosclerotic HC-aortae that revealed the highest thickness area of the arterial walls in the HC-group (596.8 ± 27.6 μm). On the other hand, treatment of HC-rats improved the architecture of the aortae, which looked similar to those of the control rats fed with a standard diet, and no lipid-containing elements were observed, the treated HC-groups showed a visible reduction in pathological changes and the vascular structure of the aorta was normal. This was reflected in the morphometric parameters of treated rats that demonstrated significant decrements in the thickness area of the aortae. The results of the histopathological study in the heart of HC-rats are in line with the work of **Sengupta and Ghosh** [109]. Combination treatment of HC-rats with omega-3 and CoQ10 results in restoration of normal structure. Finally,

we could confirm that the biochemical findings were supported by histological observations.

5. Conclusion

Overall, this study showed that anti-oxidant-based therapeutic approaches could represent an effective strategy against hypercholesterolemia-induced atherosclerosis and obesity. Our data confirmed that the combination of omega-3 and CoQ10 significantly prevented the expansion of hypercholesterolemia-induced atherosclerosis. Further work is needed to clarify the molecular mechanisms underlying the cardioprotective and atheroprotective potentials of omega-3 and CoQ10. The results of this study suggest the possible beneficial "synergistic" effects of the co-administration of omega-3 and CoQ10 on the parameters related to atherogenesis and cardiovascular alterations.

Declarations

Author contribution statement

G. Ibrahim Fouad: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Additional information

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