Synergistic Effects of Atorvastatin and Sesame Oil on the Protection Against Aortic Atherosclerosis due to High Fat Diet in Rats: Histological and Immunohistochemical study

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ABSTRACT

Background: A serious health issue that raises the possibility of strokes and cardiovascular illness is atherosclerosis. The main risk factor for atherosclerosis occurrence is the high fat diet (HFD).The elevated blood lipid levels lead to endothelial dysfunction with subsequent sub endothelial accumulation of lipids and formation of plaques. **Aim**: To clarify the preventive effect of atorvastatin and sesame oil on aortic atherosclerosis induced by HFD in rats. **Materials and methods**: Thirty two animals were split into four groups: Group I (control group), group II (atherosclerosis group): Rats were given a high-fat diet (HFD) till the end of the eighth week in order to induce atherosclerosis, group III (atorvastatin group): Rats were administered both HFD and atorvastatin orally at a dose of 20 mg/kg/day for eight weeks, and group IV (atorvastatin + sesame oil group): Rats supplemented with HFD in addition to atorvastatin (20 mg/kg/day) and 10% sesame oil for the same duration. Blood samples were withdrawn to assess the serum level of Cholesterol, Triglyceride, low-density lipoproteins (LDL), and high-density lipoproteins (HDL). The aortas were promptly excised and prepared for histological and immunohistochemical examination. **Results**: Atherosclerosis group revealed a substantial rise ($P \leq 0.05$) in cholesterol, LDL, and triglyceride serum measurements, while HDL quantity displayed a major decrease. The H&E stained sectors presented increased aortic wall thickness with intimal projection into the lumen. Foam cells appeared in the tunica media. Additionally, there was marked reduction in endothelial nitric oxide synthase (eNOS) immune expression, but there was powerful tumor necrosis factor -α (TNFα) immune reactivity in the aortic layers. Treatment with atorvastatin improves the serum lipid profile levels, the histological structure of the aortic wall, as well as the immunohistochemical results. However, combination therapy of atorvastatin and sesame oil displayed better results. **Conclusion**: The present investigation provides biochemical, histological, and immunohistochemical support of the significant prophylactic impact of atorvastatin and sesame oil combination in improving lipid profiles, amelioration of aortic damage, reduction in inflammatory indicators, and thus atherosclerosis limitation.

Keywords: Atorvastatin, sesame oil, atherosclerosis, inflammatory factors, high fat diet.

INTRODUCTION

Chronic exposure to HFD works effectively as a metabolic toxin, as it exceeds the body's lipid-handling capacity and lead to a cascade of harmful effects. HFD leads to raise free fatty acids in circulation leading to lipotoxicity, oxidative stress and inflammation in key organs (liver, pancreas and vascular endothelium) [1,2]. These changes disrupt normal metabolic signaling making insulin resistance, dyslipidaemia and endothelial dysfunction leading to atherosclerosis [3].

Atherosclerosis is a significant health concern which raises the danger of both strokes and cardiovascular illnesses. This condition is a leading factor in global mortality rates [4]. It is characterized by the buildup of calcium deposits, fibrous tissue, and lipoproteins, especially low-density lipoproteins (LDL), as well as cholesterol. These alterations cause blood vessels to constrict and trigger inflammatory responses, which ultimately contribute to the development of atheroma plaques [5]. An important part of the pathophysiology of atherosclerosis involves

inflammatory cytokines (such as interleukin (IL)-6 and tumor necrosis factor (TNF)-α) which speed up atherosclerosis in obese people. TNF-α is secreted by macrophages to stimulate endothelium and promote SMCs proliferation [6].

Statins, which are 3-hydroxy-3-methylglutaryl coenzyme-A reductase inhibitors, are used broadly in clinical practice because of their numerous cardio-protective effects and notable lipid-modifying capabilities [7]. These include the advancement of nitric oxide (NO) production, the improvement of inflammation and oxidation, and the promotion of endothelial progenitor cell migration, among others, which are now recognized as the pleiotropic effects of statin [8].

However, about 10% of individuals have complained myalgia or other statins side effects. Thus, the use of nutritional and dietary supplements has become more popular in the scientific community [9]. Medicinal herbs have shown promise in treating atherosclerosis because of their beneficial effectiveness and multifunctional actions as anti-atherosclerotic agents [10].Sesame belongs to the Pedaliaceae family, specifically classified as sesamum indicum. It has been widely utilized as a traditional food in the East, and it is also recognized for its significant medicinal properties. Numerous studies, primarily involving rodent species, provide substantial evidence of the hypolipidemic effects of various constituents found in sesame seeds and their oil [11]. According to more current research on sesamin's characteristics, sesamin and other sesame oil ingredients could enhance a number of the biochemical parameters in a lipid panel and showed anti-hyperlipidemic properties [12]. The purpose of this study was to clarify the synergistic effect of atorvastatin and sesame oil on aortic atherosclerosis due to HFD in albino rats.

MATERIALS AND METHODS

Chemicals

Cholesterol, **cholic acid, vitamins, casein, minerals, cellulose, and phosphate-buffered saline** were acquired from Elgomhoria Company for the Chemical Industry in Cairo ,Egypt.

Atorvastatin (20 mg tablets) was obtained from EIPICO (Egyptian International Pharmaceutical Industries Company). It was dissolved in phosphate-buffered saline.

Sesame oil, 250 ml bottle containing natural cold-pressed sesame oil, was obtained from Imtenan, cairo ,Egypt.

Animals

We purchased thirty-two male albino rats, aged 12-16 weeks old from the animal house at Benha University's Veterinary Faculty of Medicine. They ranged in weight from 150 to 175 grams. The rats were kept in separate plastic cages under conventional laboratory settings (temperature 24 ± 2°C, air humidity 50 ± 15%, 12-hour light-dark cycle). To aid in acclimatization, they were given unlimited access to food and water for seven days. The Benha University Faculty of Medicine's Ethical Committee gave its permission to every part of this study (Under approval number RC 8-2-2025).

Experimental design

32 animals were divided into 4 groups at random, with 8 rats assigned to each group:

Group I (Control group): is split into two subgroups as follows:

Ia: consisting of four rats that were received normal regular diet.

Ib: consisting of four rats that were administered phosphate-buffered saline (2ml/kg) orally once daily until the finish of the 8th week with normal diet.

Group II (atherosclerosis group): comprising eight rats that were fed on a high-fat diet (HFD) for the induction of atherosclerosis, continuing until the end of the 8th week **[13].** The used HFD diet (with 45% of energy from fats and 17% of energy from sucrose)

comprising cholesterol, cholic acid, mineral and vitamin mix, casein, cellulose, and sucrose was mixed properly with the normal regular diet and made in accordance with Teklad Custom Diet, (2015) [14] .

Group III (atorvastatin group): made up of eight rats were given HFD as well as atorvastatin (20mg/kg/day) liquefied in phosphate buffered saline as it was reported to be an efficient dose and does not induce toxicity in rats [15] given orally daily until the completion of the 8th week.

Group IV (atorvastatin + sesame oil group) consisting of eight rats given HFD enriched by 10% sesame oil in addition to atorvastatin (20 mg/kg/day) for the same duration. To get a final concentration of sesame oil in the animal food at a level of 10%, the proper amount of was first weighed, thoroughly mixed with the necessary amount of sesame oil, dried, and then weighed once more to achieve a final concentration of sesame oil in rat diet at a level of 10% .[11].

Blood sample collection and serum lipid profile

The animals were anesthetized at the conclusion of the investigation by intraperitoneal administration of xylazine (10 mg/kg) and ketamine (100 mg/kg). Blood samples were obtained through cardiac puncture, followed by centrifugation at 3000 rpm for fifteen minutes to isolate serum sample. Serum concentrations of cholesterol, triglycerides (TG), HDL, and LDL were deliberated utilizing enzymatic methods and specific kits (Catalog number; MAK043, MAK266 and MAK045, Sigma Aldrich, Massachusetts, USA). All the manufacturer's recommendations were adhered to [16].

Histological study

The aortas were promptly excised following the dissection of the animals. The samples were preserved in 10% formol saline for a period of 24 hours. Paraffin blocks were created, and sections of 5 micrometers in thickness were stained with Masson's trichrome as well as hematoxylin and eosin [17].

Immunohistochemichemical study

After being deparaffinized, aortic segments were treated with PBS for half an hour at room temperature. Primary anti-eNOS (1:50 rabbit polyclonal, RR-1711-R7, Neomarkers; Lab Vision, Fermont, CA, USA) and TNF-α (rabbit monoclonal #8184, Cell Signaling Technology, Massachusetts) were incubated on the slides for 30 minutes. The slices were treated with a peroxidase substrate after being incubated with a biotinylated secondary antibody for three PBS washes. After five minutes of rinsing with distilled water, the antibody-labeled specimens were dehydrated. Brown spots against the blue Mayer's hematoxylin stain background indicated a positive cytoplasmic immunological response [18].

Morphometric Analysis

The average area percentage of Masson trichome's stained and immune-histologically brown stained eNOS and TNF-α sectors was calculated from non-overlapping areas of four stained sections per group at a magnification of × 200, using the Image J program (NIH,USA).

Statistical Analysis

All statistical analyses were performed leveraging IBM SPSS software, version 20. The data are presented as the mean ± standard deviation (SD). For comparisons between groups, one-way analysis of variance (ANOVA) was used to determine whether statistically significant variations existed. A P-value of less than 0.05 was considered indicative of statistical significance.

RESULTS

Serum lipid profile

In comparison with the control rats, group II's cholesterol level displayed a substantial rise ($P \leq 0.05$). Group III exhibited an important drop in cholesterol levels compared to group II, however the combined sesame oil and atorvastatin therapy in group IV displayed a momentous decline ($P \leq 0.05$) versus both group II as well as group III. The level of triglyceride was substantially elevated in group II related to the control rats ($P \leq 0.05$)

but it displayed considerable reduction in combined therapy in group IV as related to group II and group III ($P \leq 0.05$). Group II revealed a considerably greater LDL level than the control rats ($P \leq 0.05$). Both group III as well as group IV demonstrated a momentous reduction ($P \leq 0.05$) in LDL level versus group II .On the other hand, HDL level in group II exposed a considerable diminishing as compared to the control rats ($P \leq 0.05$). It then elevated importantly in the animals of group III related to group II's animals ($P \leq 0.05$). The combination therapy of group IV revealed a substantial rise that didn't differ significantly from the control rats (Table 1, Fig. 1).

Hematoxylin and Eosin result

The aortic sections from the control group displayed typical histological architecture and thickness of the aortic wall. It is consisted of the three layers; tunica intima which demonstrated simple squamous endothelial cells, smooth muscle cells were organized concentrically forming the tunica media, and tunica adventitia (Fig. 2A) .On the other hand, group II presented increased aortic wall thickness with intimal projection into the lumen. Foam cells appeared in the tunica media. Smooth muscle cells were observed proliferating and migrating to the intima (Fig. 2B) .Administration of atorvastatin to rats in group III displayed improvement in the histological structure with decrease in the aortic wall thickness, however some foam cells were still appeared (Fig. 2C).While, treatment with both atorvastatin and sesame oil in group IV exhibited nearly similar thickness and histological appearance of the aortic wall comparable to the control group (Fig. 2D).

Masson Trichome staining

Masson trichrome's stained sectors from the control animals displayed minimal distribution of collagen fiber in the aortic wall. Conversely, group II presented marked deposition of collagen fibers in the layers of aorta. Group III displayed decreased collagen fibers deposition. While group IV demonstrated less quantity of collagen fiber (Fig. 3A-D).

eNOS immunohistochemistry

The control group displayed intense eNOS immune expression in the tunica intima. While, group II presented marked reduction in eNOS immune expression compared with the control animals. Group III exhibited moderate expression of eNOS immune stain. Interestingly, co administration of both atorvastatin and sesame oil in group IV exhibited an obvious increase in eNOS immune expression (Fig. 4A-D).

TNFα immunohistochemistry

Sections from the control group exhibited minimal TNFα immune reaction. However, there was powerful TNFα immune expression in the aortic layers of group II, in relation to the control animals. Group III moderately alleviated TNFα immune expression while, group IV displayed weak TNFα immune reactivity (Fig. 5A-D).

Morphometric findings

The average value of the collagen fiber area percentage was considerably higher ($P \leq 0.05$) in group II related to the control group. While, group III and group IV displayed a substantial reduction in the collagen area percentage ($P \leq 0.05$) relative to the control animals (table 2 & Fig. 6).

There was a major reduction in eNOS immune-reactivity area % in group II ($P \leq 0.05$) comparable to the control group. Administration of atorvastatin alone in group III produced a substantial ($P \leq 0.05$) elevation of eNOS immune expression related to group II. However, combination therapy in group IV revealed an important elevation of this percentage compared to group II but didn't differ significantly from control animals (table 2 & Fig. 7).

On the other hand, the average area percentage of TNFα immune-reactivity in group II displayed a momentous rise (P≤ 0.05) in relation to the control rats. Group III revealed a substantial (P≤0.05) reduction of TNFα immune expression. While, combination treatment in group IV exposed an important decline of TNFα immune expression that differ momentously (P≤ 0.05) from both group II as well as group III (table 2 & Fig. 8).

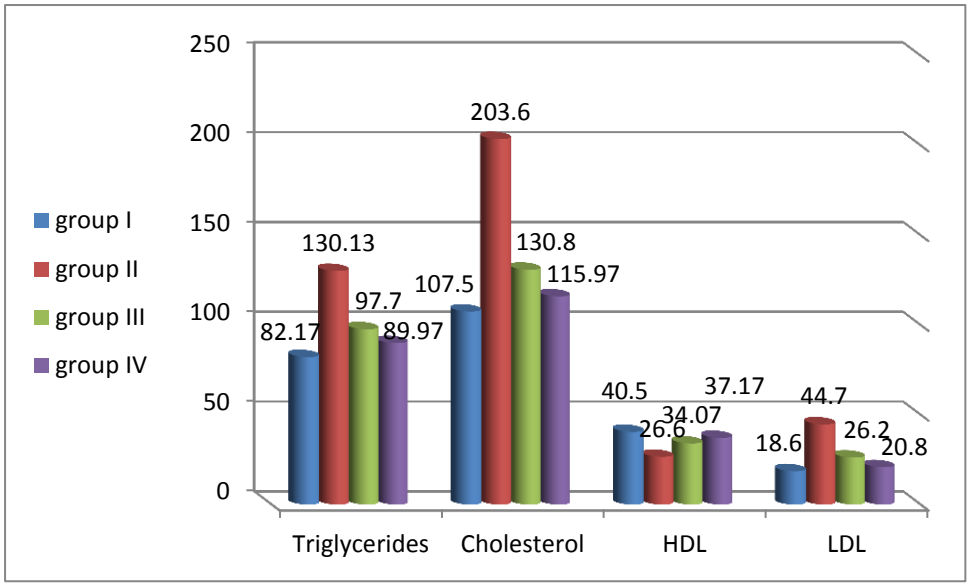

Fig. 1: Lipid profile levels in different experimental groups

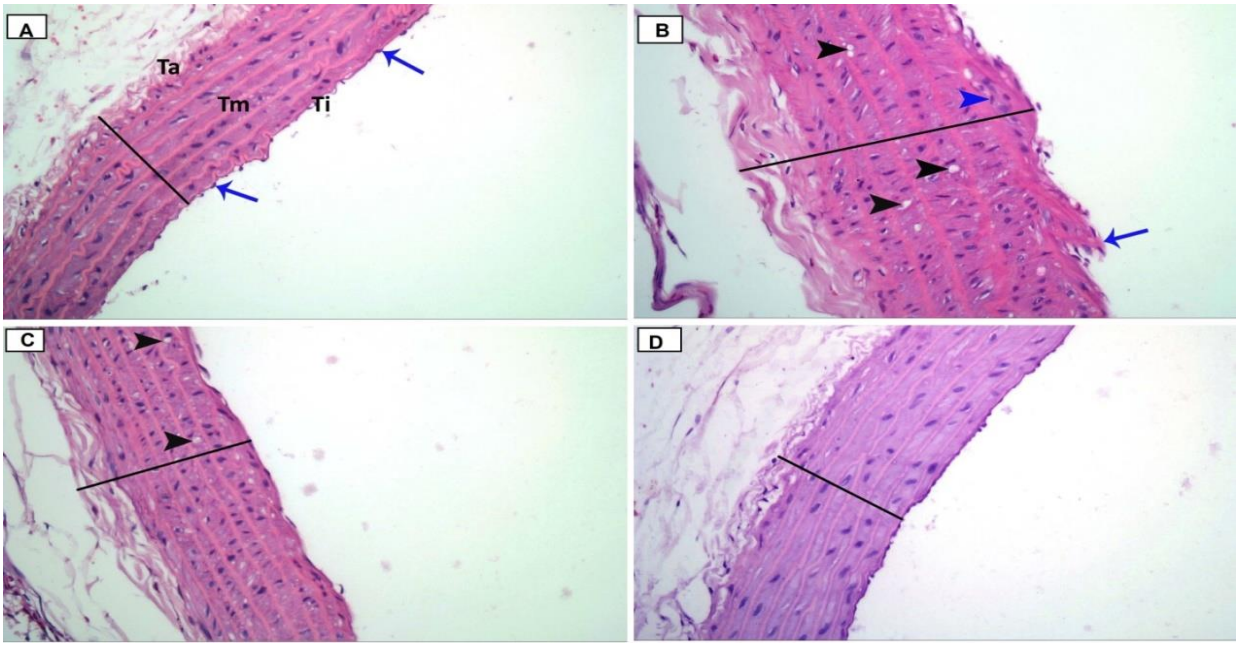

Fig. 2: Photomicrographs of H &E-stained aortic sectors: (A) Control group displays typical histological architecture and thickness (line) of the aortic wall. It is composed of three layers; tunica intima (Ti) demonstrates simple squamous endothelial cells (blue arrows), smooth muscle cells are organized concentrically forming the tunica media (Tm), in addition to tunica adventitia (Ta). (B) Group II presents intimal projection (blue arrow) into the lumen together with increased aortic wall thickness (line). Foam cells (black arrowheads) appearance, migrating smooth muscle cell to the intima (blue arrowhead). (C) Group III displays decrease in the aortic wall thickness (line) with some foam (arrow heads) still appeared. (D) Group IV exhibits nearly similar thickness of the aortic wall (line) and histological appearance comparable to the control group. (H&E x200)

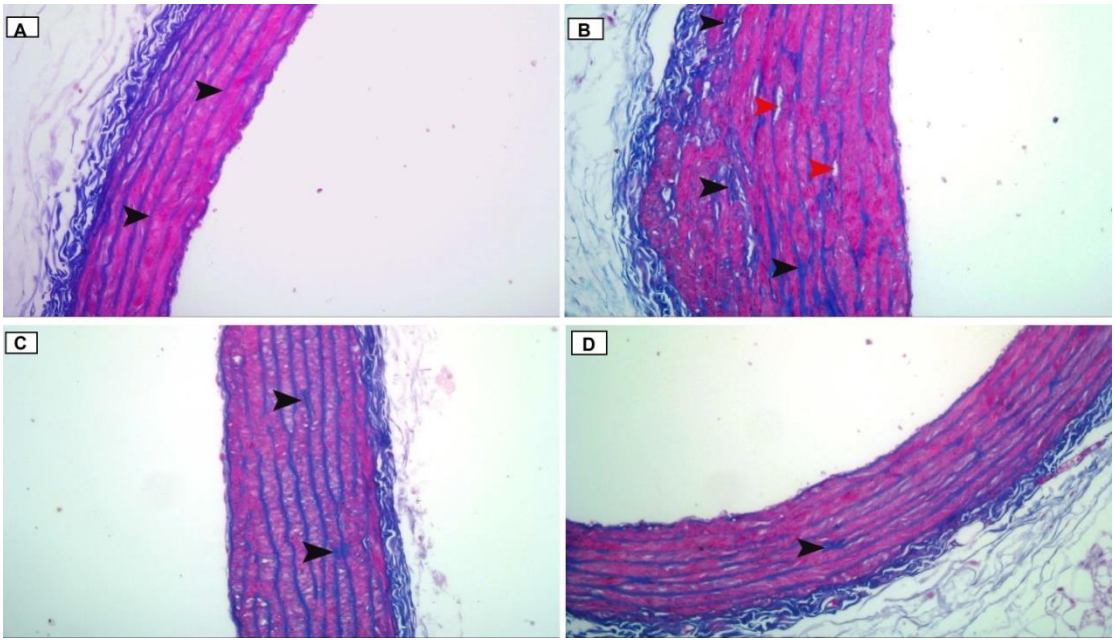

Fig. 3: Photomicrographs of Masson trichome stained aortic slices: (A) Control group displays minimal collagen fiber distribution (black arrow heads).(B) Group II presents marked deposition of collagen fibers (black arrow heads) , lipid vacuolations also seen (red arrow heads). (C) Group III displays decreased collagen fibers deposition (black arrow heads). (D) Group IV exhibits minimal quantity of collagen fibers (black arrow head). (Masson trichome x200)

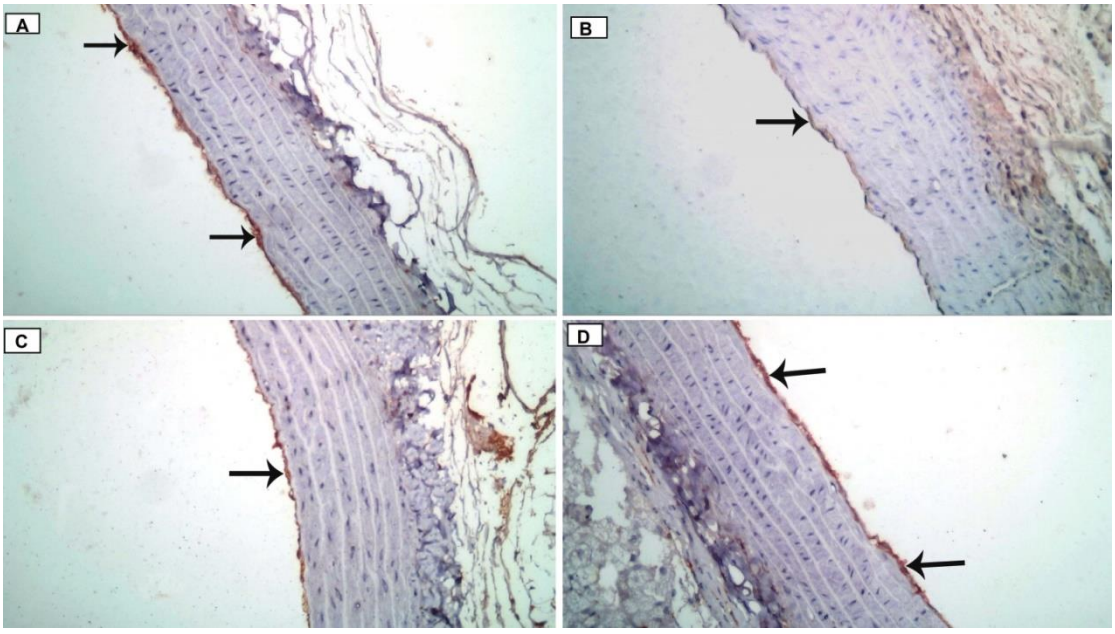

Fig. 4: Photomicrographs of eNOS immune stained aortic sectors: (A) Control group illustrates intense eNOS immune expression in the tunica intima (black arrows).(B) Group II presents weak eNOS immunoreaction in the intima (black arrow) . (C) Group III displays moderate expression of eNOS immune stain (black arrow). (D) Group IV exhibits an obvious increase in eNOS immune expression (black arrows). (eNOS immunostaining x200)

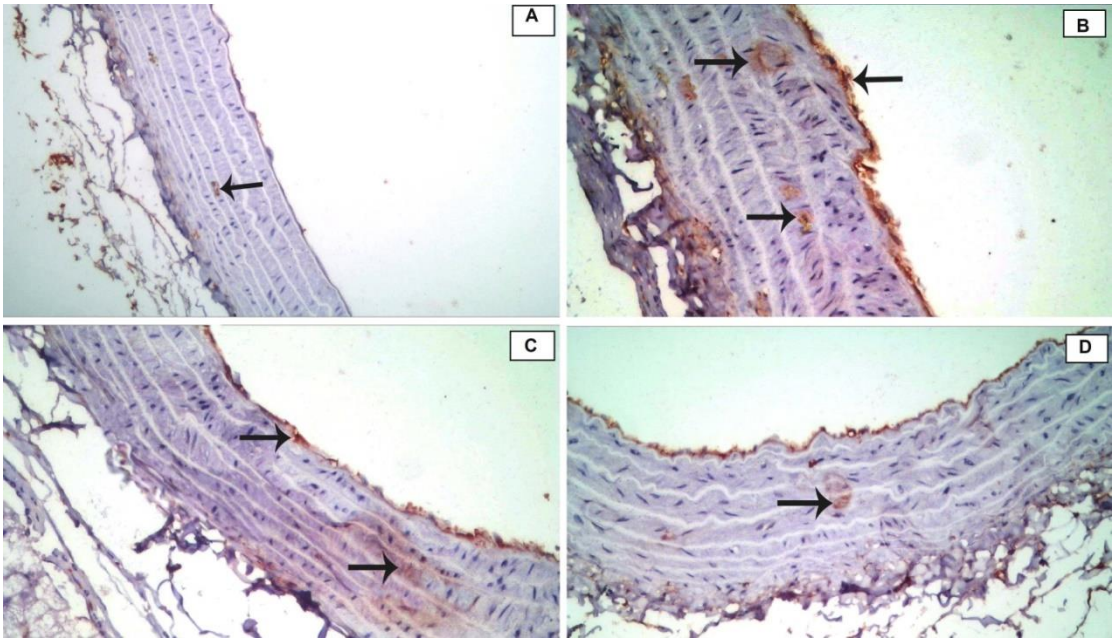

Fig. 5: Photomicrographs of TNFα immune stained aortic sectors: (A) Control animals show minimal TNFα immune reaction (arrow). (B) Group II presents extensive expression of TNFα immune stain , compared with the control animals (arrows) . (C) Group III moderately alleviates TNFα immune expression (arrows). (D) Group IV displays weak TNFα immune reactivity (arrow) . (TNFα immunostaining x200)

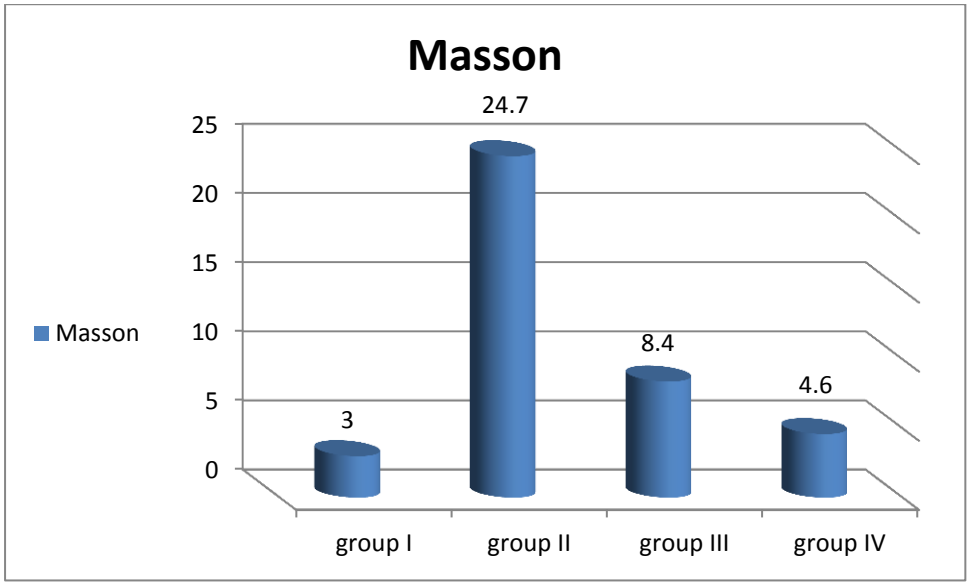

Fig. 6: The mean area percentage of Masson trichome stain in all groups.

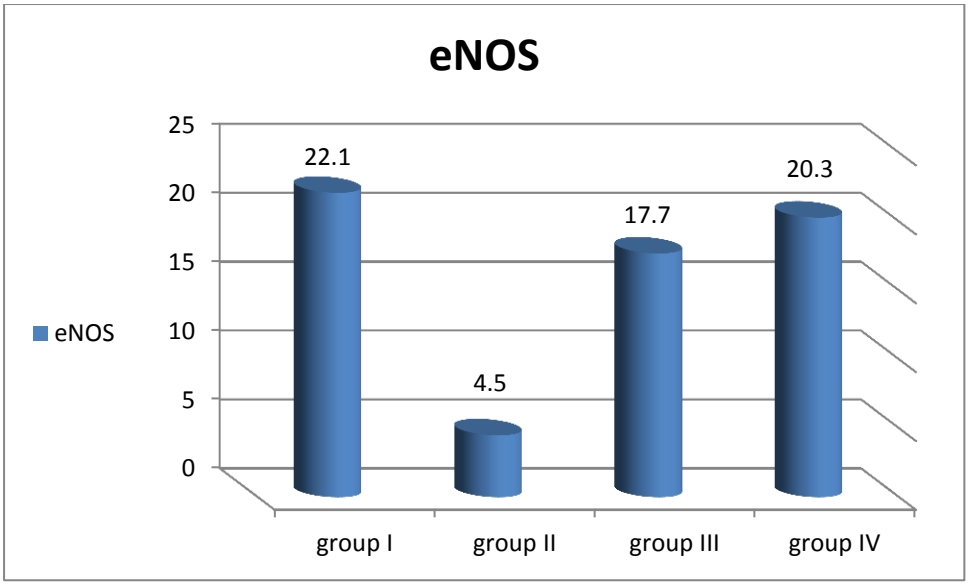

Fig. 7: The mean area percentage of eNOS immunostain in all groups**.**

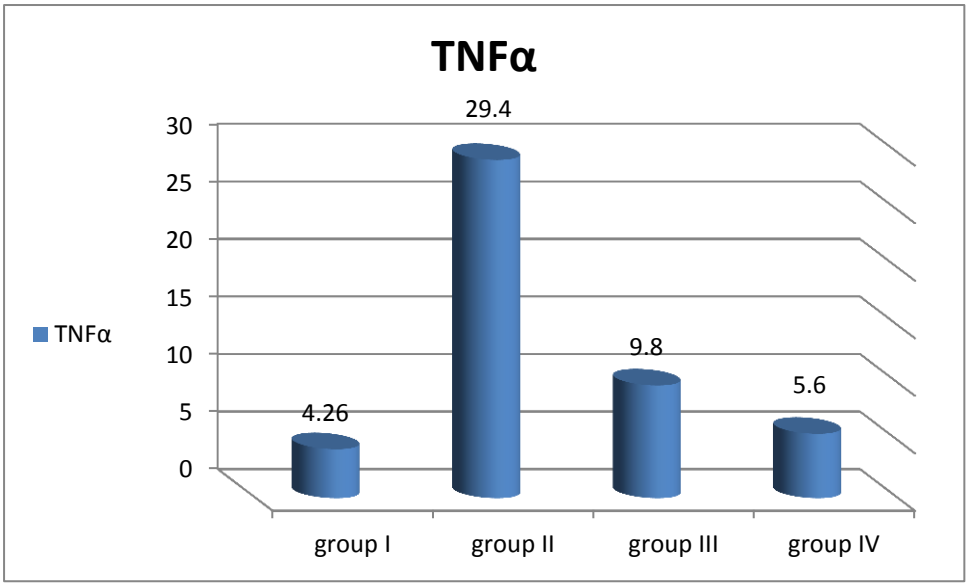

Fig. 8: The mean area percentage of TNF α immunostain in all groups.

Table 1: Serum lipid profile levels in different animal's groups

	Group I (Control)	**Group II (atherosclerosis)**	**Group III (atorvastatin)**	**Group IV (sesame oil +atorvastatin)**
Cholesterol mg/dl	82.17 ± 2	130.13 ± 5.1 [a, c & d]	97.7 ± 2.1584 [a ,b & d]	89.97 ± 2.1 [a ,b & c]
Triglyceride mg/dl	107.5 ± 2.4	203.6 ± 3.4 [a, c & d]	130.8 ± 6.9 84 [a , b & d]	115.97 ± 5.3 [a ,b & c]
LDL mg/dl	18.6 ± 1.6	44.7 ± 2.9 [a, c & d]	26.2 ± 2.584 [a & b]	20.8 ± 1.1 [b]
HDL mg/dl	40.5 ± 1.4	26.6 ± 1.3 [a, c & d]	34.07 ± 2.184 [a & b]	37.17 ± 1.2 [b]

Data in the table are expressed as "mean ± SD|, * indicates significance ≤ 0.05

a: Significance vs Control, b: Significance vs group II, c: Significance vs group III, d:Significance vs group IV.

Table 2: The average area % of Masson trichome stain, eNOS immunostain ,and TNF α immunostain across subjects

	Control Group	Group II	Group III	Group IV
Masson area %	3 ± 0.8	24.7 ± 2.6 [a, c & d]	8.4 ± 0.9 [a , b & d]	4.6 ± 0.9 [b & c]
eNOS area%	22.1 ± 1.5	4.5 ± 0.96 [a, c & d]	17.7±0.84 [a & b]	20.3± 0.8 [b]
TNF α area %	4.26 ± 0.9	29.4 ± 0.9 [a, c & d]	9.8 ± 0.9 [a , b & d]	5.6 ± 1.1 [b & c]

Data in the table are expressed as "mean ± SD|, * indicates significance ≤ 0.05

a: Significance vs Control, b: Significance vs group II, c: Significance vs group III, d: Significance vs group IV

Discussion

Atherosclerosis is a primary cause of mortality globally. From sedentary lifestyle, excessive food consumption, to other conditions like diabetes and hyperlipidemia; all contribute to the pathophysiology of the disease, which is largely caused by inflammation and oxidative stress [19]. Al-Subari *et al*., (2026) [20] suggest that the high fat diet promoted cholesterol deposition in the vascular endothelial cells and raised the level of circulating lipoproteins.

This study aimed to reveal the protective synergistic effect of atorvastatin and sesame oil on aortic atherosclerosis in albino rats.

In the present study, the levels of Cholesterol, triglyceride & LDL were significantly elevated while HDL significantly diminished in atherosclerotic group in relation to the control group. Our data are in good agreement with the previously reported study of El Zouka *et al.,* (2024) [21] which revealed a significant rise in the serum cholesterol and LDL levels as well as a significant diminution in the serum HDL level in atherosclerotic rats as compared to normal rats. Also Morsy *et al.,* (2024) [16] demonstrated significant elevation in cholesterol, triglyceride & LDL levels and significant reduction in HDL level in HFD group as compared to the control group. Moreover, Nipate *et al.,* (2023) [22] found that rats fed the high-fat diet had lower levels of high-density lipoprotein (HDL) and higher levels of serum total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL).

Meanwhile in this study, group III (Atorvastatin group) and group IV (Atorvastatin + Sesame oil group) demonstrated a momentous reduction in Cholesterol, Triglycerides & LDL level and considerable increase in HDL level as compared to group II (Atherosclerotic group). Parallel with our findings, the results of Ibrahim *et al.,* (2020) [13] who indicated that there was significant decrease in total cholesterol and LDL-cholesterol.

In atherosclerotic + sesame seeds group but HDL level was increased. Similar studies indicated that Diets containing SSO (Sesame Seed Oil) significantly reduced the serum lipid profile with elevation in HDL [23].

In the current work, H&E stained aortic sections from atherosclerotic rats showed increased aortic wall thickness with intimal projection into the lumen. Foam cells appeared in the tunica media. There was proliferation of smooth muscle cells as well as their migration to the intima. This goes in line with another author's study who observed that there was detachment developed on the inside of the tunica intima in almost endothelial cells, with no basement membrane

[24]. In certain areas of this layer, there were inflammatory holes of white blood cells caused by tissue degradation, and the tunica media looked to have disintegrated in all elastic sheets from the collagen and smooth muscle fibers and to be vacuolated in this area. Similar findings were reported by other investigators that confirmed that aorta showed hemorrhagic streak, fibrous deposition, and foam cell deposition in the endothelium layer [22]. Also, Morsy *et al.,* (2024) [16] study perceived that Aortic sections in the HFD group illustrated partial intimal desquamation, thickening of the aortic wall and muscular layer with intimal bulging and foam cells. El-Zouka *et al.,* (2024) [21] added that in atherosclerotic group, on histologic level, numerous atheromatous plaques were visible on the aortic wall. A suboptimal collection of foamy cells was seen in several of them. They were observed breaking apart the smooth muscle cells in the medium. Other regions have more advanced atheromas that took up the majority of the vessel wall. They displayed extensive dystrophic calcification and central necrosis. The atheromas' intima was thick and hyperplastic.

The current study successfully revealed that administration of atorvastatin to rats in group III displayed improvement in the histological structure with decrease in the aortic wall thickness. While, treatment with both atorvastatin and sesame oil in group IV exhibited nearly similar thickness and histological appearance of the aortic wall comparable to the control group. In line with that, Nipate *et al., (*2023) [22] proved that Experimental rats treated with atorvastatin showed reduction in foam cell deposition and fibrous deposition in endothelial layer. Correspondingly, the results of some investigators declared that when compared to the afflicted group, the histological results of the AV (Atorvastatin group) demonstrated a refinement in the aortic wall layers. Nevertheless, there was disruption was still observed in the middle of Tunica. Statin therapy lowers mortality and cardiovascular event risk mostly because it lowers cholesterol [24].

Additionally, the SO (Sesame Oil) treated group showed a significant enhancement in the aortic wall layers in the tissue sections by reducing oxidative stress due to hyperlipidemia [25]. These results were in line with Mahmoud *et al.,* (2014) [26] who attributed that Preventive treatment of hypercholesterolemic rats with simvastatin strongly alleviated aortic wall thickness. Moreover, El Zouka *et al.,* (2024) [21] declared that rats given atorvastatin exhibited a significant reduction in the quantity and size of atheromas. The intima was thin when examined at high power. Ordered smooth muscles were restored, according to the media. Occasionally, only small foci of accumulation of foamy cells were observed. Unintentionally, fine dystrophic calcifications were found. There was neither necrosis nor inflammation.

In our study, Aortic sections stained with masson trichrome in atherosclerotic group displayed marked deposition of collagen fibers between aortic layers. This was in accordance with Morsy *et al.,* (2024) [16] who stated that HFD group showed collagen deposition and lipid vacuolation. Similar results obtained by other researchers [21] who found that masson trichrome stained atherosclerosis model's aortic sections, the atheromas were covered by a thick fibrosis coating. Furthermore, fibrosis was observed within the atheroma. Smooth muscle cells were thought to create collagen, which is a unique kind of fibrous tissue [27].

Meanwhile, in the present study, group III (Atorvastatin treated group) displayed decreased collagen fibers deposition. While combination of Atorvastatin and sesame oil in group IV exhibited less amount of collagen fibers. This in accordance to El Zouka *et al.,* (2024) [21] who revealed that collagen levels declined substantially following treatment with atorvastatin. The fibrous coating

vanished. Collagen was minimal in atorvastatin treated rats.

Our results revealed that there was powerful TNFα immune expression in the aortic layers of group II (Atherosclerotic group), compared with the control animals. These outcomes were supported by the results of Morsy *et al*., (2024) [16] which showed that the immunohistochemical study revealed enhanced expression of TNFα inflammatory biomarker in the aortic tissue of the HFD group as compared to the control. This was matched with another study by Shatoor *et al.*, (2019) [28], where TNFα protein levels showed significant upregulation in the HFD group as compared to the control.

In the present work, group III (Atorvastatin group) moderately alleviated TNFα immune expression while, group IV (Sesame oil + Atorvastatin group) displayed weak TNFα immune reactivity. Correspondingly, Peng *et al*., (2018) [29] perceived that atorvastatin reduced TNFα in in-vivo models of atherosclerosis (mouse models) via autophagy upregulation, e.g. reduced secretion of TNFα in plaques. According to other researchers findings's, Wang *et al.*, (2014) [30] showed that atorvastatin suppresses TNFα mRNA and protein release in macrophages under lipopolysaccharide (LPS) stimulation, mediated via heme oxygenase-1 (HO1) induction. Moreover, yao *et al.*, (2023) [31] declared that in a rat model, particulate matter (PM2.5) exposure increased TNFα and atorvastatin treatment reduced TNFα among other inflammatory markers.

Additionally, Krithika *et al.*, (2015) [32] indictaed that the aqueous extract of sesame oil (SOAE) reduced expression of TNFα (and IL1, IL6) in LPS stimulated macrophages, and in vivo reduced TNFα in animal models. Also, Hadipour *et al.*, (2023) [33] declared that sesame and its lignans modulate inflammation, including suppression of NF-κB, COX, and reduction of proinflammatory cytokines (including TNFα) in multiple models. Aluganti *et al.*, (2018) [34] stated that pretreatment with sesame oil aqueous extract (SOAE) in animal model of atherosclerosis lowered plasma TNFα, IL6, MCP1, VCAM1.

The majority of the NO generated in this tissue is produced by eNOS, the primary NOS isoform in the vasculature. By activating soluble guanylyl cyclase and raising cyclic guanosine monophosphate (cGMP) in smooth muscle cells, vascular NO dilates all kinds of blood arteries. Platelet adhesion and aggregation are strongly inhibited by NO produced in the direction of the arterial lumen. Additionally, NO can prevent leukocyte adherence to the artery wall by either decreasing CD11/CD18 expression on leukocytes or interfering with the leukocyte adhesion molecule's capacity to produce an adhesive connection with the endothelial cell surface [35].

Since white cell adhesion is a precursor to atherosclerosis, NO may prevent atherogenesis from starting. Additionally, it was observed that NO inhibits vascular smooth muscle cell proliferation, mitogenesis, and DNA synthesis. Smooth muscle is shielded from platelet-derived growth factor exposure by the suppression of platelet adhesion and aggregation. As a result, NO also stops the development of fibrous plaque, a later stage of atherogenesis. Endothelial NO is likely the furthermost important antiatherogenic defense mechanism in the vasculature, due to its combined ability to promote vasodilation, inhibit platelet aggregation, and reduce oxidative stress [36].

Our results revealed that group II presented marked reduction in eNOS immune expression compared with the control animals. Similarly, Abdel kawi & Hashem, (2015) [27] study Rats with diet-induced hypercholesterolemia exhibited a marked decrease in aortic eNOS activity, accompanied by an increase in iNOS. These results are consistent with Mahmoud et al. (2014) [26] who reported similar changes in rats fed a high-cholesterol diet.

While, in our study, Group III exhibited moderate expression of eNOS immune stain. Interestingly, co administration of both atorvastatin and sesame oil in group IV exhibited an obvious increase in eNOS immune expression. Mahmoud *et al.,* (2014) [26] study goes with our results which stated that aortic eNOS activity was markedly elevated in hypercholesterolemic rats treated prophylactically with simvastatin. This also goes in accordance with Cebova *et al.,* (2018) [37] who found that, in Zucker rats, SO (sesame oil) and SO + SIM (sesame oil +simvastatin) treatments markedly elevated the appearance of phosphorylated eNOS protein in left ventricle. Sesame oil contains several antioxidants, especially tocopherol and lignan compounds sesamin, sesamolin, and sesamol [38]. Sesame oil and its components inhibit the inflammation signalling cascade and so help the management of inflammatory diseases [12]. Regulation of the signalling pathway involved in the expression of ECM remodelling enzymes could help to reduce the risk of atherosclerosis. The study of Aswani *et al.,* (2024) [39] declared that the dietary supplementation of sesame oil in high-fat-fed rats downregulated the inflammatory and oxidative stress markers and leukocyte-endothelial cell adhesion molecules, thereby slowing down plaque formation. Sesame oil can act on multiple targets of atherosclerosis including scavenging reactive oxygen species produced by oxidative stress, blocking the inflammatory pathways to inhibit their target expression and activation, and blocking the activity of the matrix remodelling enzymes involved in plaque formation and rupture Hsu and Parthasarathy, (2017) [12].

There were a number of limitations to this study that should be taken into account. First, Because of the substantial physiological and metabolic differences between species in lipid handling, lipoprotein metabolism, and the chronic progression of atherosclerosis, the use of a rat model naturally restricts the direct translatability of these results to humans. Second, the sample size might be inadequate and only comprised male rats, which might not represent any sex-related physiologic variability in response to dietary fats, even though it was sufficient for statistical analysis. Third, the 8-week treatment time may be insufficient to accurately examine the long-term effects of atherosclerosis, a chronic and progressive disorder; however it is sufficient to see notable changes in the rat model. Longer-term longitudinal trials would offer a more comprehensive picture of sustained safety and efficacy. Fourth, although this study assessed several important biomarker indicators related to lipid profiles, inflammatory markers, in addition to histopathological variables, more thorough mechanistic analysis including gene expression profiling and gut microbiome characterization would provide deeper insights into the mechanisms involved. Even though these limitations, the current study offers supporting evidence for the potential protective roles of both atorvastatin and sesame oil against high fat diet induced atherosclerosis in a rat model, providing a basis for more detailed future researches.

CONCLUSION

The present investigation provides a significant impact of atorvastatin and sesame oil combination in improving serum level of Cholesterol, Triglyceride, LDL and HDL, reduction of the aortic wall thickness and aortic damage, an obvious increase in eNOS immune expression with minimal TNFα immune reactivity which in turn led to a reduction in inflammatory indicators and atherosclerosis. This might serve as guidance on how to employ this combination to combat atherosclerosis and cardiovascular problems. It is recommended to have additional investigations for the beneficial effects of sesame oil on other human organs which may be harmed by increased high fat diet as other vessels in the body and the liver.

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الملخص العربى

التأثيرات التآزرية للأتورفاستاتين وزيت السمسم في الحماية من تصلب الشريان الأبهر الناتج عن نظام غذائي عالي الدهون في الجرذان: دراسة نسيجية وكيميائية مناعية

الملخص

الخلفية: .يعدّ تصلّب الشرايين مشكلة صحية خطيرة تزيد من احتمالية الإصابة بالسكتات الدماغية وأمراض القلب والأوعية الدموية. ويُعتبر النظام الغذائي الغني بالدهون (HFD) العامل الرئيسي في حدوث تصلّب الشرايين. إذ تؤدي المستويات المرتفعة من الدهون في الدم إلى خلل في وظيفة البطانة الوعائية، مما يسبب تراكم الدهون تحت البطانة وتكوّن اللويحات .

الهدف: توضيح التأثير الوقائي لكلٍ من الأتورفاستاتين وزيت السمسم على تصلّب الشريان الأبهر الناتج عن النظام الغذائي عالي الدهون لدى الجرذان.

المواد والطرق: تم تقسيم اثنان وثلاثون حيوانًا إلى أربع مجموعات: المجموعة الأولى (المجموعة الضابطه)، والمجموعة الثانية (مجموعة تصلب الشرايين): أُعطيت الجرذان نظامًا غذائيًا عالي الدهون حتى نهاية الأسبوع الثامن لتحفيز تصلب الشرايين، والمجموعة الثالثة (مجموعة الأتورفاستاتين): أُعطيت الجرذان نظامًا غذائيًا عالي الدهون وأتورفاستاتين عن طريق الفم بجرعة ٢٠ ملجم/كجم/يوم لمدة ثمانية أسابيع، والمجموعة الرابعة (مجموعة الأتورفاستاتين + زيت السمسم): أُعطيت الجرذان نظامًا غذائيًا عالي الدهون بالإضافة إلى أتورفاستاتين (٢٠ ملجم/كجم/يوم) و١٠% من زيت السمسم لنفس المدة. تم سحب عينات دم لتقييم مستوى الكوليسترول والدهون الثلاثية والبروتينات الدهنية منخفضة الكثافة (LDL) والبروتينات الدهنية عالية الكثافة (HDL) في الدم. كما تم استئصال الشرايين الأبهرية على الفور وجُهِّزت للفحص النسيجي والكيميائي المناعي.

النتائج: أظهرت مجموعة تصلب الشرايين ارتفاعًا ملحوظًا ($P \leq 0.05$) في مستويات الكوليسترول، والبروتين الدهني منخفض الكثافة (LDL)، والدهون الثلاثية في مصل الدم، بينما انخفضت كمية البروتين الدهني عالي الكثافة (HDL) بشكل كبير. وأظهرت المقاطع المصبوغة بصبغة الهيماتوكسيلين والإيوسين زيادة في سمك جدار الشريان الأورطي مع بروز الطبقة الداخلية في التجويف. وظهرت خلايا رغوية في الطبقة الوسطى. بالإضافة إلى ذلك، لوحظ انخفاض ملحوظ في التعبير المناعي لإنزيم أكسيد النيتريك البطاني (eNOS)، ولكن كان هناك تفاعل مناعي قوي لعامل نخر الورم ألفا (TNFα) في طبقات الشريان الأورطي. ان العلاج بالأتورفاستاتين يُحسّن مستويات الدهون في مصل الدم، البنية النسيجية لجدار الأبهر ، وكذلك نتائج الفحص المناعي النسيجي. ومع ذلك، فقد أظهر العلاج المركب بالأتورفاستاتين وزيت السمسم نتائج أفضل.

الخلاصة: أظهرت الدراسة الحالية تأثيرًا وقائيًا كبيرًا لمزيج الأتورفاستاتين وزيت السمسم في تحسين مستويات الدهون، وتخفيف تلف الأبهر، وتقليل المؤشرات الالتهابية، وبالتالي الحد من تصلب الشرايين.