REVIEW ARTICLE

Comparative Study on Therapeutic Effect of Wheat Germ Oil and Sesame Oil with Some Lipid-lowering Drugs in Local Male Rabbits with Hyperlipidemia, induced by Triton x-100: Physiological and Histological Study

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ABSTRACT

Hyperlipidemia is a heterogeneous group of disorders characterized by excess fat in the bloodstream, and it is considered the primary mediator of a cascade of atherosclerosis. Also, hyperlipidemia is associated with cardiovascular diseases (CVD), including coronary heart disease and stroke, and is one of the leading causes of mortality in developed and developing countries. The present study aimed to identify the therapeutic effect of wheat germ oil and sesame oil on induced hyperlipidemia by triton X-100 and compare their effect with Atorvastatin and Rosuvastatin on some biochemical parameters in local male rabbit serum, which included estimation of lipid profile levels (cholesterol, triglycerides, low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), High-density lipoprotein (HDL), HDL-C, LDL-C, VLDL-C) and lipid peroxidation marker - *Malondialdehyde* (MDA); in addition to the histological effects of wheat germ oil, sesame oil, Atorvastatin and Rosuvastatin on Aorta tissues.

Keywords: COVID-19, Health care workers, Knowledge, Mosul city

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INTRODUCTION

Hyperlipidemia is a heterogeneous group of disorders characterized by excess fat in the bloodstream.[1] Hyperlipidemia is accompanied by elevated serum total cholesterol (TC), triglycerides (TGs), low-density lipoprotein cholesterol (LDL-C), and very LDL-C (VLDL-C), and decreased high-density lipoprotein cholesterol (HDL-C) levels.[2] It is associated with cardiovascular diseases (CVD), including coronary heart disease and stroke, and is one of the leading causes of mortality in developed and developing countries, accounting for 30% of all worldwide deaths per year.[3]

Classification of Hyperlipidemia

Hyperlipidemia can be classified based on therapeutic considerations as follows:[4]

Type of dyslipidemia	*Lipoproteins involved*	*Affected serum lipids*
I. Hypercholesterolemia	LDL	Cholesterol
II. Mixed hyperlipidemia	LDL + VLDL	Cholesterol and triglyceride
III. Hypertriglyceridemia	VLDL	Triglyceride

Hyperlipidemia is considered the primary mediator of a cascade of atherosclerosis.[5] Atherosclerosis is a chronic vascular disease caused by various factors and is the pathological mechanism linked to cardiovascular and cerebrovascular diseases. Since endothelial dysfunction is the initiation factor of atherosclerosis, protecting endothelial function is essential for the early prevention and treatment of atherosclerosis.[6] Different theories have been proposed for the pathogenesis of atherosclerosis, including lipid infiltration, inflammatory reaction, damage response, thrombosis, natural immune hypothesis, etc.[7] Lipid infiltration theory concludes that lipid metabolism disorders mainly cause atherosclerosis.[8] Endothelial dysfunction, inflammation, and oxidative stress induced by high blood lipids were considered to be the pathological basis of the formation of atherosclerotic plaque.[9]

Wheat germ is a major by-product of industrial milling of wheat and represents 2.5–3% of the wheat grain, it is also a natural source of highly concentrated nutrients. Wheat germ contains about 11% oil, 30% protein, 53% carbohydrate, 12% water, and 4% ash.[10] Studies have shown that oil extracted from wheat germ is consider unsaturated and has a very high nutritional value.[11] Wheat germ oil (WGO) is rich in unsaturated

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fatty acids (83.45%), mainly linoleic acid (64.82%) and oleic acid (13.19%). While Sn-2 position fatty acids Included: linoleic acid at 87.29%, oleic acid at 12.11%, and palmitic acid 0.60%, where it can be readily absorbed during digestion.[12] WGO is an excellent source of natural vitamin E, the most powerful natural antioxidant.[13] Furthermore, WGO is rich in functional phytochemicals, mainly flavonoids, sterols, octacosanols, and glutathione.[14,15] Recently, it has been shown that WGO intake results in a rapid increase in vitamin E in different rat tissues and exerts high protection against oxidative damage.[16,17]

Sesame is a source of premium vegetable oil, and its seeds contain the highest amount of oil among other oil crops. Sesame oil SO contains antioxidants such as sucsesamin, sesamolin, sesamol, and tocopherols and is therefore very stable and has a long shelf life, so it can be blended with less stable vegetable oils to improve its stability and attractiveness.[18] SO also contains essential minerals and vitamins such as Ca, P, Fe, niacin, selenium, and thiamin.[19] SO also contains unsaturated fatty acids (85.61%), linoleic acid (47.62%), oleic acid (35.32%), saturated fat (35.32%). About 15.25%, which includes palmitic fatty acid and stearic acid, by approximately (11.49%) and (2.64%), respectively.[20]

Statins, also known as HMG-CoA reductase inhibitors, are a class of lipid-lowering drugs among the most commonly used to treat hyperlipidemia.[21] Statins include many species belonging to this category, such as Atorvastatin, Rosuvastatin, Fluvastatin, Simvastatin, Lovastatin and others.[22]

Atorvastatin AV is a class of drugs known as statins, mainly used as a lipid-lowering agent to prevent cardiovascular events and be Like all statins, AV works by inhibiting HMG-CoA reductase.[23] AV tablets are available in various doses (10, 20, 40, and 80 mg), and maybe taken with or without food at any time. AV reduction ability doses may be based on LDL-C intensity.[24] AV is rapidly absorbed after oral administration. Its peak concentration in blood plasma is within 1–2 hours, and the half-life of AV is about 14 hours, while its active metabolites have a half-life of about 20–30 hours and are highly correlated with plasma protein. More than 98% of it is bound to protein, mainly with albumin.[25]

Rosuvastatin RV is a competitive inhibitor of HMG-CoA reductase and has a mechanism of action similar to that of other statins, RV is known by the brand name Crestor is used to prevent cardiovascular disease and also to treat abnormal lipids. Crestor is a Rosuvastatin calcium salt where calcium replaces hydrogen in the carboxylic acid group.[26] RV is available in tablet form in different doses ranging from (1–80 mg) and is usually taken orally.[27] After taking a single oral dose, RV reaches its maximum concentration in the blood plasma within 3 to 5 hours. Usually, 88% of it binds to proteins, mainly with albumin protein. RV undergoes metabolism by cytochrome P450 to its metabolite N-desmethyl rosuvastatin, which possesses approximately one-sixth to one-half of the HMG-CoA reductase inhibitor activity and (90%) of RV and its metabolites is excretes in feces.[28]

Lipid peroxidation LP, a series of interactions between free radicals and fatty acids, also known as lipid rancidation, occurs when antioxidant defense systems cannot eliminate or remove free radicals as a result of their accumulation. LP is carried out in three main ways: auto-oxidation, which occurs automatically in living cells; photo-oxidation due to increased exposure of fatty acids to light, which generates free radicals; and enzyme oxidation, caused by increased activity of oxidative enzymes.[29] One of the compounds produced by LP is malondialdehyde (MDA), which is one of the final products of LP, which is self-occurring in the body and is produced by peroxidation of polyunsaturated fatty acids by the reaction of reactive oxygen species (ROS) as a result of the depletion of antioxidant systems.[30] The high concentration of MDA is good evidence of LP and is an indicator of some diseases, especially atherosclerosis.[31]

MATERIALS AND METHODS

Induction of Hyperlipidemia

Hyperlipidemia was induced in rabbit males with triton X-100 intraperitoneal injection at a concentration of (100 mg/kg) after starving animals for 18 hours; animals were left for three days.[32,33] The animals were then treated after a preliminary test to confirm the induction of hyperlipidemia.

Animals

The study is performed in the animal house unit in the College of Veterinary, University of Tikrit, Iraq for 30 days. Local males rabbits were used with weight (1000–1500) grams and age (6–8) months.

Distribution of Study Animals

The study included 25 adult males divided randomly into (5) groups; each group contains (5) rabbits; these groups were given daily food and water throughout the study period. These groups included:

G1: Normal control group;
G2: Hyperlipidemic group induced by triton X-100;
G3: Treated with (1 mL/Kg) WGO only;
G4: Treated with (1 mL/Kg) SO only
G5: Treated with (0.3 mg/Kg) AV only;
G6: Treated with (0.3 mg/Kg) RV only

Biochemical Assays

Serum total cholesterol (TC) and Triglyceride (TG) concentration were estimated following the principle described by using a special and equipped analysis kit from the French company Biolabo.[34] High-Density Lipoprotein (HDL-C) was estimated following the principle described by using a special and equipped analysis kit from the French company Biolabom,[35] while serum low-density lipoprotein (LDL-C) and (VLDL-C) was calculated using Burtis & Ashwood formula.[36] Lipid peroxidation was determined by measuring the formation of (MDA) according to the method of Guidet and Shah.[37]

Histopathology

Small tissue piece of the aorta was collected in neutral buffered formalin for routine histoprocessing by paraffin embedding

technique, and sections was stained with hematoxylin and eosin.[38]

RESULTS

Physiologically

As shown in Table (1), results showed a significant increase (P≤0.05) in the concentrations of total cholesterol, triglycerides, LDL-C, VLDL-C. At the same time, HDL-C significantly decreased in the G2 (hyperlipidemic group) when intraperitoneal injected by (100 mg/Kg) of triton X-100 in compared with the G1 (normal control group). Whereas concentrations of total cholesterol, triglycerides, LDL-C, VLDL-C are significantly decreased, while HDL-C levels increased significantly in the G3 (WGO treated group), G4 (AV treated group) and G5 (WGO+AV treated group); in comparison with the hyperlipidemic affected group. The lipid peroxidation marker (MDA) also increased significantly (P≤0.05) in G2 (hyperlipidemic group) when compared with the G1 (regular control group). At the same time, MDA level decreased significantly in the G3 (WGO treated group), G4 (AV treated group), and G5 (WGO + AV treated group) in comparison with the hyperlipidemic affected group, as shown in Table 1.

Aorta

G2 (Control Group) As shown in figure 1 and 2 the aortic wall consists of the tunica intima layer of simple squamous cells based on the basement membrane with a sub-endothelial layer of soft connective tissue, and then the Tunica media, which consists of several flexible winding sheets of elastic fibers and between these sheets found collagen fibers in addition to the presence of smooth muscle fibers, and from the outside surrounded by a soft layer of connective tissue called tunica adventitia.

G2 (Hyperlipidemic group) As shown in Figures 3 and 4, an alienation appeared on the internal surface of tunica intima in almost endothelial cells, with the absence of a basement membrane. Tunica media appeared disintegrated in all elastic sheets from the smooth muscle fibers and collagen fibers and appeared vacuolation in the region of this layer; also, this layer showed inflammatory hole of white blood cells that results from the degeneration of tissue in some regions of this layer. Tunica adventitia showed infiltration of some white blood cells between collagen fibers, and widespread fatty tissue continued with this layer.

G3 (WGO Treated Group) As shown in figures 5 and 6, the aorta appears in three layers, tunica intima appeared the endothelial cells and the basement membrane in the internal surface of this layer. Tunica media appeared like normal tissue with a few disintegrations of the elastic sheets; tunica adventitia appeared a few white blood cells between collagen fibers.

G4 (SO Treated Group): As shown in figure 7, the aorta wall appeared in three layers: the tunica intima consisted of simple squamous cells based on the basement membrane and a sub-endothelial layer continuously with the middle tunic composed of large numbers of elastic plates continuously with smooth muscle fibers and colloidal fiber bundles as usually. The tunica adventitia consisted of bundles of colloidal fibrous tissue with some elastic fibers with blood capillaries in the region with the spread of limited white blood cells and the presence of fat cells in the outer part of the region tunica.

G5 (AV Treated Group): As shown in figure 8, the aorta appears in three layers, tunica intima appeared the endothelial cells and the basement membrane; tunica media appeared several vacuolation between smooth muscle fibers and elastic sheets. Tunica adventitia showed infiltration of white blood cells bet

G6 (RV Treated Group): As shown in figures (9,10), the basement of the aorta was evident and had thickened and underneath the endothelial layer, which contained connective tissue fibers and smooth muscle cells stretched longitudinally. The middle layer or tunica stretched flexible circular plates with

Table 1. Lipid profile and MDA concentrations in serum of hyperlipidemic rabbits

Groups / *Parameter*	*G1*	*G2*	*G3*	*G4*	*G5*	*G6*
Total cholesterol (mg/dL)	70.58 ± 4.33 b	99.80 ± 6.72 a	62.45 ± 3.94 b	58.74 ± 4.55 b	66.54 ± 2.86 b	65.04 ± 3.81 b
Triglycerides (mg/dL)	57.08 ± 7.68 b	85.54 ±8.25 a	54.48 ± 4.62 b	50.13 ± 5.34 b	65.66 ±6.87 b	62.05 ± 2.94 b
LDL-C (mg/dL)	26.22 ± 1.68 b	57.19 ± 4.38 a	12.014±1.29 c	5.984 ± 1.21 d	18.36 ± 2.11 c	15.82 ± 1.78 c
VLDL-C (mg/dL)	11.416±1.12 b	17.108±1.65 a	10.896±1.11 bc	10.006±1.31 c	13.132±2.03 b	12.41 ± 2.41 b
HDL-C (mg/dL)	32.94 ± 1.89 b	25.50 ± 2.08 c	39.54 ± 4.37 a	42.75 ± 3.15 a	35.04 ± 3.35 b	37.81 ± 4.10 a
MDA (K/mL)	4.741 ± 0.93 b	8.024 ± 1.24 a	3.582 ± 0.87 b	3.346 ± 0.90 c	5.031 ± 1.18 b	3.769 ± 0.73 b

Result expressed as Mean ± SD.
Numbers followed by different small letters indicate significant differences at (P≤ 0.05)

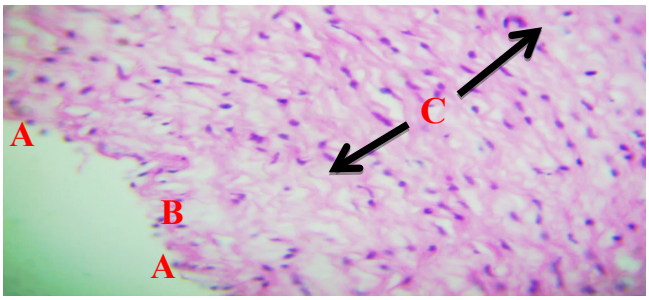

Figure 1: Section of G 1 Aorta, showed tunic intima (A) sub-endothelial layer (B) and tunica media (C) (H and E, 100×)

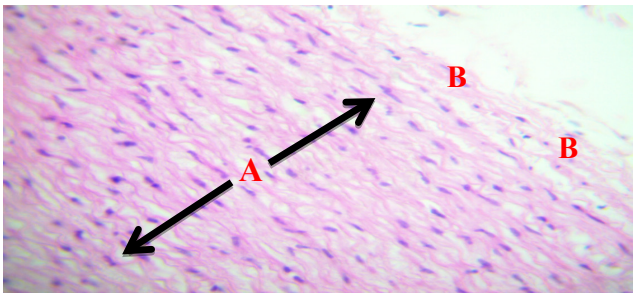

Figure 2: Section of G 1 Aorta, showed tunic media (A) and tunica media (B) (H and E, 100×)

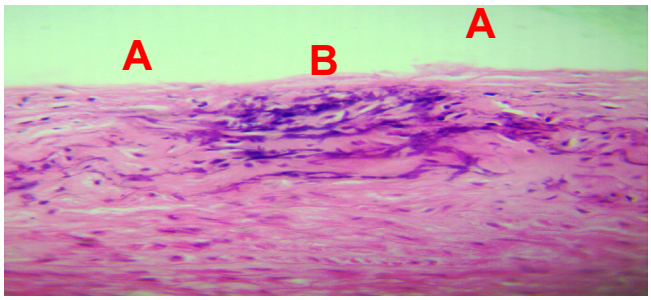

Figure 3: Section of G 2 Aorta, showed tunic intima (A) and degeneration of tunica media (B) (H and E, 100×)

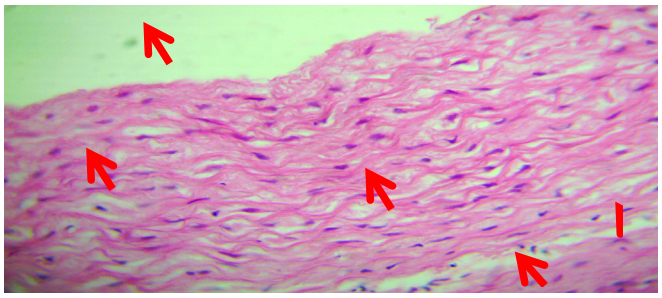

Figure 4: Section of G 2 Aorta, showed vacuolation in tunica media (H and E, 100×

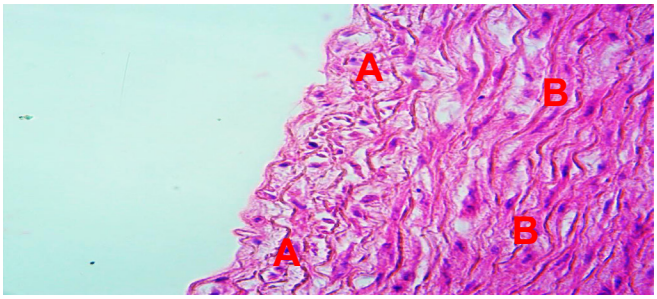

Figure 5: Section of G 3 Aorta, showed tunic intima (A) and tunica media (B) (H and E, 100×)

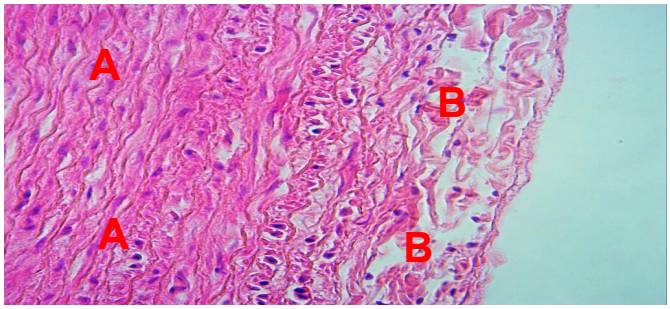

Figure 6: Section of G3 Aorta, showed part of tunic media (A) and tunica adventitia (B) (H and E, 100×)

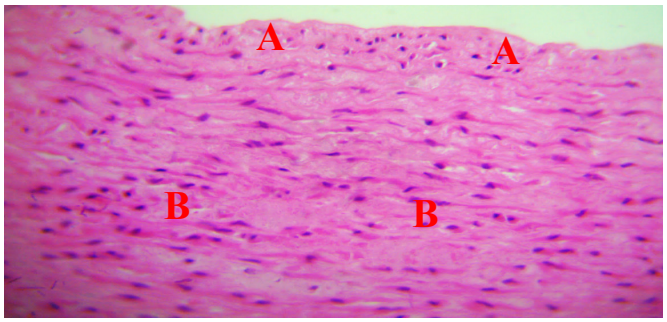

Figure 7: Section of G 4 Aorta, showed tunica intima (A), tunic media (B) (H and E, 100×)

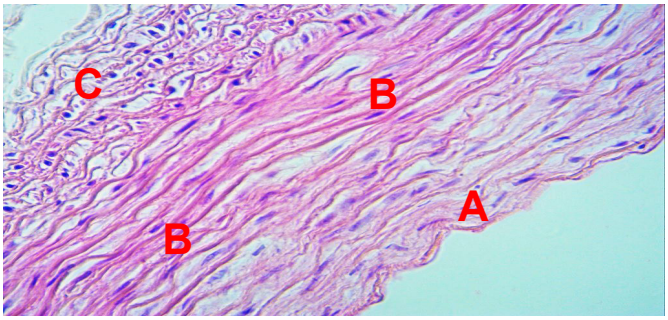

Figure 8: Section of G 4 Aorta, showed tunica intima (A), tunic media with vacuolation (B), and tunica adventitia (C) (H and E, 100×)

the presence of smooth muscle cells in addition to colloidal fibers, the outer part of the middle tunic has irregularity of the wavy shape of the elastic sheet, and the tunica adventitia has appearance disintegration.

DISCUSSION

The present study results agree with Parwin *et al.*[39] in the induction of hyperlipidemia by Triton X-100, resulting in increased levels of total cholesterol, triglycerides, LDL-C VLDL-C in addition to decreased levels of HDL-C, which are risk factors for cardiovascular disease. The role of triton is illustrated by inhibiting the action of the enzyme LPL as it prevents the absorption of TG-rich lipoproteins from circulation by extracellular tissues, which leads to increased blood lipid concentrations and results in hyperlipidemia in animals.[40] Treatment with WGO for rabbits with high

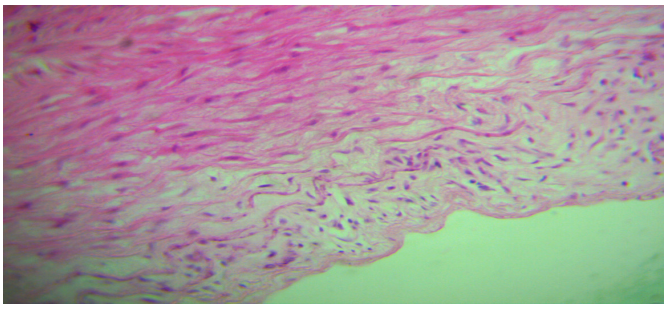

Figure 9: Section of G 6 Aorta shows the basal membrane of the tunica intima (A), the middle tunic shows elastic fibers and smooth muscle fibers (B) (H&E 40X).

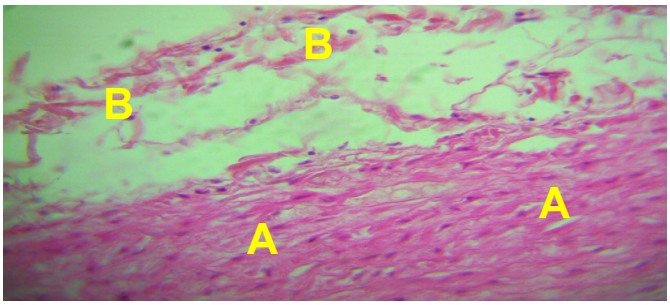

Figure 10: Section of G 6 Aorta shows a part of the middle tunica (A), tunica adventitia (B) (H&E 40X)

cholesterol, triglycerides, LDL-C, and VLDL-C in serum resulted in a significant decrease in lipid profile and consequently, a decrease in atherosclerosis, which was consistent with the results of Rezq and Mahmoud, 2011;[41] the phytosterols, Octacosanol and polyunsaturated fatty acids found in the WGO has a prominent role in reducing cholesterol absorption.[42] Also, WGO works to inhibit the generation of free radicals and increase the levels of autoantigenic antioxidants, the improved effect of WGO is due to its high vitamin E (alpha tocopherols) content which has the potential to inactivate reactive free radicals, thus avoiding the spread of the radical chain reaction.[43] WGO also contains fat-soluble carotenoids such as lutein, zeaxanthin and beta-carotene, all of which have an antioxidant effect.[44]

The present study results showed that treatment with sesame oil reflected induced hyperlipidemia by Triton X-100, which led to a significant decrease in the concentrations of cholesterol, triglycerides, LDL-C, and VLDL-C. In addition to increasing the concentration of HDL-C, which corresponded With a study of Chandrakala *et al*.;[45] this is due to the presence of sesamin, which is the main lignan in sesame seeds that have a lipid-lowering effect as sesamin prevents the absorption of cholesterol from the intestine.[46] The role of SO in increasing the inhibition of intestinal absorption of cholesterol is also due to interference in the production of lipoprotein, which increases the expression and protection of hepatic LDL-C receptors, leading to increased removal of LDL-C from the blood and increased cholesterol breakdown and destruction in the bod.[47]

Results of G5 and G6 (treated with AV and RV, respectively) are in agreement with Kashyapa *et al.* 2018 that showed a significant decrease in cholesterol, triglycerides, LDL-C and VLDL-C levels while increased levels of HDL-C.[48] The mechanism of action of statins is by blocking the active site of the first and main enzyme (HMG-CoA reductase) in the mevalonate pathway.[49] Inhibition of this site prevents the arrival of the substrate and the conversion of HMG-CoA to mevalonic acid in liver. It reduces the manufacture of hepatic cholesterol, leading to increased production microsomal HMG-CoA reductase and increased expression of LDL-receptors on the cell surface. It facilitates increased LDL-C clearance from the bloodstream and subsequent reduction of LDL-C levels in the blood plasma by 20% to 50%.[50] Many studies have shown that statins have antioxidant properties through their ability to reduce the production and/or activity of ROS, which prevent lipid and lipoproteins oxidation and thus inhibit the formation of ROS and reduced the harmful effects of these radicals.[51]

The histological results of the present study showed damage to the aortic wall layers of the hyperlipidemic group, especially in the tunica intima, as the lipid disturbance caused by Triton X-100 leading to almost total damage, the sub-endothelial layer thickness, and the disintegration and damage of the middle tunic layer, which corresponded to a study Naik *et al*. (2018).[52] This is due to the oxidative stress caused by hyperlipidemia, especially the oxidation of LDL-C lipoproteins in the arterial walls, which leads to the weakening of the endothelium, which contributes to the formation of atherosclerotic lesions,[53] also the decreased levels of HDL-C due to hyperlipidemia that observed in the affected group contributes to an increase atherosclerotic lesions and cardiovascular disease.[54] Increased lipid peroxidation resulting from ROS is a crucial mechanism for developing atherosclerosis and inflammatory vessels damage; lipid peroxidation leads to the formation of highly reactive mediators that contribute to cell injury or damage, change in cell function and generate biologically active compounds.[55] The histological results of the WGO treated group showed improvement in the aortic wall layers compared with the affected group with some disintegration between the elastic sheets forming the middle tunica; This is due to the antioxidant effectiveness of wheat germ oil.[56] WGO is known to contain important compounds such as: tocopherols, phenols, phytosterols mainly (camp sterol, β-cytosterol), flavonoids in addition to polyunsaturated fatty acids, and all of these compounds have an antioxidant activity which contributes to lower cholesterol levels leading to reduce Oxidative stress thus improving the structure of the aortic wall.[57]

The SO treated group showed a significant improvement in the aortic wall layers in the tissue sections of the present study by reducing oxidative stress due to hyperlipidemia. SO can reduce LDL-C levels while maintaining HDL-C levels making it effective in reducing atherosclerosis and risk of cardiovascular disease.[58] The main phenolic compounds of SO (sesamin, sesamolin, and sesamol), sesamol acts as a metabolic regulator, antioxidant, anti-atherosclerotic, anti-aging, antibacterial, and

antifungal agent in addition to its anticancer activity; sesamin and sesamolin also have a wide range of pharmacological effects, including Anti-inflammatory, anti-hypertension, and cardiac protective effects.[59] Studies have shown that SO is instrumental in reducing atherosclerotic lesions by inhibiting gene expression of inflammatory cytokines such as (Il-1, Il-6, TNF-α); these cytokines mediate the inflammatory process and thus develop atherosclerosis, which indicates the anti-inflammatory property of sesame oil.[60]

For the AV and RV treated groups, the histological results of the present study showed an improvement in the aortic wall layers compared to the affected group. However, there is some disintegration in the middle tunica treatment of statins reduces the risk of cardiovascular events and mortality mainly due to improved cholesterol levels.[61] The main effect of these drugs (i.e., statins) is to inhibit the synthesis of cholesterol and isoprenoid, leading to upregulation of endothelial nitric oxide synthase, an enzyme involved in endothelial function. In addition, inflammation indicators such as C-reactive protein and NF-κB have been reduced by statins, leading to the hypothesis that statins possess anti-inflammatory properties.[62]

CONCLUSION

We conclude from the present study that wheat germ oil has an influential role in controlling hyperlipidemia and protecting the body against oxidative stress and thus reduce the risk and development of CVD, as well as the preventive effectiveness in protecting the tissues of the aorta from the damage caused by Triton X-100. WGO, and SO were more effective than statins (Atorvastatin and Rosuvastatin) in improving the biochemical variables and histologically through their antioxidant and anti-inflammatory activities.

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