International Journal of Medical Sciences; May 2022; 5(2):44-54;
ISSNe 2522-7386; DOI: http://doi.org/10.32441.ijms.5.2.6

The Effect of Hydroxytyrosol (HXT) and Local Olive Oil (LOO) on Lipid Profile and Histopathological Changes in The Heart as Outcome of Induced Hyperlipidemia in Male Rats

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Received: 31/10/2021 Accepted: 14/12/2021 Published: May 2022

Abstract
Background: Hydroxytyrozole is a polyphenol found in olive oil with antioxidant activity. It may be with a protective role against oxidative stress.
Aim: To investigate the role of both Local olive oil (LOO) and Hydroxytyrosol (HXT) in controlling the lipid profile and histopathological changes of the heart in male albino rats with experimental hyperlipidemia and comparison of their effect with atorvastatin.
Materials and methods: In this study 30 males from white rats were used. They were divided into 6 groups with close weights. The first group (control group) was given a standard diet and the second group (cholesterol group) was given a diet containing 2% cholesterol throughout the eight-week trial period, while the third, fourth, fifth and sixth groups were given a high-cholesterol diet for two weeks and then gavages with LOO only, HXT only, LOO + HXT and ATOR respectively for six weeks while continuing on the diet rich in cholesterol.
Results: There was a significant increase ($P<0.05$) in the level of total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), very low-density lipoprotein cholesterol (VLDL-C) and atherogenic index (AI). While there was a significant decrease ($P<0.05\leq$) in the level of high-density lipoprotein cholesterol (HDL-C), As well as many of the imbalances histopathological in the heart, included the presence of fibrosis (F), coronary vessels thickening (CoVT), and myocardial fibers hypertrophy (MFH) in a moderate ratio with inflammatory infiltration (II) and hemolysis (He) between myocardial fibers degeneration (MFD) at a low ratio and disintegrates (DIS) cardiac muscle fiber by high rate in the treated group compared to the healthy control group. While the groups of treated animals that were gavage with LOO, HXT, LOO + HXT, and ATOR showed positive improvement in all the above variables, LOO + HXT surpassed all treatments.
Conclusion: Current results suggest that the synergistic effect of HXT with LOO may enhance the antioxidant system and thus reduce the negative effects of hyperlipidemia.
Keywords: Local Olive Oil, Hydroxytyrosol, Lipid Profile, Heart.

Introduction

The term hyperlipidemia refers to an abnormally high level of lipids or lipoproteins due to imbalance in the metabolism and function of lipids, due to dietary disorders, obesity, and genetic diseases such as familial hypercholesterolemia and diabetes diseases [1,2]. Hyperlipidemia patients are more likely to develop a cardiovascular disease. Therefore, hyperlipidemia is a very important risk factor for predicting atherosclerosis (AS) and coronary artery diseases [3,4]. This is a disease, as categorized by the world health organization (WHO) [5] . The number one cause of death in the world, changes in lipid parameters associated with atherosclerosis include elevated (TC), (TG), (LDL-C), low (HDL-C). As high cholesterol in the blood contributes to the development of atherosclerosis [6].

A high-fat diet directly damages the heart muscle through the accumulation of excess cholesterol within the heart tissue and lipid disorders in the blood in addition to myocardial striation depletion and apoptosis elevation [7]. Functional nutrients and functional nutrients beneficial to vascular health may be beneficial compounds that can reduce the overall cardiovascular risk caused by fat disorders by acting in parallel with statins or as auxiliary substances [8]. Olive oil is an oil that is commonly used in the food and has many beneficial health effects [9]. The properties of virgin olive oil have been associated with the presence of prominent fatty acids and phenols that change in shape with the ripening stages of the olives. Oleic acid predominates throughout the ripening period, and palmitic acid is found in the immature stage, while the palmitoleic, stearic, and linoleic acids are found mainly in ripe olives [10,11]. Phenols, the compound oleuropien is predominant in the early stages of olive ripening, and as the ripening progresses, it decomposes into hydroxytyrosol. The current study aims to investigate the efficacy of LOO extract and HXT in improving the lipid profile, and histological dysfunctions of the heart in rats with experimental hyperlipidemia.

Materials and Methods:

Materials

LOO was obtained from the Kamaran laboratory in Kirkuk governorate, and the rats were gavage with a concentration of 1/2 ml/kg of body weight. HXT was purchased from Shaanxibolin Biotechnology - Shaanxi of China, and the rats were gavage with a concentration of 50µl / kg of body weight. The atorvastatin drug used in this experiment was a product of the international Pharmaceutical Industries / Amman / Jordan, and the rats were gavage with a concentration of 2.06 mg/kg [12]**.**

The Animals

A 30 Sprague dawley male albino rats, with age of (16-18) weeks and weigh of (200-260) grams. Animals were placed in cages designed for this purpose. These animals were subjected to laboratory conditions that included 12 hours of light and 12 hours of darkness. The temperature was established at (25 ± 2) ^{0}C. Cages were taken into account, clean, and sterilized. The animals were left for two weeks to adapt to the new breeding conditions and to ensure that they were free of diseases, and they fed on the diet (25% wheat, 45% yellow corn, 20% soybeans, 10% concentrated animal protein, 1% powdered milk added to it 50 g / 100 kg vitamins and preservatives and anti-fungal materials) [13]. And given food and water continuously throughout the experiment.

Experiment Design

This study used 30 male from mature albino rats distributed to 6 groups, each group included 5 animals with close weights. Healthy animals were fed on the standard diet during the eight-week trial period, while the treated animals fed on the diet containing 2% cholesterol [14] for two weeks, then administered LOO, HXT, and ATOR for six weeks while continuing on the cholesterol-rich diet as follows.

1. The first group (control group): This group was given a standard cholesterol-free diet and gavages with distilled water.
2. The second group (the cholesterol group): This group was given a standard diet plus cholesterol (2%) and gavages with distilled water.
3. The third group (the group of cholesterol and LOO): This group was given a standard diet plus cholesterol (2%) and gavage with LOO at a concentration of (1/2 ml/kg) of body weight.
4. The fourth group (cholesterol group and HXT): This group was given a standard diet plus cholesterol (2%) and gavage with HXT at a concentration of (50µl / kg) of body weight.
5. The fifth group (cholesterol group and LOO + HXT): This group was given a standard diet plus cholesterol (2%) and gavage with LOO at a concentration of (2/1 ml/kg) + HXT at a concentration of (50µl / kg) bodyweight.
6. The Sixth group (the cholesterol group and ATOR): This group was given a standard diet plus cholesterol (2%) of the weight of the diet and was gavages with ATOR at a concentration of (2.06 mg/kg) of body weight.

Liver Tissue Extract Preparation:

After 8 weeks of starting the experiment . The animals were starved for 12 hours and then drugged with ketamine and xylazine in doses of 5-35 mg/kg of body weight by intramuscular injection [15]. Then the liver tissue extract was prepared as described previously [16]. The serum is kept at a temperature of 20°C until the required biochemical analyses are performed.

Biochemical Tests in Serum:

The TC concentration was estimated as described before [17], TG, and HDL-C using the commercial kits manufactured by the French company (BIOLABS SA, France) and calculating the LDL-C according to previous description [18]. VLDL – C concentration was calculated as described before [18]. While the AI index is calculated as previously reported [19].

Histological Preparations:

After the animals were dissected, the heart was removed and washed with a physiological solution. Samples were prepared using microscopic tissue sections [20], using hematoxylin and eosin and they were examined by optical microscopy.

Statistical Analysis:

Statistical analysis of the results was conducted by ANOVA analysis of variance. The significant differences were determined according to Duncan's multiple ranges and at a significant level of ($P<0.05$)[21].

Results and Discussion:

Lipid Profile in Liver Extract:

Table 1 showed a significant increase ($P \leq 0.05$) in the level of TC, TG, LDL-c, VLDL-c, and AI and low HDL-c level in the treated animal group compared to the healthy control group. It is noted that the groups of treated animals that were gavage with LOO, HXT, LOO + HXT and ATOR showed a significant increase ($P \leq 0.05$) in the level of HDL-c and a significant decrease in the level of TC, TG, LDL-c, VLDL- c and AI compared to the treated group. Olive + HXT was the most effective treatment followed by ATOR, HXT and LOO.

These results are consistent with other studies [22] in rabbits. The reason is due to an imbalance in the metabolism of lipids, or an imbalance in the absorption and excretion of steroids, or perhaps due to a decrease in the concentration of bile salts [23]. The reason for the high values of the atherogenic index in serum is due to the deposition of macrophages and fats in major organs and blood vessels such as the liver, kidney, heart, aorta, and the coronary artery [24]. Regarding the role of LOO in reducing lipid profile, the results of the current study were consistent with other study [25], when diabetes induced and the use of olive oil

lead to a significant decrease in the level of lipid profile. The role of olive oil in preventing high cholesterol in the blood is because it contains monounsaturated fatty acids (MUFA) and its effect on the cholesterol synthesis, which causes its prevention or contributes to the process of metabolizing cholesterol, with subsequent reducing its level in the body [26]. Olive oil acts to reduce or control the TG via containing of olive oil high amounts of unsaturated fats that lead to prevention of AS, especially in the coronary arteries [27]. Regarding the role of HXT, our results are consistent with other study [28], when using HXT to treat hyperlipidemia in rats, which reduced the lipid profile and increased the HDL level and inhibited lipid peroxide by increasing the activity of CAT and SOD in serum compared to the affected control group. Moreover, HXT also exerts a beneficial effect on HDL-C [29]. Our results also showed a significant improvement in the lipid profile in the group of gavage with LOO + HXT together as compared to other treatments through a decrease in lipid profile and AI values and a significant increase in HDL-C level. This positive change may be attributed to the LOO possessing several bioactive compounds in addition to HXT that can regulate the different mechanisms associated with cholesterol metabolism and hypocholesterolemic by building the molecular mechanisms responsible for these changes [30]. The role of ATOR is to inhibit cholesterol production by regulating the build-up of LDL-C receptors on the surface of liver cells which leads to the removal of LDL-C from circulation [31]. Statins lower cholesterol levels through selective and competitive inhibition of the HMG-CoA reductase, in addition to this it works indirectly by increasing the median receptors and absorbing LDL-C and thereby reducing their level of blood plasma [32].

Table (1) HXT, LOO, and ATOR drug in lipid profile and atherogenic index in the serum of rats male treated with cholesterol.

Groups	Total Cholesterol	Triglyceride mg/dl	HDL-c mg/dl	LDL-c mg/dl	VLDL-c mg/dl	Atherogenic index
Control	95.18±9.48b	116.24±9.45b	33.24±8.43a	38.69±0.84d	23.25±1.89b	2.94±0.45c
HFD	137.92±8.92a	153.27±9.48a	24.55±5.88b	82.72±1.85a	30.66±1.90a	5.77±0.93a
Olive Oil + HFD	105.32±8.46b	121.20±9.61b	38.24±7.43a	42.84±0.90c	24.24±1.92b	2.79±0.32c
HXT +HFD	98.24±9.43b	121.93±9.52b	35.05±4.37a	38.81±6.58d	24.39±1.90b	2.82±0.34c
Olive Oil +	89.84±8.35b	108.87±8.63b	35.65±4.48a	32.42±2.46d	21.77±1.73b	2.52±0.1c
ATOR + HFD	97.88±9.59b	118.31±8.02b	25.62±4.03b	48.60±4.00b	23.66±1.60b	3.84±0.22b

•Values are expressed in mean ± standard deviation.
•The number of rats (5) in each group.
• The numbers followed by vertically different letters indicate a significant difference at the probability level ($P \leq 0.05$).

Histological Study of The Heart:

The results of the current study of the healthy control group showed the normal shape of myocardial fibers (MF), which was characterized by the branching of the bundles that

formed it and having one nucleus in the center. It was also possible to distinguish the transverse layout of the cardiac muscles and coronary vessels (CoV) as in figure (A). Whereas in the group fed a high-fat diet, there were several histological changes, including fibrosis (F), coronary vessels thickening (CoVT), and myocardial fibers hypertrophy (MFH) moderately with inflammatory infiltration (II), hemolysis (He) between cardiac muscle fibers, myocardial fibers degeneration (MFD) at a low rate, and disintegrates (DIS) of cardiac muscle fibers with a high percentage, Figures. (B, C, D).

The group that was fed a high-fat diet and gavage with LOO only showed improvement compared to the high-fat group. However, cases of myocardial fibers degeneration continued at a moderate rate, with myocardial fibers hypertrophy and degeneration and rare coronary vessel wall thickening (Trace), With no noticing myocardial fibers fibrosis, hemolysis, and inflammatory infiltration, Figure (E). The microscopic examination of the cardiac sections of the group that was fed a high-fat diet and gavage with HXT only showed a significant improvement compared with the high-fat group HFD, as it decreased the breakdown of myocardial fibers to a low percentage and myocardial fibers hypertrophy to a rare rate (Trace), with no noticed myocardial fibers fibrosis within the heart muscle, thickening of the coronary vessel wall, hemolysis, and inflammatory infiltration, Figure (F).

The histological examination of the heart sections of the group that was fed a high-fat diet and gavage with LOO + HXT showed a very significant improvement compared with all the treated groups, as it reduced the disintegrate of myocardial fibers to a rare rate (trace) with the end of the presence of hypertrophy and degeneration of myocardial fibers. In addition to fibrosis within the heart muscle, thickening of the coronary vessel wall, hemolysis, Moreover inflammatory infiltration between Myocardial Fibers, as in Figure (G).And in the group fed on a high-fat diet and gavage with ATOR showed improvement compared with the group fed on a high-fat diet, as it reduced the disintegrate of myocardial fibers to a low rate, hypertrophy, and degeneration of myocardial fibers and thickening of the coronary vessel wall to a rare rate with no observation of fibrosis within the heart muscle, hemolysis and inflammatory infiltration between the myocardial fibers, Figure (H), showing the presence of fibrosis (F) within the heart muscle and coronary vessels thickening (CoVT) with inflammatory infiltration (II) between the muscle fibers, the presence of hemolysis(He) between the cardiac muscle fibers with myocardial fibers hypertrophy (MFH), the presence of disintegrates (DIS) between myocardial fibers with myocardial fibers degeneration (MFD).Figure (E) Section of the heart of the treated group gavage with LOO . Figure (F) Section of the heart of the treated group gavage with HXT. Figure (G) Section of the heart of the treated group gavage with LOO + HXT. Figure (H) Cardiac section of the treated group gavage with ATOR showing cardiac muscle fibers, stained with H&E 400X.

The results of the current study showed that giving a high-fat diet led to a set of tissue changes in the heart, including the presence of fibrosis within the heart muscle, thickening of the coronary vessel wall, myocardial fibers hypertrophy, inflammatory infiltration, hemolysis between myocardial fibers, with degeneration of myocardial fibers and disintegration between heart muscle fibers. The results of the current study are in agreement with others [33], when treating rabbits with 1% of cholesterol with the standard diet for 42 days, as it led to tissue changes in the heart muscle that were characterized by the occurrence of disintegration. In addition, vacuolation between some cardiac muscle fibers, hemorrhage and congestion. The cause of tissue dysfunctions is due to myocardium in the current study led to a high level of lipids, as hyperlipidemia affects the systolic function and the physiological response of the heart directly, which is related to the progressive accumulation

of fats in the heart. In addition, systemic oxidative stress, pro-inflammatory state, and mitochondrial dysfunction was reported [34].

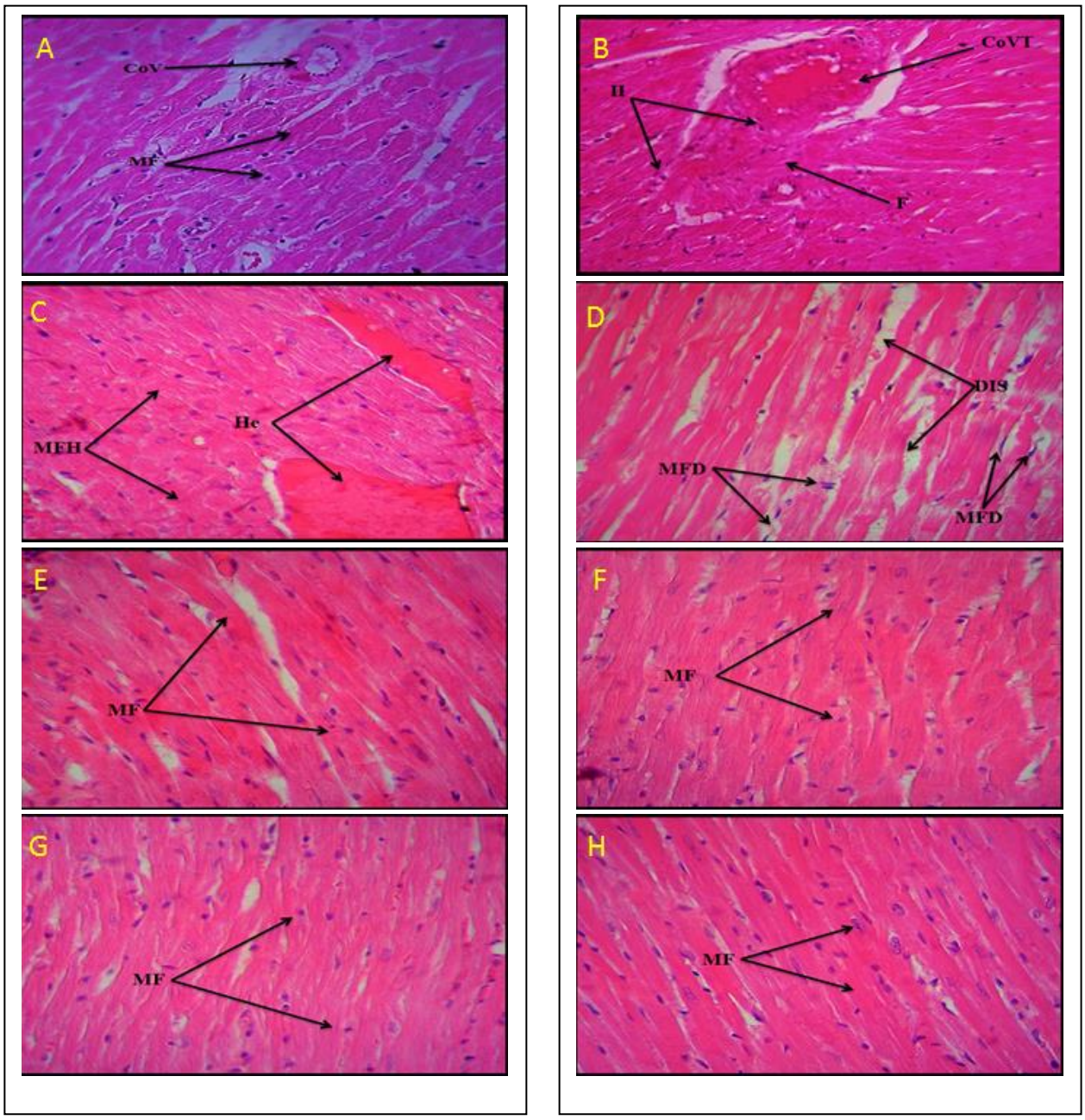

Figure A: Heart section of the control group showing myocardial fibers (MF) and coronary vessels (CoV).Figure (B, C and D), a section of the heart of the group treated with HFD,

As for the role of olive oil, it showed improvement of tissue lesions in the cardiac tissue, which included a decrease in the rate of disintegrate between the cardiac muscle fibers, cardiac muscle hypertrophy, and degeneration, and thickening of the coronary vessel wall with the end of hemolysis, inflammatory infiltration and fibrosis within the heart muscle. The results of the present study are in agreement with others [35], when a male rats were treated with virgin olive oil, sunflower oil, or fish oil at a dose of 25 g per animal up to the

age of 24 months. Olive oil showed better activity than fish oil in the prevention of papillary muscle calcification, and coronary hyalinosis was less in the virgin olive oil group compared to other oils. This activity was attributed to the high percentage of oleic acid in the mitochondrial membrane of cardiac cells and the activity of higher catalase of the olive oil group compared to the other groups. MUFA in olive oil has a protective effect in mitochondria of cardiac cells [36]. As dietary fatty acids are incorporated when digested into biological membranes, including the mitochondrial membrane, which contributes to the structure of the cell and at the same time modifies many of its properties, in addition to that it is used to produce energy or convert it into long-chain and unsaturated fatty acids, which may lead To other compounds that affect a variety of biological processes [37,38].

As for the role of HXT in improving the cardiac tissue, it had the effect of reducing the disintegrate and hypertrophy of cardiac muscle fibers with the end of the presence of cardiac muscle fibrosis degeneration, fibrosis within the heart muscle, thickening of the coronary vessel wall, hemolysis and inflammatory infiltration, The results of this study are in agreement with other study [39] when treating male rats with experimental myocardial infarction with isoproterenol, the administration of HXT at a dose of (2 and 5 mg/kg) led to a significant improvement in myocardial fibrosis degeneration and necrosis and inflammatory infiltration. Lowered serum levels of ALT and troponin-T parameters, pancreatic lipase activity, associated with a significant decrease in TC, TG, and LDL-C, with a significant increase in HDL-C. In addition, a significant decrease in the activity of angiotensin-converting enzyme (ACE), compared with rats with untreated myocardial infarction. HXT has been shown to have many pharmacological properties as anti-oxidant, anti-inflammatory, anti-thrombotic, anti-diabetic effects, hypolipidemic effect, and protection against cardiovascular disease [40].

Local olive oil + HXT, showed improvement in tissue disorders in the heart to a very large extent, as it reduced the disintegrate of cardiac muscle fibers and ended the presence of hypertrophy and degeneration of cardiac muscle fibers, fibrosis within the heart muscle and thickening of the coronary vessel wall. In addition, control hemolysis and inflammatory infiltration, which attributed to the high percentage of active compounds for each group of LOO and HXT separately, that led to a decrease in the concentration of TC, TG and an increase in the concentration of HDL-C. Olive oil contains Gallic acid, which inhibit lipid formation by increasing the activity of the lipoprotein lipase enzyme [41]. The tissue and cellular repair is due to the active compounds, as each compound has its therapeutic benefit, and the flavonoids present in olive oil can affect the properties of the cell membrane. The function of the receptors present in this way may prevent and protect the damage to myocardial cells as well as inhibit the production of TNF-α [42].

ATOR was improving the tissue lesions, represented by reduced disintegration and hypertrophy of cardiac muscle fibers and thickening of the coronary vessel wall with the termination of hemolysis, inflammatory infiltration, and fibrosis within the heart muscle. These results are in agreement with another study [43] when inducing hyperlipidemia in male rats for six weeks and treated with (2.1 mg/kg) of ATOR, which led to a decrease in bleeding in the heart muscle tissue of the treated group compared with the gavage group. In addition, the normalization of histopathological imbalances caused by hyperlipidemia. These changes may be attributed to that ATOR reduced TC, TG, LDL-C, VLDL-C, AI levels, and increasing HDL-C, as evidence of maceration indicates deposition of foam cells or fat deposits in the heart and coronary artery. As the AI increases, the risk of organ damage has increased and the evidence of cross-linking correlates with the size of pro- and anti-atherogenic lipoprotein particles, and which is a predictor of cardiovascular disease risk [44]. In a study [45], ATOR improved cardiac function and restored left ventricular inhibition in

mice with heart failure. Moreover, the effect of anti-myocardial for drug statins reduces cholesterol and may help in the anti-fibrotic mechanism. For example, ATOR can relieve myocardial hypertrophy and remodeling in rats with high blood pressure spontaneously by inhibiting apoptosis [46].

In conclusion, the current study indicated that LOO and HXT have a very effective control of lipid profiles with subsequent significant prevention of histopathological changes. A finding may suggest the production of combined drugs (LOO and HXT) for clinical trials to clarify its therapeutic effect in humans.

ETHICAL APPROVAL: Kirkuk University College of Education for Pure Science (KUCOEPS) Ethical Committee

CONSENT TO PARTICIPATE: Informed consent was taken from each subject before their enrolment in the study.

HUMAN AND ANIMAL RIGHTS: The study conducted in adherence with Helsinki Ethical standards.

CONSENT FOR PUBLICATION: Authors transfer the copyright to the International Journal of Medical Sciences.

FUNDING: No funding

CONFLICT OF INTEREST: The authors declare no conflict of interest, financial or otherwise.

DATA AVAILABILITY: the data was available in Kirkuk University College of Education for Pure Science (KUCOEPS), Kirkuk, Iraq, information set and available on request.

References

1. Sudhakaran S, Bottiglieri T, TecsonK. M, KlugerA. Y, McCullough P. A. Alteration of lipid metabolism in chronic kidney disease, the role of novel antihyperlipidemic agents, and future directions. Reviews Cardio Medicine.2018; 19(3): 77-88.
2. Yao YS, Di Li T, Zeng ZH. Mechanisms underlying direct actions of hyperlipidemia on myocardium: an updated review. Lipids Health Dis. 2020; 19(1): 1-6.
3. Elhissi JH, Sukkar DN, El-Sakka MA. Nutritional attributes as predictors of coronary heart disease. Pharmacol Pharm. 2014; 5(13):1171-1179.
4. Zhang Q, Qian ZY, Zhou PH, Zhou XL, Zhang DL, He N, et al. Effects of oral selenium and magnesium co-supplementation on lipid metabolism, antioxidative status, histopathological lesions, and related gene expression in rats fed a high-fat diet. Lipids Health Dis. 2018; 17(1):1-12.
5. WHO, World Health Organization (2017). Cardiovascular diseases (CVDs). Retrieved September 15. From https://www.who.int/mediacenter/factsheets/fs317/en/.
6. Schwingshackl L, Hoffmann G. Comparison of the long-term effects of high-fat v. low-fat diet consumption on cardiometabolic risk factors in subjects with abnormal glucose metabolism: a systematic review and meta-analysis. British Journal of Nutrition. 2014; 111(12): 2047-2058.
7. Han Q, Yeung S. C, IpM. S, MakJ. C. Dysregulation of cardiac lipid parameters in high-fat high-cholesterol diet-induced rat model. Lipids in health and disease. 2018; 17(1):1-10.
8. Scicchitano P, Cameli M, Maiello M, Modesti PA, Muiesan ML, Novo S, et al. Nutraceuticals and dyslipidaemia: beyond the common therapeutics. Journal of Functional Foods. 2014; 6: 11-32.

9. Buckland G, Gonzalez C. A. The role of olive oil in disease prevention: a focus on the recent epidemiological evidence from cohort studies and dietary intervention trials. British Journal of Nutrition. 2015; 113(2): 94-101.
10. Peyrol J, Riva C, Amiot MJ. Hydroxytyrosol in the prevention of the metabolic syndrome and related disorders. Nutrients. 2017; 9(3): 306.
11. Wani TA, Masoodi FA, Gani A, Baba WN, Rahmanian N, Akhter R, et al. Olive oil and its principal bioactive compound: Hydroxytyrosol–A review of the recent literature. Trends in Food Science and Technology. 2018; 77: 77-90.
12. Nair A. B, Jacob S. A simple practice guide for dose conversion between animals and human. Journal Basic Clinical Pharmacy. 2016; 7(2):27–31.
13. Balducci-Roslindo E, Silvério K. G, Jorge M. A, Gonzaga H. F. D. S. Effect of isotretinoin on tooth germ and palate development in mouse embryos. Braz Dent J. 2001; 12(2): 115-119.
14. Yang R, Wang C, Ye H, Gao F, Cheng J, Zhang T, Guo M. Effects of feeding hyperlipidemia rats with symbiotic oat-based frozen yogurt on serum triglycerides and cholesterol. Food science and nutrition. 2019; 7(3): 1096-1103.
15. Jaber SM, Hankenson FC, Heng K, McKinstry-Wu A, Kelz MB, Marx JO. Dose regimens, variability, and complications associated with using repeat-bolus dosing to extend a surgical plane of anesthesia in laboratory mice. Journal of the American Association for Laboratory Animal Science. 2014; 53(6): 684-691.
16. Graham J. Homogenization of mammalian tissues. The Scientific World Journal 2. 2002; 1626–1629.
17. Deacon AC, Dawson PJ. Enzymic assay of total cholesterol involving chemical or enzymic hydrolysis--a comparison of methods. Clinical chemistry. 1979; 25(6): 976-984.
18. Burtis C, Ashwood E. Teitz Text Book of Clinical Chemistry, 3rded., W.B. Saunders Company, London,UK. 1999; pp: 840.
19.Dobiasova M. Atherogenic index of plasma: theoretical and practical implications. J. Clin. Chem. 2004; 50: 1113-1115.
20. Suvarna KS, Lyton C, Bancroft JD. Bancroft,s theory and practice of histological techniques.7th edition .Churchill Livingston Elsevier . 2013; pp:187-214.
21. Elsahookie MM, Wuhaib KM. Design and Analysis of Experiments, Dar Al-Hekma for Printing and Publishing Mosul, Iraq. 1999; pp: 250.
22. Al-Obaidy MHM, Al-Obaidy SMR. Comparison of the effect of the alcoholic extract of leaves and fruits of hawthorn plant Crataegusazarolus with the drug simvastatin in its effect in reducing cardiovascular disease in male rabbits with experimental fat disorder. Tikrit Journal of Pure Science. 2018; 23(8): 30-37.
23. Al-Ashlash HT. Effect of cholesterol and boiling extract of red pepper on a number of physiological, chemical and textile parameters in local male rabbits. Master Thesis, College of Science, University of Mosul, Iraq, 2012.
24. Garg G, Patil A, Singh J, Kaushik N, Praksah A, Pal A, Chakrabarti A. Pharmacological evaluation of Convolvulus pluricaulis as hypolipidaemic agent in Triton WR-1339-induced hyperlipidaemia in rats. Journal of Pharmacy and Pharmacology. 2018; 70(11): 1572-1580.
25. AL-Azawiy SN. the protective and therapeutic effect of some vegetable oils of Biochemical and Physiological Parameters in normal on health localized rabbits with

Diabetes mellitus and atherosclerosis. Master Thesis, College of Education Pure Science, University of Tikrit, Iraq, 2018.

26. Pignatelli P, Carnevale R, Pastori D, Cangemi R, Napoleone L, Bartimoccia S, et al. Immediate antioxidant and antiplatelet effect of atorvastatin via inhibition of Nox2. Circulation. 2012; 126(1): 92-103.
27. Orsavova J, Misurcova L, Ambrozova JV, Vicha R, Mlcek J. Fatty acids composition of vegetable oils and its contribution to dietary energy intake and dependence of cardiovascular mortality on dietary intake of fatty acids. International journal of molecular sciences. 2015; 16(6): 12871-12890.
28. Jemai H, Fki I, Bouaziz M, Bouallagui Z, El Feki A, Isoda H, Sayadi S. Lipid-lowering and antioxidant effects of hydroxytyrosol and its triacetylated derivative recovered from olive tree leaves in cholesterol-fed rats. Journal of agricultural and food chemistry. 2008; 56(8): 2630-2636.
29. Berrougui H, Ikhlef S, Khalil A. Extra virgin olive oil polyphenols promote cholesterol efflux and improve HDL functionality. Evidence-Based Complementary and Alternative Medicine. 2015; Volume 2015, Article ID 208062, 9 pages.
30. Meneses ME, Martínez-Carrera D, Torres N, Sánchez-Tapia M, Aguilar-López M, Morales P, et al. Hypocholesterolemic properties and prebiotic effects of Mexican Ganodermalucidum in C57BL/6 mice. PLOs one. 2016; 11(7): e0159631.
31. Gotto Jr A. M. Statins: powerful drugs for lowering cholesterol: advice for patients. Circulation. 2002; 105(13): 1514-1516.
32. Raghow R. Statins redux: A re-assessment of how statins lower plasma cholesterol. World J Diabetes. 2017; 8(6): 230–234.
33. Al-Haddad S. T.A. Comparison of some preventative and therapeutic effects of somebotanical extracts and Medications Antihypertensive to reduce Atherosclerosis Induced by Cholesterol in Male rabbits. Doctoral Thesis, College of Education Pure Science, University of Tikrit, Iraq. 2019.
34. Yao Y. S, Di Li T, Zeng Z. H. Mechanisms underlying direct actions of hyperlipidemia on myocardium: an updated review. Lipids in Health and Disease. 2020; 19(1): 1-6.
35. Navarro-Hortal MD, Ramírez-Tortosa CL, Varela-López A, Romero-Márquez JM, Ochoa JJ, Ramírez-Tortosa M, et al. Heart Histopathology and Mitochondrial Ultrastructure in Aged Rats Fed for 24 Months on Different Unsaturated Fats (Virgin Olive Oil, Sunflower Oil or Fish Oil) and Affected by Different Longevity. Nutrients. 2019; 11(10): 2390.
36. Quiles JL, Pamplona R, Ramirez-Tortosa MC, Naudí A, Portero-Otin M, Araujo-Nepomuceno E, et al. Coenzyme Q addition to an n-6 PUFA-rich diet resembles benefits on age-related mitochondrial DNA deletion and oxidative stress of a MUFA-rich diet in rat heart. Mechanisms Ageing Development. 2010; 131(1): 38-47.
37. Desnoyers M, Gilbert K, Madingou N, Gagné MA, Daneault C, Des Rosiers C, et al. A high omega-3 fatty acid diet rapidly changes the lipid composition of cardiac tissue and results in cardioprotection. Canadian journal of physiology and pharmacology. 2018; 96(9): 916-921.
38. Veno SK, Schmid EB, Bork CS. Polyunsaturated fatty acids and risk of ischemic stroke. Nutrients. 2019; 11(7): 1467.
39. Mnafgui K, Hajji R, Derbali F, Khlif I, Kraiem F, Ellefi H, et al. Protective effect of hydroxytyrosol against cardiac remodeling after isoproterenol-induced myocardial infarction in rat. Cardiovascular Toxicology. 2016; 16(2): 147-155.
40. Colica C, Di Renzo L, Trombetta D, Smeriglio A, Bernardini S, Cioccoloni G, et al. Antioxidant effects of a hydroxytyrosol-based pharmaceutical formulation on body

composition, metabolic state, and gene expression: a randomized double-blinded, placebo-controlled crossover trial. Oxidative Medicine Cellular Longevity. 2017; Volume 2017, Article ID 2473495, 14 pages.

41. Liu F, Kim JK, Li Y, Liu X. Q, Li J, Chen X. An extract of Lagerstroemia speciosa L. has insulin-like glucose uptake–stimulatory and adipocyte differentiation–inhibitory activities in 3T3-L1 cells. J Nutrition. 2001; 131(9): 2242-2247.
42. HeissC, Dejam A, Kleinbongard P, Schewe T, Sies H, Kelm M. Vascular effect of cocoa rich in flavan-3-ols. JAMA. 2003;290(8): 1030-1031.
43. Munshi RP, Joshi SG, Rane BN. Development of an experimental diet model in rats to study hyperlipidemia and insulin resistance, markers for coronary heart disease. Indian J Pharm. 2014; 46(3):270–276.
44. Frohlich J, Dobiasova M. Fractional esterification rate of cholesterol and ratio of triglycerides to HDL-cholesterol are powerful predictors of positive findings on coronary angiography. Clin Chem. 2003; 49(11): 1873-1880.
45. Cheng G, Xu G, Cai HW, Wang HH, Bao XF. Effect of atorvastatin on non-ischemic heart failure and matrix metalloproteinase-2 and 9 in rats 1. Acta Pharmacologica Sinica. 2007; 28(4):511-517.
46. Chen Y, Chang Y, Zhang N, Guo X, Sun G, Sun Y. Atorvastatin Attenuates Myocardial Hypertrophy in Spontaneously Hypertensive Rats via the C/EBPβ/PGC-1α/UCP3 Pathway. Cell Physiol Biochem. 2018; 46(3): 1009-1018.