AAJMS [Formerly IJMS], January 2022;5(1):43-54;ISSNe2522-7386;
DOI: http://doi.org/10.32441/aajms.5.1.6

The Effect of Hydroxytyrosol (HXT) and Local Olive Oil (LOO) on Oxidative Stress and Histopathological Changes in the Liver Resulting from Induced Hyperlipidaemia in Male Rats

Qays Assi Ahmed, Kirkuk University College of Pure Science (KUCOPS), Kirkuk, Iraq. Email: qaysassi@gmail.com; Mobile: +9647701248538
ORCID: http://orcid.org/0000-0001-8450-211X
Saleh Mohamed Rahim, Kirkuk University College of Pure Science (KUCOPS), Kirkuk, Iraq. Email: saleh_mr57@yahoo.com, Mobile: +9647701991262,
ORCID: http://orcid.org/0000-0001-5773-7570
Aziz Khalid Hameed, Tikrit University College of Science, Tikrit, Iraq
Correspondence author: Qays Assi Ahmed, Kirkuk University College of Pure Science (KUCOPS), Kirkuk, Iraq. Email: qaysassi@gmail.com; Mobile: +9647701248538
ORCID: http://orcid.org/0000-0001-8450-211X

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

Abstract
Background: Reactive oxygen species are produced through many internal and external processes. Its negative effects are neutralized by the antioxidant defenses.
Aim: To study the role of local olive oil and Hydroxytyrosol in improving the level of oxidation - antioxidants balance.
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: The results of the study showed a significant increase ($P \leq 0.05$) in malondialdehyde level and a significant decrease in the level of glutathione and catalase. Additionally, liver histological changes induced, which include fibrosis (F), central vein well thickening (CVWT), lymphocytes infiltration (LI) with bile ducts sclerosis (BDS), congestions (CON), note degeneration (D) and necrosis (N) in infected cholesterol group compared to the healthy control group. While the groups of infected animals that were gavages with OO, HXT, OO + HXT and ATOR drug showed an improvement in all the above variables, as LOO + HXT substance exceeded all treatments.
Conclusion: oxidative stress is associated with many chronic diseases and that the synergistic role of LOO and HXT played a key role as an antioxidant and positively influence the liver damages induced by oxidative stress.
Keywords: Olive Oil, Hydroxytyrosol, ATOR, GGT, MDA.

Introduction

The increase in total cholesterol in the blood is a major cause of impairment in TG metabolism, which leads to the accumulation of free fatty acids (FFAs) in the liver, leading to

a disorder known as fatty liver [1]. Since cholesterol in the body is derived from dietary intake and biosynthesized de novo, 70% of the daily total cholesterol requirements are synthesized in the liver, and the rest comes from dietary intake [2]. Under certain conditions, cholesterol obtained from diet with cholesterol that is synthesized may exceed the body's requirements to produce cellular membranes, steroids, or bile and this excess cholesterol accumulates in the blood vessels and leads to the formation of plaques that contribute to the development of various cardiovascular diseases [3]. The expansion of the accumulation of fatty acids in the liver leads to an increase in peroxisome and mitochondrial β-oxidation, which results in the production of reactive oxygen species (ROS), which in turn may stimulate the generation of a local inflammatory condition that causes progression in liver injury [4]. When the body's ability to store fat in white adipose tissue was limited, the fat spreads to non-adipose tissue, such as muscles, liver, kidneys, and pancreas [5]. Excessive feeding with a high amount of saturated fat is the leading cause of nonalcoholic fatty liver disease (NAFLD) and is the most common chronic liver disease in the world [6,7]. This disease is characterized by liver steatosis due to the abnormal accumulation of fat, especially triglycerides in the liver cells [8]. Although anti-fat medications have a rapid effect in reducing fat, their use is limited due to their side effects [9]. And still are the ability of statins to prevent cardiovascular events is limited by 30% to 40% in treated patients even when using an intense statin treatment [10, 11]. Moreover, it has recently been shown that taking supplements containing olive-oil rich in anti-oxidant polyphenols reduces the harmful effects of a high-fat diet in rat liver, modifies oxidative stress and maintains tissue levels of long-chain polyunsaturated fatty acids long chain polyunsaturated fatty acids (LC - PUFA) [12]. In the same way, daily intake of HXT, by consuming olive oil and table olives, demonstrated anti-proliferative, anti-cancer and anti-apoptotic activity [13,14]. Thus the present study was conducted to evaluate the protective antioxidant effect of LOO, HXT and GGT.

Materials and Methods

Materials

Local olive oil (LOO) was obtained from the Kamaran laboratory in Kirkuk governorate, and the rats were gavages with a concentration of 1/2 ml/kg of body weight. Hydroxytyrosol (HXT) was obtained from Shaanxi Bolin Biotechnology - Shaanxi of China, and the rats were gavages with a concentration of 50µl / kg of body weight. The Atorvastatin (ATOR) used in this experiment was a product of the International Pharmaceutical Industries / Amman / Jordan, and the rats were gavages with a concentration of 2.06 mg/kg [15].

The Animals

Thirty Sprague dawley male white rats were used in the study with age of (16-18) weeks and weight of (200-260) grams. Animals were placed in cages designed for this purpose (clean and sterilized). The animals were subjected to laboratory conditions that included 12 hours of light and 12 hours of darkness. The temperature was established at (22 ± 2) ^{0}C. 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 with adding of vitamins (50 g /100 kg), preservatives and anti-fungal materials [16]. And given food and water continuously throughout the experiment.

Experiment Design

This study used 30 male from adult albino rats distributed into 6 groups, each group included 5 animals with about the same weights. Healthy animals were fed on the standard diet during the eight-week trial period, while the treated animals feed on the diet containing cholesterol (2%) [17] 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 gavages 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 gavages 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 gavages 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.

Collection of Blood Samples:

Blood samples were collected 8 weeks after the start of the experiment. The animals were starved for 12 hours and then anesthetized with ketamine and xylazine in doses of 5-35 mg/kg of body weight by intramuscular injection [18]. Blood samples were drawn from the heart, the blood was placed in plastic tubes free of anticoagulant and left for 15 minutes at room temperature until blood coagulation, and then the tubes were placed in a centrifuge at a speed of 3000 r / min for 15 minutes to obtain a serum. The serum was kept at a temperature of -20°C until the chemical analysis. Liver was extracted for histological study.

Biochemical Tests in Serum:

The malondialdehyde (MDA) concentration was estimated using the thiobarbituric acid (TBA) reaction method [19]. Glutathione (GSH) was determined by using axis Elman's method [20]. The efficacy of catalase (CAT) was estimated as described before [21]. The efficacy of gamma-glutamyltranspeptidase (GGT) was estimated using a kit purchased from BIOLABO (France).

Histological Preparations:

After the animals were dissected, the liver was extracted and washed with a physiological solution. Samples were prepared using microscopic tissue sections [22]. Using hematoxylin and eosin as staining dye. After completing the preparation of the microscopic tissue sections, 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 ($P<0.05$) [23].

Results and Discussion:

Antioxidant in serum:

The results in Table 1 shows a significant increase ($P<0.05$) in srum MDA level and a significant decrease in serum GSH level and catalase (CAT) enzyme in the cholesterol group compared to the healthy control group. Additionally, It is noted that the administration of LOO, HXT, LOO + HXT and ATOR to the animals induced a significant ($P<0.05$) reduction in serum MDA concentration and a significant increase in the level of GSH and CAT as compared to cholesterol group. The best effect was induced by treatment with LOO + HXT, followed by HXT, LOO, and ATOR.

The results of the current study are consistent with others findings [24], as they reported that hypercholesterolemia in rabbits caused liver toxicity by producing free radicals,

an increase in MDA concentration, a decrease in enzymatic antioxidants such as SOD and CAT in addition to a decrease in GSH concentration. The decrease in GSH concentrations is due to increased demolition and reduced formation inside the liver cells, as it may be due to the high concentrations of MDA. In addition, it may be due to the low concentration of high-density lipoproteins (HDL-c) in the serum, as the relationship is inverse between the concentration of both MDA and HDL-c [25]. As for the role of LOO, the present study results are consistent with a study [26] that studied the effect of virgin olive oil as treat for oxidative stress in male rats. They [26] found that rats treated with virgin olive oil showed a decrease in the MDA level and increase in the level of GSH, CAT, and SOD compared to treated group. To overcome oxidative stress, antioxidants and plant phenols are chemical agents against stress-related illnesses. Virgin olive oil has anti-oxidant properties and positive effects against oxidative stress [27]. As for the antioxidant effect of HXT, the present study results are consistent with others findings [28]. It was found that HXT was able to reduce the concentrations of hydroperoxide, MDA and increased plasma antioxidant capacity of the rat hyperlipidaemia model, also demonstrated that HXT increases SOD expression, and CAT activity in cellular culture, indicating a decrease in the oxidation state [29,30]. LOO + HXT together administration reduced serum concentration of MDA and increased the level of GSH and CAT compared to the affected group. A study performed in 45 healthy adults [31] shows that regular consumption of virgin olive oil rich in phenolic compounds increases the plasma antioxidant capacity and activity of its enzymes (CAT and SOD) and reduces the manifestations of oxidative stress.

Oleic acid being the main fatty acid found in olive oil is less prone to oxidation than polyunsaturated acids that are found in seed oils, and the high content of the anti-oxidant polyphenols (HXT, Oleuropein) makes virgin olive oil relatively stable and resistant to oxidation [32].

As for the role of ATOR and its effect on oxidative stress factors, the present study finding agrees with other study [33] that reported a reduction of hyperlipidaemia and MDA with an increase in glutathione concentration. Also agrees with other study [34] that observed a significant decrease in MDA concentration after ATOR administration to treat hyperlipidaemia in rats.

Table (1) The effect of HXT and LOO and ATOR on MDA, GSH, and CAT concentration in serum of albino rats with cholesterol treatment.

Groups / Parameters	Control	Hyperlipidaemia (HLD)	Olive Oil +HLD	HXT +HLD	Olive Oil +HXT +HLD	ATOR +HLD
MDA (µmol/l)	168.4±7.99 b	212.6±3.82 a	161.7±3.99 b	155.2±4.38 c	145.2±4.46 d	167.6±3.61 b
GSH (µmol/l)	220.6±7.61b	177.6±6.81 d	219.2±5.01 b	251.5±10.54 a	263.7±3.68 a	204.1±5.59 c
CAT (IU/ml)	142.7±7.32 a	91.2±6.24 d	100.2±2.76 c	112.6±4.05 b	144.2±5.45 a	108.6±1.98 b

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

Effectiveness of GGT

The results in table 2 showed a significant increase (P<0.05) in the efficacy of serum gamma-glutamyltranspeptidase (GGT) in the treated animal group in comparison with the healthy control group. The groups of treated animals that were gavaged with LOO, HXT, and LOO + HXT showed a significant decrease (P<0.05) in the effectiveness of GGT compared with the treated group whereas the gavaged group with ATOR was not significantly decreased. The potent effect induced by administration of LOO + HXT, followed by HXT and LOO.

Table.2. Effect of HXT, LOO, and ATOR on GGT effectiveness in serums of albino rats with treated cholesterol.

Groups Parameters	Control	HLD	Olive Oil +HLD	HXT +HLD	Olive Oil +HXT +HLD	ATOR +HLD
GGT(IU /L)	5.410±1.50 c	9.32±1.70 a	7.31± 1.17 b	6.61± 0.79 b	5.53±0.55 c	8.24±1.70 a

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

The results showed that the induction of hyperlipidaemia in rats led to an increase in the effectiveness of serum GGT compared to the healthy control group and this is consistent with others [35] as there was an increase in the effectiveness of serum GGT when treating rats with a high-fat diet compared to the healthy control group. The reason for structural and functional damage to hepatocytes was due to hyperlipidaemia with the subsequent occurrence of fatty degeneration of cells [36]. GGT is considered as an indicator of organ imbalance and cellular damage, cellular contents leakage and loss of cell membrane integrity in the liver and other organs. A high cholesterol diet caused liver injury in animal and human models, and leads to hepatic fibrosis and lipid peroxidation, increasing internal oxidative stress, causing damage to cells and increased blood lipids, and the increases in the GGT level are thought to be caused by oxidative stress associated with hyperlipidaemia [37].

The present study finding for the role of LOO was in consistent with other study [38], as they confirmed the low level of GGT caused by poisoning in rats when using olive oil, and may be due to their antioxidant effects of olive oil that can prevent or delay oxidation of sensitive cellular substrates and thus prevent oxidative stress. Phenolic compounds such as flavonoids, phenolic acids, diterpenes, saponins, and tannins have received much attention for their antioxidant activity [39]. For the role of HXT, our results are consistent with other study [40]. HXT reduced GGT expression caused by hyperlipidaemia in mice. It showed an anti-steatosis effect, while reducing liver fat and TG contents. The anti-steatosis effect of HXT is linked to strengthening anti-oxidant potential in the liver; this partially restores the levels of polyunsaturated fatty acids and thus favors oxidation of fatty acids, while reducing lipogenic formation within the body [41]. The LOO + HXT also showed a greater role in reducing the effectiveness of GGT compared to the control group and other treatments. This improvement is due to the presence of phenol compounds at a higher rate compared to HXT and olive oil separately, as most phenol and flavonoids have been described as having an antioxidant effect

in living systems, as they act as a scavenger of free radicals [42]. Polyphenols in olive leaves have interesting effects such as anti-oxidant, anti-hypertensive, hypoglycemic, and hypocholesterolemic efficacy in the blood [43,44] It was reported that treating diabetic rats with olive leaf extract induced a significant increases in GGT levels. Olive oil, HXT, and tyrosol have also shown protective effects against hepatotoxicity in male rats, restoring ALT, AST, and ALP content. Also, treatment with olive oil and its phenolic compounds reduced apoptosis [45].

ATOR drug had no significant effect on reducing the effectiveness of GGT as compared to healthy control and hyperlipidaemia, and this was inconsistent with the results reported by others [46]. They confirmed a significant decrease in the effectiveness of GGT when using the ATOR at a dose of 5 mg/kg in hyperhipidemic mice. The reason for the variation in the studies outcomes may be due to the high statin dose and the low cholesterol concentration used as compared to the present study.

Histological study of the liver

The present study showed the normal shape of the central vein (CV) with the hepatocytes (HC), sinusoids (S) and kupffer cells (KC) in controls, Fig. (A). While in the fed group with a high-fat diet there was several histological changes, including fibrosis (F), central vein well thickening (CVWT) and lymphocytes infiltration (LI) with bile ducts sclerosis (BDS) and congestion (CON) at a moderate rate while degeneration (D) and necrosis (N) at a low rate (Figs. B and C).

In the infected group treated with LOO only, a moderate improvement was observed, as it reduced the infiltration of the inflammatory cells to an average rate. The central vein wall thickness was reduced to a low rate and bile ducts sclerosis, congestion, degeneration, and necrosis to a low ratio with no fibrosis observed, as in Figure (D). Liver sections of the treated group gavage with HXT only, it showed a great improvement. It reduced the infiltration of inflammatory cells to a low rate and the thickening of the central vein wall and degeneration to a low ratio with no noticeable presence of fibrosis, necrosis, bile ducts sclerosis, and congestion, Figure (E). In the affected group that was treated with LOO + HXT, it showed a very significant improvement compared to all treated groups, as it reduced the infiltration of inflammatory cells and degenerated to low ratio without noticing the presence of fibrosis, thickening of the central wall of the vein, necrosis, bile ducts sclerosis and congestion, Figure (F). While the group treated with ATOR drug showed a slight improvement compared to the affected group. It reduced the infiltration of inflammatory cells to an average rate, fibrosis and thickening of the central vein wall, degeneration to a low rate and necrosis of bile ducts sclerosis, and low rate congestion, Figure (G) and (H).

The results of the present study were consistent with others finding [24]. When they induced hyperlipidaemia in rabbits, the histological results of the liver of a group of animals treated with cholesterol showed the presence of degeneration in most hepatocytes with loss of radial structure of cells, as well as cases of necrosis in cells and thickening of the central veins wall. These histological changes may be attributed to cholesterol administration, which resulted in higher levels of TC, LDL, VLDL and TG and a decrease in HDL level in the hypercholesterol group compared to the control group. Moreover, exposure to hyperlipidaemia resulted in the current study to increase MDA and decrease in antioxidant activity such as CAT and GSH and increase the effectiveness of serum GGT as a result of damage to the liver cells. High levels of saturated fatty acids encourages greater activation of NADPH oxidase, lead to less formation of antioxidant enzymes, and accordingly, it leads to

an imbalance between the formation of reactive oxygen species and the protection of antioxidants characterized by oxidative stress [47].

The role of olive oil extract in combating histological imbalances, our results are consistent with other study [48] that indicated the role of olive oil in promoting and maintaining the natural structure of the liver tissue, and manage the toxic effects and acts as free radical scavengers. The use of olive oil to treat histological changes in the liver caused by hyperlipidaemia in mice, significantly reduced lipid droplet area and lipid accumulation percentage in the liver with improvement of ALT and ALP levels, and serum lipid profile compared with the affected control group [49].

The effect of HXT material on the improvement of histopathological abnormalities in the liver, the results of the present study were in agreement with others [40] when hyperlipidaemia was induced in rats, it reduced HXT Liver steatosis, in conjunction with the overall restoration of the condition of serum glutathione, liver steatosis was significantly associated with hepatic TG and fatty content and inversely with antioxidant capacity compared to the control group.

The use of LOO + HXT improved histological disorders of the liver to a very large degree compared to other treatments, a results that are consistent with others study [50], when hyperlipidaemia was induced in rats, HXT supplementation reduced hepatitis and apoptosis and had effects to lower blood fat and protecting the liver against metabolic disorders caused by hyperlipidaemia by strengthening the anti-oxidant defense system and preventing the expression of proteins involved in inflammation and damage to the liver, as it reduces infiltration of inflammatory cells and inflammations in the liver tissue and steatosis. The enhancement of the antioxidant status represented by the decrease in the level of MDA and the increase in the levels of CAT and GSH in the serum as a result of using LOO + HXT in the present study led to the normalization of histological imbalances caused by hyperlipidaemia.

In regard to the role of ATOR in improving tissue dysfunctions in the liver, our results agreed with other study [51] as reported that treatment with ATOR significantly reduced hepatocyte lipid degeneration and ruptured lobular structures of hepatocytes infiltration of inflammatory cells, it also greatly improved the levels of TC, LDL, ALT, and AST In the serum and raised the total cholesterol level in the stool compared to the affected control group.

In conclusion, the present study indicated a role of LOO and HXT in the treatment of biochemical variables and improvement of histological changes in the liver comes through the antioxidant role that suppresses the negative effects of reactive oxygen species.

ETHICAL APPROVAL: Kirkuk University College of Pure Science (KUCOPS) Ethical Committee

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

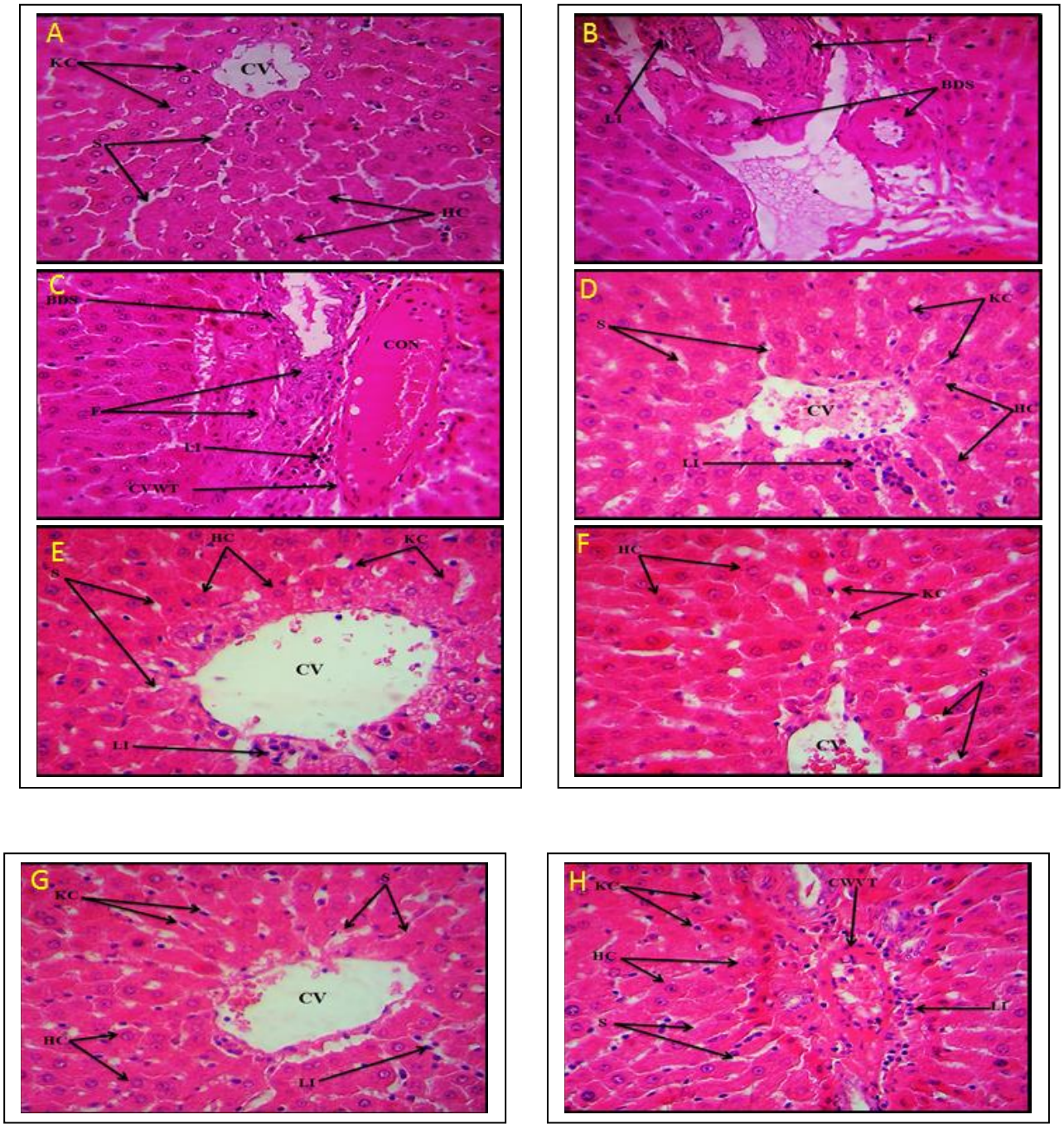
HUMAN AND ANIMAL RIGHTS: The study conducted in adherence with Helsinki Ethical standards.

CONSENT FOR PUBLICATION: Authors transfer the copyright to Aalborg Academy 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 Pure Science (KUCOPS), Kirkuk, Iraq, information set and available on request.



Figures. A to H. Liver histological studies.

Figure (A): A control group liver section showing the central vein (CV), hepatocytes (HC),Sinusoids (S) and kupffer cells (KC) as normal.

Figures (B) and (C) liver section hyperlipidaemia transaction group. It shows lymphocytes infiltration (LI), fibrosis (F), bile ducts sclerosis (BDS), congestion (CON), and central vein wall thickness (CVWT).

Figure (D): The liver of the treatment group with hyperlipidaemia and LOO. It shows a central vein (CV), hepatocytes (HC), sinusoids (S), and kupffercells (KC) with lymphocytes infiltration (LI).

Figure (E) treatment group liver section with hyperlipidaemia and HXT. It shows the central vein (CV), hepatocytes (HC), Sinusoids (S), and kupffer cells (KC) with presence lymphocytes infiltration (LI).

Figure (F) treatment group liver section with hyperlipidaemia and LOO + HXT, the central vein (CV), hepatocytes (HC), sinusoids (S), and kupffer cells (KC) are shown.

Figure (G) and (H) the liver section of the treatment group with hyperlipidaemia and ATOR the central vein (CV), hepatocytes (HC), Sinusoids (S) and kupffer cells (KC) are shown with presence lymphocytes infiltration (LI) and the thickness of the central vein wall. H & E 400X.

References

1. Wang YM, Zhang B, Xue Y, Li ZJ, Wang JF, Xue CH, et al. The mechanism of dietary cholesterol effects on lipids metabolism in rats. Lipids Health Dis, 2010;9(1):4.
2. Iqbal J, Hussain MM. Intestinal lipid absorption. Am J Physiol Endocrinol Metabol, 2009;296(6):E1183-E1194.
3. Whitney E, Rolfes SR. Understanding Nutrition. 14th ed. Nutrition Reviews. United States of America: Cengage Learning., 2016.
4. Schwimmer JB, Pardee PE, Lavine JE, Blumkin AK, Cook S. Clinical Perspective. Circulation, 2008;118(3):277-283.
5. Samuel VT, Shulman GI. Mechanisms for insulin resistance: common threads and missing links. Cell, 2012;148(5):852-871.
6. Townsend SA, Newsome PN. Non-alcoholic fatty liver disease in 2016. Brit Med Bul 2016;119(1):143.
7. Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease—meta-analytic assessment of prevalence, incidence, and outcomes. Hepatology, 2016;64(1):73-84.
8. Tilg H, Moschen AR. Evolution of inflammation in nonalcoholic fatty liver disease: the multiple parallel hits hypothesis. Hepatology, 2010;52(5):1836-1846.
9. Rutishauser J. The role of statins in clinical medicine-LDL-cholesterol lowering and beyond. Swiss Med Weekly, 2006;136(3-4):41-49.
10. Nelson RH. Hyperlipidemia as a risk factor for cardiovascular disease. Primary Care: Clinics in Office Practice, 2013;40(1):195-211.

11. Keyamura Y, Nagano C, Kohashi M, Niimi M, Nozako M, Koyama T, et al. Add-on effect of probucol in atherosclerotic, cholesterol-fed rabbits treated with atorvastatin. PLoS One,2014; 9(5):e96929.
12. Rincón-Cervera MA, Valenzuela R, Hernandez-Rodas MC, Marambio M, Espinosa A, Mayer S, et al. Supplementation with antioxidant-rich extra virgin olive oil prevents hepatic oxidative stress and reduction of desaturation capacity in mice fed a high-fat diet: Effects on fatty acid composition in liver and extrahepatic tissues. Nutrition, 2016;32(11-12):1254-1267.
13. Hashim YZY, Rowland IR, McGlynn H, Servili M, Selvaggini R, Taticchi A, et al. Inhibitory effects of olive oil phenolics on invasion in human colon adenocarcinoma cells in vitro. Int J Cancer, 2008;122(3):495-500.
14. Warleta F, Quesada CS, Campos M, Allouche Y, Beltrán G, Gaforio JJ. Hydroxytyrosol protects against oxidative DNA damage in human breast cells. Nutrients, 2011;3(10):839-857.
15. Nair AB, Jacob S. A simple practice guide for dose conversion between animals and human. J Basic Clin Pharm, 2016;7(2):27.
16. Balducci-Roslindo E, Silvério KG, Jorge MA, Gonzaga HFDS. Effect of isotretinoin on tooth germ and palate development in mouse embryos. Braz Dent J, 2001;12(2):115-119.
17. Yang R, Wang C, Ye H, Gao F, Cheng J, Zhang T, Guo M. Effects of feeding hyperlipidaemia rats with symbiotic oat-based frozen yogurt on serum triglycerides and cholesterol. Food Sci Nutr, 2019;7(3):1096-1103.
18. 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. J Am Assoc Lab Animal Sci, 2014;53(6): 684-691.
19. Dobiasova M. Atherogenic index of plasma: theoretical and practical implications. J Clin Chem, 2004;50: 1113-1115.
20. Al-Zamely OY, Al-Nimer MS, Al-Muslih RK. Detection the level of proxy nitrite and related antioxidant status in the serum of patients with acute myocardial infarction. Nation J Chem, 2001;4:625-637.
21. Goth L. A simple method for determination of serum catalase activity and revision of reference range. Clinica Chimica Acta, 1991;196(2-3):143-151.
22. Suvarna KS, Lyton C, Bancroft JD. Bancroft's theory and practice of histological techniques.7th edition .Churchill Livingston Elsevier, 2013, pp:187-214.
23. Elsahookie MM, Wuhaib KM. Design and Analysis of Experiments, Dar Al-Hekma for Printing and Publishing Mosul, Iraq, 1999, pp: 250.
24. Al-Obaidy MH. Effect of alcoholic Extract of leaves and fruits of wild hawthorn plant Crataegusazarolus and Simvastatin drug in reduction of risk factors on liver and heart and improvement of oxidative stress in male rabbits with experimental Lipid Disorder. Master Thesis, College of Education Pure Science, University of Tikrit, Iraq, 2018.
25. 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.
26. Ghorbel I, Elwej A, Fendri N, Mnif H, Jamoussi K, Boudawara T, et al. Olive oil abrogates acrylamide induced nephrotoxicity by modulating biochemical and histological changes in rats. Renal Failure, 2017;39(1):236-245.
27. Fito M, de la Torre R, Covas MI. Olive oil and oxidative stress. Mol Nutr Food Res, 2007;51(10):1215-1224.

28. Hmimed S, Belarbi M, Visioli F. Hydroxytyrosol augments the redox status of high fat diet-fed rats. Pharm Nutr, 2016;4(4):139-142.
29. Zhu L, Liu Z, Feng Z, Hao J, Shen W, Li X, et al. Hydroxytyrosol protects against oxidative damage by simultaneous activation of mitochondrial biogenesis and phase II detoxifying enzyme systems in retinal pigment epithelial cells. J Nut Biochemistry, 2010;21(11), 1089-1098.
30. Zrelli H, Matsuoka M, Kitazaki S, Araki M, Kusunoki M, Zarrouk M, et al. Hydroxytyrosol induces proliferation and cytoprotection against oxidative injury in vascular endothelial cells: role of Nrf2 activation and HO-1 induction. J Agricul Food Chem, 2011;59(9):4473-4482.
31. Oliveras-López MJ, Berná G, Jurado-Ruiz E, de la Serrana HLG, Martín F. Consumption of extra-virgin olive oil rich in phenolic compounds has beneficial antioxidant effects in healthy human adults. J Fun Foods, 2014;10:475-484.
32. Caramia G, Gori A, Valli E, Cerretani L. Virgin olive oil in preventive medicine: From legend to epigenetics. Eur J Lipid Sci Technol, 2012;114(4):375-388.
33. Kafi LA, Al-Ezzi NTN. Comparative study among aqueous, hexane extracts of sweet almond (Prunusamygdalus) with Atorvastatin for treating hyperlipidaemia induced in mice. Kufa J Vet Med Sci, 2015;6(1):176-183.
34. Safaeian L, Zolfaghari B, Karimi S, Talebi A, Ghazvini MA. The effects of hydroalcoholic extract of Allium elburzense Wendelbo bulb on dexamethasone-induced dyslipidemia, hyperglycemia, and oxidative stress in rats. Res Pharmaceut Sci, 2018;13(1):22.
35. Nasr LR, Saber ME, Hussam B, Salma AR, Muzaffar I. The effect of vanadium on rat hypercholesterolemia in the presence and absence of statins. Afr J Pharm Pharmacol, 2017;11(39):509-516.
36. Beyegue CN, Ngangoum RC, Kuate D, Ngondi JL, Oben JE. Effect of Guibourtia tessmannii extracts on blood lipids and oxidative stress markers in triton WR 1339 and high fat diet induced hyperlipidemic rats. Biology and Medicine, 2012;4(1):1.
37. Uluisik D, Keskin E. Hepatoprotective effects of ginseng in rats fed cholesterol rich diet. Acta Scientiae Veterinariae, 2016;44:1-5.
38. Al-Attar AM, Elnaggar MH, Almalki EA. Protective effect of some plant oils on diazinon induced hepatorenal toxicity in male rats. Saudi J Bio Sci, 2017;24(6):1162-1171.
39. Rice-Evans CA, Miller NJ, Paganga G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free radical biology and medicine, 1996;20(7):933-956.
40. Echeverría F, Valenzuela R, Bustamante A, Álvarez D, Ortiz M, Soto-Alarcon SA, et al. Attenuation of high-fat diet-induced rat liver oxidative stress and steatosis by combined hydroxytyrosol-(HT-) eicosapentaenoic acid supplementation mainly relies on HT. Oxidative Med Cell Longevity, 2018;Volume 2018, Article ID 5109503, 13 pages.
41. Piro S, Spadaro L, Russello M, Spampinato D, Oliveri CE, Vasquez E, et al. Molecular determinants of insulin resistance, cell apoptosis and lipid accumulation in non-alcoholic steatohepatitis. Nut Metab Cardio Dis, 2008;18(8):545-552.
42. Rice-Evans CA, Sampson J, Bramley PM, Holloway DE. Why do we expect carotenoids to be antioxidants in vivo?. Free Rad Res, 1997;26(4):381-398.
43. Vogel P, Machado IK, Garavaglia J, Zani VT, de Souza D, Dal Bosco SM. Polyphenols benefits of olive leaf (Oleaeuropaea L) to human health. Nut Hosp, 2015;31(3):1427-1433.
44. Al-Attar AM, Alsalmi FA. Influence of olive leaves extract on hepatorenal injury in streptozotocin diabetic rats. Saudi Journal of Biological Sciences. 2017;26(7).

45. Kalaiselvan I, Samuthirapandi M, Govindaraju A, Sheeja Malar D, Kasi PD. Olive oil and its phenolic compounds (hydroxytyrosol and tyrosol) ameliorated TCDD-induced heptotoxicity in rats via inhibition of oxidative stress and apoptosis. Pharmaceut Bio,2016;54(2):338-346.
46. Li G, Wu XW, Lu WH, Ai R, Chen F, Tang ZZ. Effect of atorvastatin on the expression of gamma-glutamyltransferase in aortic atherosclerotic plaques of apolipoprotein E–knockout mice. BMC cardiovascular disorders, 2014;14(1):145.
47. Carmiel-Haggai M, Cederbaum AI, Nieto N. A high-fat diet leads to the progression of non-alcoholic fatty liver disease in obese rats. FASEB J, 2005;19(1):136-138.
48. Al-jubori AS. The protective effect of aqueous and alcoholic extracts of Alhagimaurorum plant against experimentally created liver damage in male rats using CCl4. Master Thesis, College of Education Pure Science, University of Tikrit, Iraq, 2015..
49. Wani FA, Albahrawy AZ, Rahiman S. Hypolipidemic activity of olive oil (oleaeuropaea) against high fat diet-induced nonalcoholic fatty liver disease (NAFLD) in mice. Open J Path, 2015;5(3):720-726.
50. Fki I, Sayadi S, Mahmoudi A, Daoued I, Marrekchi R, Ghorbel H. Comparative Study on Beneficial Effects of Hydroxytyrosol-and Oleuropein-Rich Olive Leaf Extracts on High-Fat Diet-Induced Lipid Metabolism Disturbance and Liver Injury in Rats. BioMed Research International, 2020.BioMed Research International Volume 2020, Article ID 1315202, 15 pages.
51. Huang J, Wang Y, Ying C, Liu L, Lou Z. Effects of mulberry leaf on experimental hyperlipidaemia rats induced by high fat diet. Experimental and therapeutic medicine, 2018;16(2):547-556.