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The Effect Of Olive Oil (Oo) And Hydroxytyrosol (Hxt) In Improving The Level Of Sex Hormones And Suppressing Oxidative Stress And Histopathological Of The Testes Caused By Hyperlipidemia In Male Rats

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Abstract

Hypercholesterolemiais a major contributor to production of free radical,as impairs the biosynthesis of steroid hormones, and causes many tissue lesions of the testes, and their negative effects are prevented by antioxidant defenses.Therefore, this study was designed to search for the effect of both olive oil(OO) and hydroxytyrosol(HXT) in amelioration the scaleof sex hormones and antioxidants in the blood serum and histopathological disorders of testes inmale albino ratsOf the Sprague Dawleytype, infected experimental hyperlipidemia and comparing the results with atorvastatin(ATOR).In this study, 50 male albino rats were used, distributed into 10 groups with close weights.The first group (control group) was given the normal diet, as for the groups from the second to the fifth, they were given the normal diet for two weeks, and then they were gavage (OO), (HXT), (OO) + (HXT), and (ATOR), respectively, for six weeks.While the sixth group (cholesterol group) was given a diet containing cholesterol in the rate of 2% throughout the experiment period of eight weeks,The groups are from seven to ten were given ahypercholesterolemiadiet for two weeks, then they were gavage(OO), (HXT), (OO)+ (HXT), and (ATOR) , respectively, for six weeks.The results of the study showed a significant decrease($P \leq 0.05$) in the level of testosterone, folliclestimulating hormone (FSH), luteinizing hormone (LH) , the activity of superoxide dismutase (SOD), and glutathione peroxidase (GPx) and a significant increase in the level of Peroxynitrite (ONOO-),as well as many histological abnormalities in the testes, which included a decrease in the number of seminiferous tubules and their destruction, reduction in the number of germ cells and the separation of spermatogonia from each other and the basement membrane, degeneration and atrophy of germ cells with degeneration of spermatids and a decrease in the number of sperms,degeneration of Sertoli cells and a decrease in their numbers, degeneration of the cytoplasm of leydig cells and a reduction in their numbers, and the occurrence of congestion and hemorrhage between the seminiferous tubules significantly in the affected group compared to the control group.While groups of infected animals that were administered (OO),(HXT), (OO)+ (HXT), and (ATOR) showed a positive improvement in all the above-mentioned variables, as (OO) + (HXT)outperformed all treatments.We conclude

from the current study that hyperlipidemia leads to a decrease in the level of sex hormones and the occurrence of oxidative stress, which is associated with many histological disorders in the testes, and that the role of (OO)+(HXT) together plays a key role in suppression the negative effects caused by hyperlipidemia.

Keywords: olive oil,hydroxytyrosol,atorvastatin, sex hormones, antioxidants, testis.

1.Introduction

Hypercholesterolemia is considered a major health problem worldwide, as it results from a disorder in the metabolism of fats that can cause a range of cardiovascular diseases and thus threaten human health. Hyperlipidemia is linked to the consumption of foods containing saturated fats and cholesterol[1,2]. The mechanisms underlying the metabolic disturbances associated with hypercholesterolemia include excessive production of free radical and oxidative stress.In addition, studies have confirmed that oxidative stress leads to inflammation that is associated with histological disorders including necrosis, apoptosis, and DNA damage during elevated blood cholesterol levels[3].Several experimental studies have also reported that hypercholesterolemia significantly increases reactive oxygen species and redox imbalances in tissues[4].Studies indicate that obesity leads to a decrease in the level of testosterone in the blood and the production of oxidative stress, which acts as a mediator between obesity and related diseases.Dyslipidemia due to high reactive oxygen and/or inflammatory substances is a cause of metabolic disorders such as infertility and creating an inappropriate environment, which leads to a decrease in the effectiveness of testicular cells for the formation of sperm[5,6].Olive oil plays a major role in the mediterranean diet, which is based on low saturated fats and increased intake of vegetable oils [7]. (HXT) is a prominent polyphenolic active ingredient in olive fruits [8]. (HXT) has gained increasing attention during previous studies due to its significant biological properties, such as antioxidant, cytoprotective [9].The bio compound present in olive oil leads to changes in redox signaling and reduces molecular damage by protecting against the excessive generation of reactive oxygen species and suppressing oxidative stress, which is defined as an increased level of redox systems compared to antioxidants[10].

2.Materials and Methods

2.1Material

Olive oil (OO) was obtained from one of the kirkuk governorate laboratories, and rats were treated with it at a dose of 1/2 ml per kg of body weight. hydroxytyrosol (HXT) was obtained from Shaanxi bolinbiotechnology–Shaanxi, China, and rats were treated with it at a dose of 50 µlper kg of body weight. atorvastatin (ATOR) used in this experiment is manufactured by the International Company for Pharmaceutical Industrie/Amman/Jordan, and rats were treated with it at a concentration of 2.06 mgper kg of body weight[11].

2.2Animals:

Sprague–Dawley male albino rats were used at 16-18 weeks of age and weighed (200-260) grams.The animals were placed in cages designed for this purpose, and these animals were subjected to laboratory conditions that It included stabilizing the temperature at (22±2)C°, and the hours of illumination at 12 hours for light and 12 hours for darkness. The cages were cleaned and sterilized. Then the animals were left to adapt to the conditions and to ensure that they were free of diseases for two weeks with free access to water and food.

2.3Experiment Design:

In this study, 50 male adult albino rats were used and divided by every 5 animals in collection with close weights.Healthy animals feed the normal diet throughout the experiment period, while infected animals feed a diet containing 2% cholesterol [12].for two weeks, then they were gavage with (OO) and (HXT) for six weeks With continuing on the cholesterol diet,as follows.

1. The control group: This group treated with a standard diet free of cholesterol and gavage with distilled water.

2. The control group and(OO): This group was treated with a standard diet and gavage with (OO) at a dose of (1/2 ml per kg).

3. The control group and (HXT): This group was treated with a standard diet and gavage with (HXT) at a dose of (50 µl per kg).

4. The control group and ((OO)+ (HXT)): This group was treated with a standard diet and gavage with (OO) at a doseof (1/2 ml per kg) + (HXT) at a dose(50 µl per kg).

5. The control group and (ATOR): This group was treated with a standard diet and gavage with (ATOR) at a doseof (2.06 mg per kg).

6. Thecholesterol group: This group was treated with a standard diet, adding 2% cholesterol to it and gavage with distilled water.

7. Thecholesterol and (OO): This group was treated with a standard diet plus 2% cholesterol and gavage with (OO)at a doseof (1/2 ml per kg).

8. The cholesterol and (HXT): This group was treated with a standard diet plus 2% cholesterol and gavage with (HXT) at a doseof (50 µl per kg).

9. The cholesterol and ((OO)+ (HXT)): This group was treated with a standard diet plus 2% cholesterol and gavage with (OO)at a dose(1/2 ml per kg) + (HXT) at a dose(50 µl per kg).

10. The cholesterol and (ATOR): This group was treated with a standard diet plus 2% cholesterol and gavagewith (ATOR) ata doseof (2.06 mg per kg).

2.4 BloodSample Collection:

Blood samples were collected 8 weeks after the start of the experiment. The animals were starved for 12 hours and then anesthetized by ketamine and xylazine at doses 5-35 mg/kg of body weight by intramuscular injection.Then blood samples were drawn from the heart, and the blood was placed in plastic tubes free of anticoagulant and left for 15 minutes at room temperature until blood clotting, then the tubes were placed in a centrifuge to obtain the blood serum and the serum was kept by freezing, until biochemical analyzes are performed, Then the testes were extracted for the histological study.

2.5 Biochemical Tests in Blood Serum:

The levels of Follicle-stimulating hormone (FSH), Luteinizing hormone (LH) and Testosterone estimate according to the instructions of Bio ChecK- U.S.A. by ELISA technology. Peroxynitrite radical was also estimated using the method [13].and estimation of superoxide dismutase (SOD) using the method [14].Estimation of the activity of the enzyme glutathione peroxidase (GPx) based on [15].

2.6 HistologicalPreparations:

After the anatomy of the animals, the testes were extracted and washed with normal saline.Then, techniques of preparing microscopic tissue sections were conducted on the samples[16] . using hematoxylin and eosin stain, after completing the preparation of microscopic tissue sections, they were examined under a light microscope.

2.7 Statistical Analysis:

Statistical analysis of the results was carried out using the Analysis of Variance (ANOVA) test, and significant differences were determined according to Duncan's multiple ranges and at a level of significance ($P \leq 0.05$).

3.Results and discussion:

3.1Level of Sex Hormonesin Blood Serum:

The results showed in the affected group a decrease in the level of Testosterone, FSH and LH compared to the healthy control group, and at a significant level ($P \leq 0.05$).It is noted that the infected animals gavage with OO, HXT, OO + HXT, and ATOR showed a significant increase in the level of Testosterone, FSH, and LH compared to the infected group.OO + HXT outperformed all treatments, followed by HXT and OO in the second place, then ATOR drug (Table1).

Table (1) Effect of HXT, OO, and ATOR on the level of Testosterone, FSH, and LH in the blood serum of male rats, the experimental groups.

Parameters / Groups	Testosterone (ng/L)	FSH (mlu /ml)	LH (mlu /ml)
Control	0.559±0.021e	7.173±0.052e	5.696±0.021e
Olive Oil only	0.689±0.021c	7.655±0.018c	6.470±0.045c
Hydroxytyrosol only	0.791±0.022b	7.778±0.017b	6.774±0.020b
Hydroxy + Oil only	0.957±0.026a	8.561±0.021a	7.256±0.022a
Atorvastatin only	0.621±0.029d	7.351±0.021d	5.981±0.012d
hyperlipidemic diet- HLD	0.316±0.017i	4.755±0.015j	3.876±0.016j
Olive Oil +HLD	0.391±0.010g	5.575±0.016h	4.266±0.017h
Hydroxytyrosol +HLD	0.407±0.006g	5.787±0.013g	4.377±0.016g
(Hydroxy +Oil) +HLD	0.503±0.012f	6.472±0.022f	5.0416±0.090f
Atorvastatin+HLD	0.355±0.013h	5.233±0.017i	3.966±0.017i

The values are mean ± standard deviation.

• The numbers followed by vertically different letters indicate a significant difference at level (P ≤0.05).

Our results, which showed a decrease in the level of testosterone, FSH, and LH in the affected group, agree with the study [17] When they induced hyperlipidemia in rats using (1% cholesterol and 15% palm oil) for four weeks, they noticed that the level of testosterone decreased significantly compared to the normal control group.It is hypothesized that the decrease in serum testosterone and LH levels in affected rats,It may be due to the accumulation of fat in the leydig cells or their low number[18].Regarding the role of OO and HXT in raising the level of testosterone, FSH and LH compared with the infected and normal control group, our results converge with the study [19],which showed that OO gavage at a concentration of (2.5 ml/kg) per day for 8 weeks for rats It led to a significant increase in the level of Testosterone compared to both the infected and normal group,The strongest effect is due to OO and HXT together ,The latter being polyphenols, as well as the oil's content of unsaturated fatty acids and phenolic antioxidants [20]. Reactive oxygen species (ROS) are suppressed that mediates degenerative diseases by the protective effects of phenols from natural sources [21]. Regarding the role of ATOR in raising the level of testosterone, our results agree with the study [22].When they administered atorvastatin to rats, it led to a significant increase in the levels of FSH, LH and HDL and a significant decrease in the levels of TC, TG, LDL, and VLDL compared with normal group. Also, the study [23] showed a decrease in the level of TC, TG, LDL, VLDL and an increase in the level of HDL. When rats were gavage Simvastatin at a concentration of (3 mg/kg) per day for 8 weeks

compared to the hyperlipidemic group, and thus we conclude that the high level of testosterone is due to the role of atorvastatin in improving lipid parameters.

3.2 The Level of Antioxidants in The Blood Serum:

The results in Table 2 showed an increase in the level of (ONOO-) and a decrease in the activity of SOD and GPx in the infected group compared to the healthy control group with a significant level (P≤0.05). It is noted that the infected animal's gavage with OO, HXT, OO + HXT, and ATOR showed a significant decrease in ONOO- concentration and a significant increase in the level of SOD and GPx compared to the infected group. OO + HXT was superior to all treatments, followed by HXT and OO in second place, then the drug ATOR, and for ONOO- there were no significant differences between the HXT and OO groups, each separately. The healthy group's gavage with OO, HXT, OO + HXT, and ATOR exhibit a decrease in the level of ONOO- with a significant increase SOD and GPx activity compared to the healthy control group, as OO + HXT outperformed all the treatments, followed HXT and OO in second place, then ATOR drug. Regarding the level of ONOO- between the HXT and OO group, each separately, there were no significant differences.

Table (2) Effect of HXT, OO and ATOR on ONOO-, SOD and GPx in the blood serum of male rats, the experimental groups.

Parameters	ONOO- (μmol/l)	SOD IU/ml	GPx (U/L)
Control	75.40±3.44e	12.38±0.30d	6.36±0.23d
Olive Oil only	66.00±2.92f	13.46±0.38c	7.70±0.16b
Hydroxytyrosol only	63.60±3.58f	14.24±0.27b	7.58±0.19b
Hydroxy + Oil only	48.00±4.30g	16.14±0.34a	8.74±0.24a
Atorvastatin only	69.20±1.92f	12.72±0.26d	6.90±0.16c
hyperlipidemic diet- HLD	149.70±5.45a	6.00±0.71h	2.68±0.13h
Olive Oil +HLD	128.60±6.11c	8.76±0.50g	3.12±0.13g
Hydroxytyrosol +HLD	127.60±4.83c	9.54±0.36f	3.52±0.19f
(Hydroxy +Oil) +HLD	110.80±5.12d	11.70±0.21e	4.72±0.28e
Atorvastatin+HLD	137.00±2.55b	8.36±0.30g	2.84±0.21h

•The values are mean ± standard deviation.

• The numbers followed by vertically different letters indicate a significant difference at level (P ≤0.05).

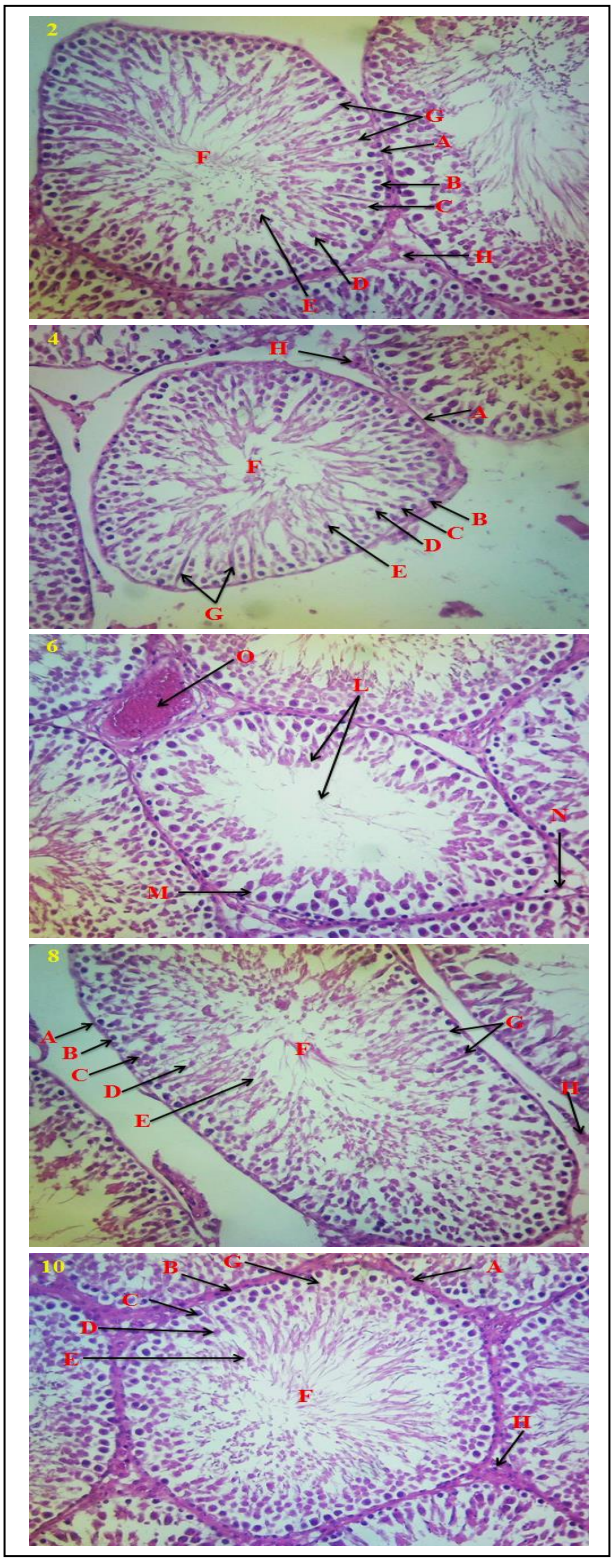
Our results showed that in the affected group, the level of testosterone, FSH, and LH This is in agreement with the findings of a study[24]when hyperlipidemia was induced using 1% cholesterol in rats for six weeks,

as the concentration of nitric oxide (NO) increased in the blood serum,the concentrations of superoxide dismutase (SOD), glutathione oxidase (GPx), glutathione (GSH) and catalase (CAT) were decreased in the liver extract contrast to the healthy control group.One of the main sources for the production of free radicals is the increase in NADPH oxidase activity in cholesterol-fed rats. One study showed an association between NADPH oxidase activity with diet-induced hypercholesterolemia in rats [25] .The combination of superoxide with nitric oxide, which is the main product of NADPH oxidase, leads to the formation ofperoxynitrite (ONOO-).As for the role of OO and HXT in decreasing the concentration of peroxynitrite (ONOO-)and increasing the activity of SOD and GPx compared with the infected control, it agrees with the study [26] ,Which confirmed that OO protects the body from oxidation resulting from oxidative stress when it contains 5 mg or more of hydroxytyrosol and its derivatives per 20 g of OO, also enhances OO the antioxidant properties of the blood, brain, muscles, and small intestine due to its high content of monounsaturated fatty acids (MUFA), specifically oleic acid, which is the highest, followed by palmitic acid and linoleic acid. [27].In addition to MUFA, OO contains bioactive compounds including non-saponifiable compounds (such as squalene, sitosterols, triterpenes, pigments), and hydrophilic compounds (such as α-tocopherol, sterols, carotenoids). and phenolic compounds especially hydroxytyrosol (HXT), oleuropein, and oleocanthal, tyrosol, caffeic acid, vanillic acid, and hydroxytyrosol esters It is considered one of the most important components that act as powerful antioxidants[28] Regarding the role of ATOR in decreasing the concentration of peroxynitrit(ONOO-) and increasing the activity of SOD and GPx compared with the affected group, our results agree with the study [17] , wheninduced hyperlipidemia in rats using (1% Cholesterol and 15%Palm oil) for four weeks andgavage animals withATOR at a concentration of 5 mg per kg Two weeks after the start of the experiment,that showed decreased MDA levels in the rate of 54% and increased glutathione reductase activity in the rate of 112% compared with the infected control group.ATOR also reduced the atherogenicindex in the rate of 18% compared to the affected control, Thus, ATOR reduces oxidative stress, which represents The main cause of complications resulting from obesity[29]. Thus, dyslipidemia caused by high reactive oxygen levels is reduced [5].

3.3 Histological Study of The Testes:

The results of the current study for the control group and groups gavage with OO , HXT, OO + HXT, and ATOR, respectively, showed the normal shape of the testes sections,The seminiferous tubules are healthy and enlarged,the basement membrane of the seminiferous tubule (A), spermatogonium (B), primary spermatocytes (C), secondary spermatocytes (D), spermatids (E), mature spermatogonia (F), sertoli cells (G), and leydig cells (H) as in the figure (1, 2, 3, 4, 5) and table (3), respectively.While in the group that was fed a high-fat diet, there were several histological changes, including a decrease in the number of seminiferous tubules to trace degree and the destruction of some of the tubules (I),Reducing the number of germ cells and the separation of spermatogonium from each other and the basement membrane (J) to a large degree, degeneration and atrophy of germ cells (K) with degeneration of the spermatids and reducing

the number of sperms (L) to a large degree,Degeneration of Sertoli cells and a decrease in their numbers (M) to a large degree, and degeneration of the cytoplasm of leydig cells and a decrease in their numbers (N) to a large degree, with congestion and hemorrhage between the seminiferous tubules (O) to a large degree, as in figure (6,7) and table (3).As for the affected group that was treated with OO only, it was noticed that the seminiferous tubules, germ cells, sertoli cells, and leydig cells improved to a small degree, and congestion and hemorrhage between the seminiferous tubules decreased to a medium degree as in figure (8) and table (3).As for the testicular sections, the infected group treated with HXT only showed improvement in the seminiferous tubules, germ cells, and sertoli cells, as well as reduced leydig cell degeneration with congestion and hemorrhagebetween the seminiferous tubules to a moderate degree, as shown in figure (9) and table (3).In the affected group that was treated with OO + HXT, it showed a significant improvement compared to all treated groups, as it reduced the damage of seminiferous tubules, germ cells, and Sertoli cells to a moderate degree, and the degeneration of leydig cells with congestion and hemorrhage between the seminiferous tubules to a large degree, as in figure (10) and table (3).While the group gavage with ATOR drug showed a slight improvement compared to the infected group, it improved the damage of seminiferous tubules and germ cells, degeneration of sertoli cells and leydig cells with congestion and hemorrhage between the seminiferous tubules to a slight degree as in figure (11) and table (3).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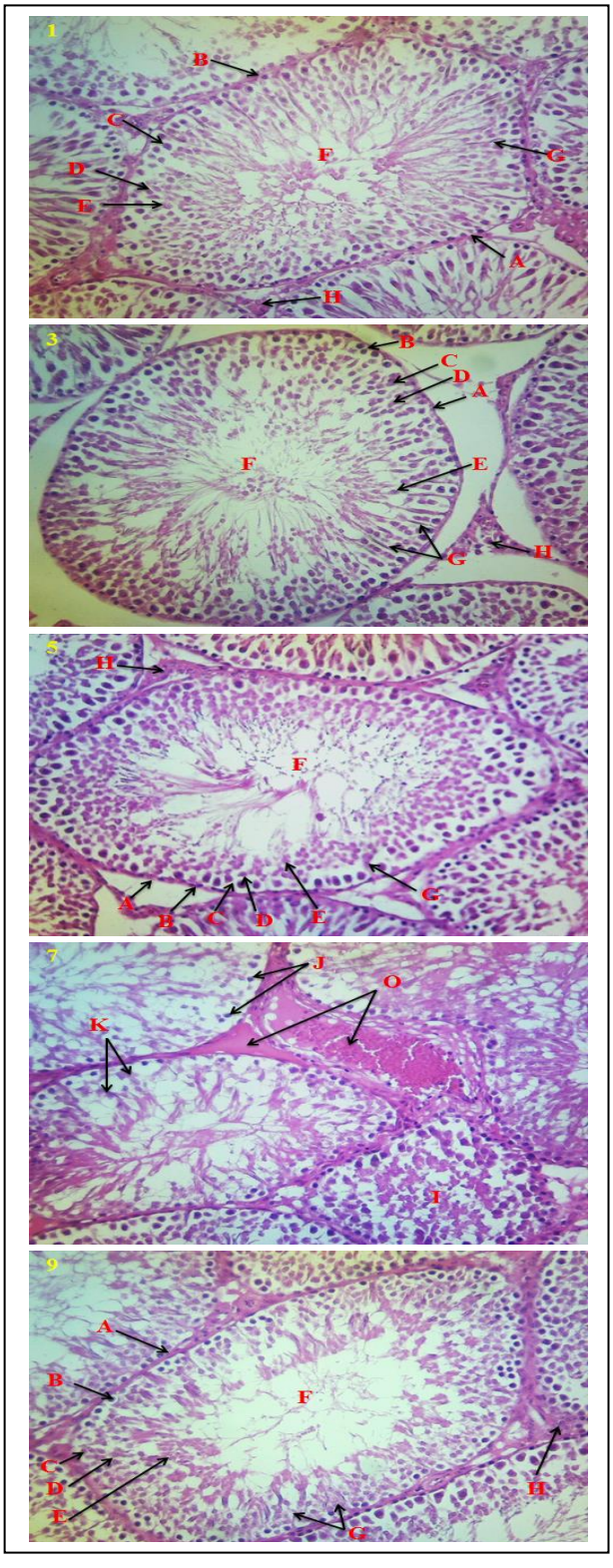
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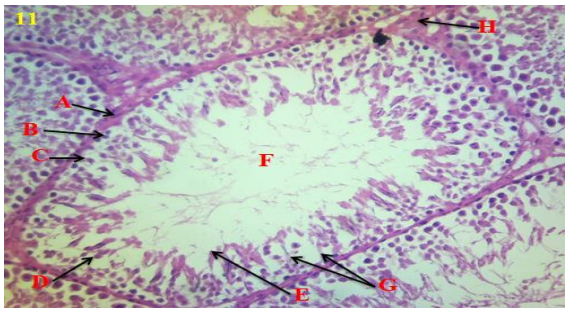

Figure (1) shows the testes section of the control group The seminiferous tubules are healthy and enlarged, Basement membrane of the seminiferous tubule (A), spermatogonium (B), Primary spermatocytes (C), Secondary spermatocytes (D), spermatids (E), mature spermatogonia (F), Sertoli cells (G), and Leydig cells (H).figures (2, 3, 4, 5) sections of the testes of groups treated with OO, HXT, OO + HXT, and ATOR, respectively, shows the composition of the parts mentioned in Figure (1).figures (6) and (7) sections of the testes of the group treated with HLD show the decrease in the number of seminiferous tubules and the destruction of some tubules (I), reduction in the number of germ cells, and the separation of spermatozoa from each other and the basement membrane (J), degeneration and atrophy of germ cells (K), there is a degeneration of the spermatids and a decrease in the number of sperms (L), a degeneration of the sertoli cells and a decrease in their numbers (M), and degeneration in the cytoplasm of leydig cells and a decrease in their numbers (N), congestion and hemorrhage between the seminiferous tubules (O).figure (8) section of the testes of the group treated with HLD and OO shows a slight improvement in seminiferous tubules, germ cells, Sertoli cells, and Leydig cells.figure (9) section of the testes of the group treated with HLD and HXT showed a slight improvement in seminiferous tubules, germ cells, sertoli cells, and leydig cells to a moderate degree.figure (10) section of the testes of the group treated with HLD and OO + HXT showed a moderate improvement in seminiferous tubules, germ cells, and sertoli cells, while leydig cells improved significantly.Figure (11) section of the testes of the group treated with HLD and ATOR, showing a slight improvement in seminiferous tubules and germ cells, and trace degree in sertoli cells and lydig cells. H & E 200X.

Table (3) Effect of HXT, OO, and ATOR on histological changes in testes of male rats of experimental groups.

Parameters	seminiferous Tubules Number	Germ Cells Number	Germ cell Degeneration	Sertoli Cell Number	Sertoli Cell Degeneration	Leydig Cell Number	Leydig Cell Degeneration	Congestion and Hemorrhage
Control	+++	+++	Nil	+++	Nil	+++	Nil	Nil
Olive Oil (OO) only	+++	+++	Nil	+++	Nil	+++	Nil	Nil

HTX only	+++	+++	Nil	+++	Nil	+++	Nil	Nil
HTX + (OO) only	+++	+++	Nil	+++	Nil	+++	Nil	Nil
ATOR only	+++	+++	Nil	+++	Nil	+++	Nil	Nil
HLD	Trace	Trace	+++	Trace	+++	Trace	+++	+++
Olive Oil (OO) +HLD	+	+	++	+	++	+	+	++
HTX +HLD	+	+	+	+	++	++	+	++
(HTX + OO) +HLD	++	++	Trace	++	Trace	+++	Trace	Trace
ATOR +HLD	+	+	++	Trace	++	Trace	++	++

• **Marks (+ means little), (++ means medium), (+++ means high).**

The results of the current study, which showed histological disorders in the testes of the affected group, agreed with the findings of the study [30] , when induce hyperlipidemia in rats.The histological results of the testes of the cholesterol-treated group showed the scattering and lysis of the seminiferous tubules With an increase in the thickness of the basement membrane and its irregularity, the dissociation, and irregularity of sperm cells, in addition to vacuolization within the cytoplasm, the sloughing of the germinal epithelial tissue and the degenerated of connective tissue between the tubules and the leydig cells With congestion and hemorrhage between the tubules.Also with the study [31] ,which confirmed that induce of hyperlipidemia causes atrophied seminiferous tubules, disruption of spermatogenesis and vacuolization of the germinal epithelium.The reason for this is that hyperlipidemia leads to damage to mitochondria, accumulation of myelin vesicles within the mitochondrial matrix of leydig cells, and a gradual increase in the size of myelin vesicles, which leads to a decrease in the functional efficiency of leydig cells [32].In addition, exposure to hyperlipidemia in the current study caused the elevated level of ONOO- and a decrease in the activity of antioxidants such as SOD and GPx, and this is agree with the study [33], which confirmed that hyperlipidemia leads to oxidative stress.As for the role of OO and HXT in suppressing histopathological disorders, it is due to the role of OO in lowering blood cholesterol levels and prevent Oxidative stress in blood lipids due to polyphenols present in OO.Consuming20 g of OO that containing 5 mg of HXT and their derivatives (such as oleuropein and Tyrosol) can protect against hyperlipidemia and oxidative stress[34].The protective effects of natural phenols against degenerative diseases that are mediated by (ROS)[21].The study [35] , also showed the protective effects of OO in protecting the kidneys from Ethephon-induced toxicity in male rats through the improvement of histopathological disorders and level of oxidative stress.As for the role of ATOR in improving histological abnormalities in the testes, our results agree with the study [36], which showed a significant increase in the level of Testosterone, reduced glutathione, superoxide dismutase, catalase, anti-apoptotic level, sperm count and reduced activity The level of malondialdehyde and nitric oxide when rats were gavage with ATOR at a concentration of (1 mg per

kg) daily for 65 days compared to the hyperlipidemic group.Thus, we conclude that the high level of testosterone is due to the role of ATOR in raising the level of antioxidants and scavenging free radicals.

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