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# Studying the possibility of using olive oil and its active components together with atorvastatin in the treatment of biochemical parameters and iron deposition in heart tissue with lung histopathology conditions induced by hypercholesterolemia in male rodents

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## Abstract

**Background** Hyperlipidemia is one of the leading causes of the inflammatory response due to damage to the lung tissue, and the antioxidant activity of olive oil and hydroxytyrosol curbs these disorders. Therefore, the current study aimed to investigate the effect of Hydroxytyrosol (HXT) and Olive Oil (OO) on increased levels of Adiponectin, decreased Tumor Necrosis Factor Alpha (TNF-α) and Interleukin 6 (IL-6) in blood serum with the improvement of histological lung disorders in experimental hyperlipidemia in male Sprague Dawley rats and an assessment of outcomes with atorvastatin.

**Method** Thirty albino male rats were used in this study, divided into six groups of approximately identical weight. The initial group received a typical diet devoid of cholesterol. The two groups were fed a diet including 2% cholesterol for the length of the experiment for eight weeks, and the 3rd, 4th, 5th, and 6th teams were placed on a two-week high-cholesterol diet. They were given a dose of (OO) extract just, (HXT) just, (OO) extract + (HXT), and a drug. Atorvastatin (ATOR), consecutively, from the third week to the eighth week of the experiment.

**Results** The results of the study showed a significant decrease (P≤0.05) in the concentration of Adiponectin and a significant increase in the concentration of TNF-α and IL-6 in the blood serum, In addition to a rise in the scale of malondialdehyde (MDA), iron deposition, lower rates in the level of glutathione (GSH), and the activity of the catalase (CAT) enzyme in heart tissue; additionally, numerous lung histopathological disorders, that also comprised damage to the alveolar (DA) and damage to the alveolar sacs (DAS), Thickening Vessels (TV), Thickening Bronchioles (TB), Fibrosis (Fi), Inflammatory Infiltration (II), Congestions (CON), Hemorrhage (H), Degeneration (D), necrosis (N) and karyolysis (KL) in the group with hyperlipidemia food contrasted to the group of healthy controls. Whereas the teams of high-fat rats, which were also gavage by (OO) extract just, (HXT) just, (OO) extract + (HXT), and (ATOR) drug demonstrated an enhancement in each of the factors above, as (OO) extract + (HXT) outperformed all treatments.

**Conclusions** As a result, we conclude the present study that hyperlipidemia causes a significant lowering in the concentration of adiponectin and a significant rise in the concentration of TNF-α and IL-6 in serum from the blood, In addition to lower rates in the scale of MDA, iron deposition, lower rates in the level of GSH, and the activity of the CAT enzyme in heart tissue, as well as many histological disorders in the lung, and that mixing olive oil extract and hydroxytyrosol plays an essential role in Inhibition of adverse consequences of hypercholesterolemia.

**Keywords:** Hypercholesterolemia; Olive Derivatives; Cytokines; Oxidative Stress; Iron Deposition; Heart; Lung.

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## 1. Introduction

Obesity has many adverse effects on health and the economy, contributing significantly to long-term diseases and related disorders worldwide. In recent decades obesity has increased rapidly worldwide and is now affecting people of all ages and socioeconomic classes (Haththotuwa *et al*., 2020). Since obesity is associated with chronic inflammation of tissues and increases the risk of many diseases, it is also a public health problem from an epidemiological perspective. Inflammation thus affects the function of the high-density lipoprotein (HDLs), endothelial cells, and adiponectin, and increases the risk of cardiovascular disease Adiponectin also regulates energy metabolism and body composition (Zocchi *et al*., 2022).

Obesity reduces the lung's essential capacity and causes a decline in activity. Chronic inflammation in the tissues responsible for metabolism is also connected to it. As more than a result, type 2 diabetes, metabolic syndrome, and insulin resistance are all directly caused by the chronic inflammation linked to obesity as well as a host of other illnesses like cancer, autoimmune and neurodegenerative disorders, long-term kidney disease, long-term non-alcoholic fatty liver disease, and cardiovascular disease (Furman *et al*., 2019; Huang *et al*., 2019).

Moreover, adiponectin levels are decreased in obesity, which has an anti-inflammatory function, and histopathological disorders (Achari and Jain, 2017). Adiponectin promotes intimal disease, the first step in the inflammatory response. Endothelial cells affect lipoprotein metabolism and function, which is the initial action through the expression of many lipases that bind to the extracellular matrix, lipoprotein triglycerides, and several lipoprotein receptors. (Justine *et al*., 2018; Abumrad *et al*., 2021). In addition, endothelial cells are essential in controlling arterial elastic modulus, activation of platelets, thrombosis, proliferation of inflammatory response, and vascular smooth muscle cells. More specifically, inflammatory cytokines are produced and targeted by endothelial cells. A reciprocal relationship between endothelial cells and adipocytes within adipose tissue was also demonstrated to play a role in regulating metabolism through the release of nitric oxide (NO), which controls vascular tension and stimulates the liver, skeletal muscle, and adipose tissue to use glucose regulated by insulin. The mesenchymal-epithelial lining of the blood vessels is transferred to the fatty tissue, which reduces blood vessels and causes fibrosis (Cook, 2020).

Adipose tissue surrounds all vessels and functions as a fundamental regulator of vascular function by secreting adipokines, such as adiponectin, that impact endothelial function and vascular interaction (Nava and Llorens, 2019). While perivascular adipose tissue is vasoprotective under normal circumstances, it contributes to vascular dysfunction in obese individuals because Inflammatory cytokines are released by perivascular adipose tissue, including (TNF-α), which increases free radicals and the development of a phenotype of inflammation in the endothelial (Li *et al*., 2021). Additionally, obese people produce less adiponectin from their adipose tissue than healthy people because adiponectin concentrations are directly linked to insufficient vascular endothelium vasodilator in both healthy people and people with high blood pressure and type 2 diabetes (Hui *et al*., 2014). HDLs which are known to protect blood vessels, are altered by the relationship between obesity and inflammation because they are dysfunctional in obesity, which causes an increase in oxidative stress and the release of inflammatory cytokines (Pérez *et al*., 2019; Stadler *et al*., 2021).

In several previous studies, hypercholesterolemia has been linked to lipid peroxidation, which shifts the balance of pro-oxidant antioxidants towards oxidative stress in serum and various tissues. Hypercholesterolemia causes oxidative stress in the serum, aorta, liver, and heart (Alfarisi *et al*., 2020). Oxidative stress results from an imbalance between the production of reactive oxygen species and the antioxidant tolerance of affected tissues (Hamad Zubi and Hamad Alfarisi, 2021). Despite the extensive research about the beneficial consequences of olive oil, there are few research projects on the outcomes of antioxidants in olive oil. Therefore, this study aimed to know the effects of olive oil and hydroxytyrosol in olive leaves in reducing the complications of hyperlipidemia.

## 2. Materials and Method

### 2.1. Material

The rats were given (OO) extracted by thermal pressing method at a dosage of 0.5 ml per kilogram of body mass. Shaanxi Bolin Biotechnology's . (HXT) was administered to rats at a concentration of 50 µl/kg body weight. Rats were given the medication (ATOR) at a dose of 2.06 mg per kg of body mass (Nair and Jacob, 2016).

### 2.2. Experimental Animal

Thirty healthy male albino Wistar rats weighing 200-260g, aged 16 to 18 weeks, were used for the study. The animals were procured from the animal rearing facility of the University of Tikrit, and housed in well-ventilated cages. Animals

were acclimatized for two weeks, fed with standard pellets with access to water ad libitum, and maintained at different standard laboratory conditions, temperature (25±2°C), humidity (50±5%), and 12 hours of light and dark cycle. This study was carried out following ethical guidelines for animal welfare by the Department of Biological, the Faculty of Sciences, University of Kirkuk.

### 2.3. Experimental Design

The experimental animals were distributed into six groups, with five animals in each group with relative weights, and the experimental groups were divided as follows:

- The first group (the healthy control): Was awarded a conventional meal for two weeks of treatment, and from the third week through the eighth week, orally administered normal deionized water at a dose of 1ml/kg body weight.
- The second group High-Fat Diet (HFD): They were administered the 2% cholesterol-containing diet for two weeks; the next step was to administer deionized water from the third week until the eighth week while continuing on (HFD).
- The third group High-Fat Diet (HFD) and (OO): They were administered with the 2% cholesterol-containing diet for two weeks; the next step was to administer (OO) at a concentration of (0.5 ml/kg) of body weight from the third week to the eighth week with Continue (HFD).
- The fourth group (HFD) and (HXT): They were administered with the 2% cholesterol-containing diet for two weeks; the next step was to administer (HXT) at a percent (50 µl/kg) of body mass from the third week to the eighth week, and continue (HFD).
- The fifth group (HFD) and ((OO) + (HXT)): They were administered with the 2% cholesterol-containing diet for two weeks; the next step was to administer them by (OO) at a concentration (1/2 ml/kg) + (HXT) at a percent (50 µl /kg) of body weight from the third week until the eighth week, while continuing (HFD).
- The sixth group (HFD) and (ATOR): Were administered with the 2% cholesterol-containing diet for two weeks; the next step was to administer them by (ATOR) at a concentration (2.06 mg/kg) of body weight from the third week to the eighth week, while continuing to (HFD).

### 2.4. Blood Sample Collection

After the eighth week of starting the experiment, the animals were starved for half a day and then sedated by Ketamine with Xylazine at a percent of 5-35 mg/kg of body mass via injection into the muscle. After the autopsy, blood samples were withdrawn from the heart to procure plasma serum. At -20°C, the serum was maintained until the necessary biochemical analyses could be done. The lungs and the heart were then removed for histopathological examination. Part of the heart was also placed in plastic containers containing 0.9% Normal saline until the crushing was carried out using the crushing and homogenizer to obtain the heart extract for biochemical examinations.

### 2.5. Biochemical Examinations in Plasma Serum and Heart Extract:

The concentrations of Adiponectin, Tumour necrosis factor α (TNF-α), and Interleukin 6 (IL-6) were determined using ELISA technology according to the instructions of Kit from (MyBioSource) USA in serum. The concentration of Malondialdehyde (MDA) was estimated according to the method of (Guidet and Shah, 1989) and Glutathione (GSH) using the modified Elman's reagent method (Al-Zamely *et al*., 2001) and the activity of Catalase (CAT) according to the method of (Goth, 1991) in the heart extract.

### 2.6. Histological Examination

The lungs of the animals were removed and cleaned with a physiological solution after being dissected. Samples were then prepared using hematoxylin and eosin staining for microscopic tissue slides. At the same time, tiny histological sections of the heart were prepared using a blue Iron stain. The prepared microscopic slides were then viewed under a light microscope, and pictures were taken.

### 2.7. Analysis of The Data

The data were statistically analyzed using the Analysis of Variance (ANOVA) test, and Duncan's multiple ranges test was used to identify significant differences at a major degree ($P \leq 0.05$).

## 3. Results and analysis

### 3.1. Concentration of Adiponectin, TNF-α and IL-6 in Serum.

The outcomes in Figure 1 (a,b,c) and Table (1) demonstrated a significant reduction in the rate of (Adiponectin) and a significant rise in the rate of (TNF-α and IL-6) in the (HFD) group treated with cholesterol comparison with the healthy standard group treated with the standard diet, as for the group's Treatment with the high-fat diet gavage by (OO) only, (HXT) only, (OO)+ (HXT) and (ATOR) increased (Adiponectin) concentration and decreased (TNF-α) and (IL-6) concentrations significantly than the (HFD) group treated with cholesterol. The group treated with (OO) + (HXT) together had the best effect on improving the parameters mentioned above, followed by the group treated with (HXT), then the group treated with (OO). Finally, the group was treated with (ATOR).

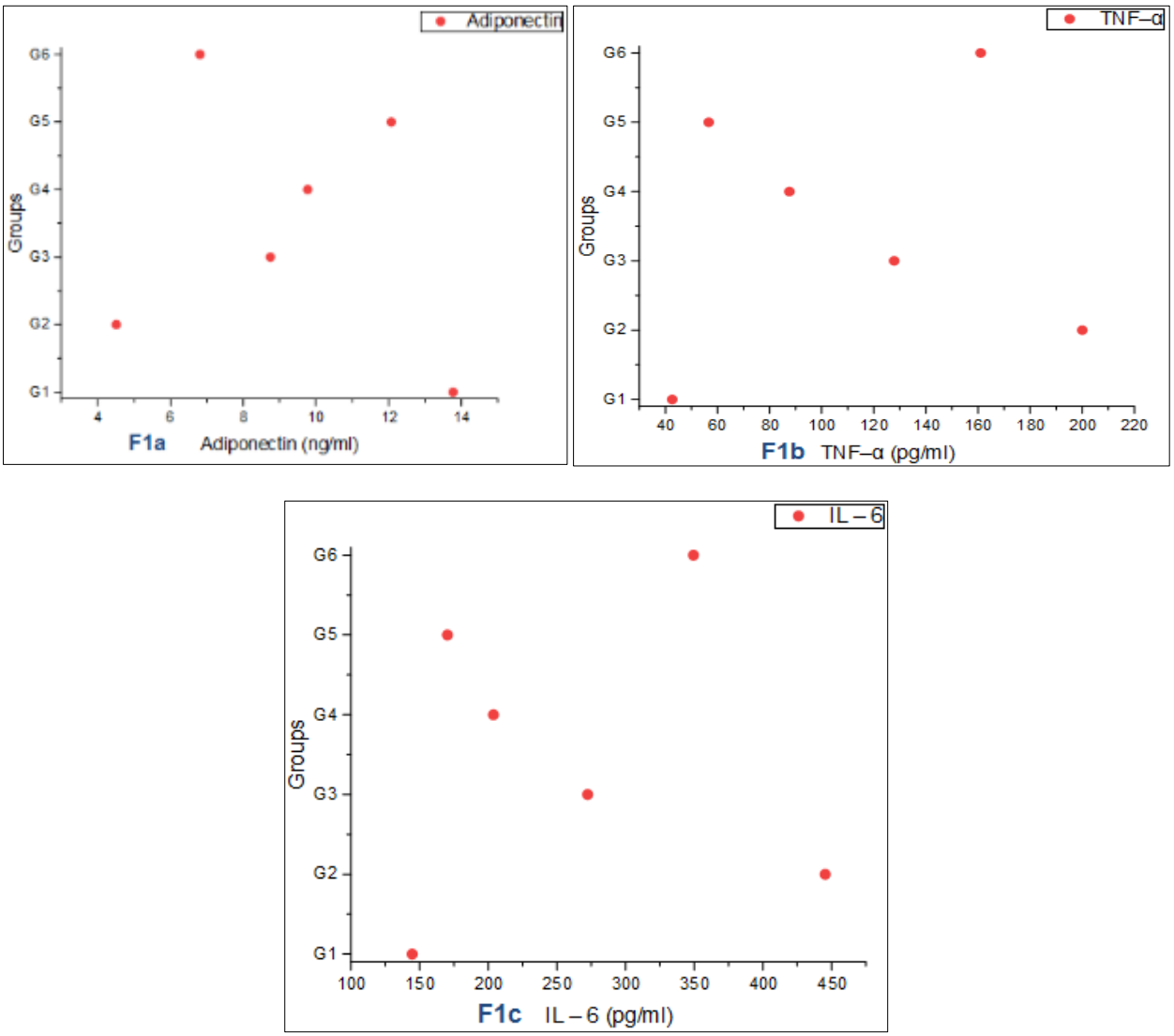


**Figure 1** (a) Adiponectin concentration, (b) TNF-α concentration, and (c) IL-6 concentration in blood serum

Our outcomes in Figure 1 (a,b,c) and Table (1) demonstrated a significant reduction in the rate of Adiponectin with a major rise in the rate of TNF-α and IL-6 in the (HFD) group compared to the healthy standard group, and this is due to the role of fat in increasing body weight and the accumulation of fatty tissue because Adiponectin regulates mitochondrial function and muscle mass. Blood adiponectin concentration is oppositely associated to the thickening of white adipose tissue, insulin resistance, and serum glucose, insulin, and triglyceride levels (Nigro *et al*., 2014). The increase in the concentration of TNF-α and IL-6, is due to the role of hyperlipidemia in triggering the inflammatory response that is inversely proportional to the concentration of adiponectin (Achari and Jain,2017). Adiponectin regulates mitochondrial function and muscle mass. It also has anti-inflammatory actions, which help to maintain

vascular balance by boosting the immune system's effectiveness (Hafiane *et al*., 2019; Cohen *et al*., 2022). Adiponectin secretion is mediated by a peroxisome proliferator-activated receptors (PPARs), as adiponectin is a major regulator of fat cell physiology (Astapova and Leff, 2012). PPARs controls fatty acid metabolism and glucose tolerance and is crucial for forming new blood vessels and the vascular system (Kotlinowski and Jozkowicz, 2016). Additionally, PPARs has an anti-inflammatory impact; as a result, pro-inflammatory cytokines, such as TNF-α, block the expression of the adiponectin genotype in adipose tissues (Chang *et al*.,2014).

**Table 1** Impact of Olive Oil (OO), Hydroxytyrosol (HXT) and Atorvastatin (ATOR) on the concentration of Adiponectin, TNF-α and IL-6 in the serum of experiment rats

| Parameters/ Groups | Adiponectin (ng/ml) | TNF–α (pg/ml) | IL – 6 (pg/ml) |
|---|---|---|---|
| Control | 13.774±0.537[a] | 42.620±5.332[f] | 144.600±9.370[f] |
| HFD | 4.510±0.635[f] | 200.000±12.748[a] | 445.400±20.182[a] |
| HFD + OO | 8.750±0.546[d] | 127.800±8.198[c] | 272.400±16.682[c] |
| HFD + HXT | 9.774±0.288[c] | 87.600±7.301[d] | 203.800±12.194[d] |
| HFD + (HTX + OO) | 12.074±0.705[b] | 56.600±6.348[e] | 170.200±7.596[e] |
| HFD + ATOR | 6.814±0.537[e] | 161.000±7.176[b] | 349.400±20.599[b] |

The values represent the arithmetic average ± standard deviation (SD); Numbers with various letters following them vertically denote statistically significant differences (P≤0.05).

As far as the function of (OO) with (HXT) in raising the levels of adiponectin and reducing the concentration of TNF-α and IL-6 compared to the (HFD) group treated with cholesterol, the reason is due to the function of (OO) with (HXT) in reducing the levels of total cholesterol, triglyceride, and LDL-c and raising the levels of HDL-c level, and thus reduce inflammation and iron deposition in tissues, which is one of the causes of tissue disorders and inflammatory response (Ahmed *et al*., 2020). The use of olive oil and its derivatives when inducing hyperlipidemia in male rats leads to an increase in the level of antioxidants such as GSH and CAT and a decrease in the level of free radicals such as MDA compared to the high-fat diet (HFD), which leads to a reduction in the severity of inflammatory responses and the recovery of tissue disorders (Ahmed *et al*., 2022[a]).

The function of (ATOR), in increasing the levels of adiponectin and decreasing the concentration of TNF-α and IL-6 contrasted to the (HFD) group treated with cholesterol, the reason is due to the role of statins in the inhibition of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase because it is a lipid-lowering medication that can be utilized as a substitute therapy for those with chronic respiratory disorders, acting through multiple mechanisms as antioxidant, inflammatory and immunomodulatory. Along with the growing usage of these medications, statins' immunomodulating properties are becoming to be understood. Because they have a high affinity for the active site of HMG-CoA reductase, synthetic statins are more effective than other statins at reducing enzyme activity and cholesterol production in vitro. Compared to other statins, (ATOR) possesses immunomodulatory capabilities in addition to its anti-inflammatory and antioxidant effects (Capra and Rovati, 2014; Bradbury *et al*., 2018).

### 3.2. MDA and GSH Concentrations, CAT Enzyme Activity, and Iron Deposition in Heart Tissue

The results in Figure2 (a,b,c,d) and Figure3 (b,c,d), and Table (2) showed a significant increase in MDA concentration and iron deposition and an essential reduction in GSH concentration and CAT activity in (HFD) group treated with cholesterol, comparison with the healthy standard group treated with the usual diet. As for the groups treated with a high-fat diet dosed with olive oil only, hydroxytyrosol only (olive oil + hydroxytyrosol), and the drug atorvastatin. MDA concentration, and iron deposition decreased, and GSH concentration and CAT enzyme activity increased significantly for the high-fat diet group treated with cholesterol. The group treated with olive oil and hydroxytyrosol had the best effect on improving the criteria mentioned above, followed by the group treated with HXT and OO, and finally, the group treated with Atorvastatin.

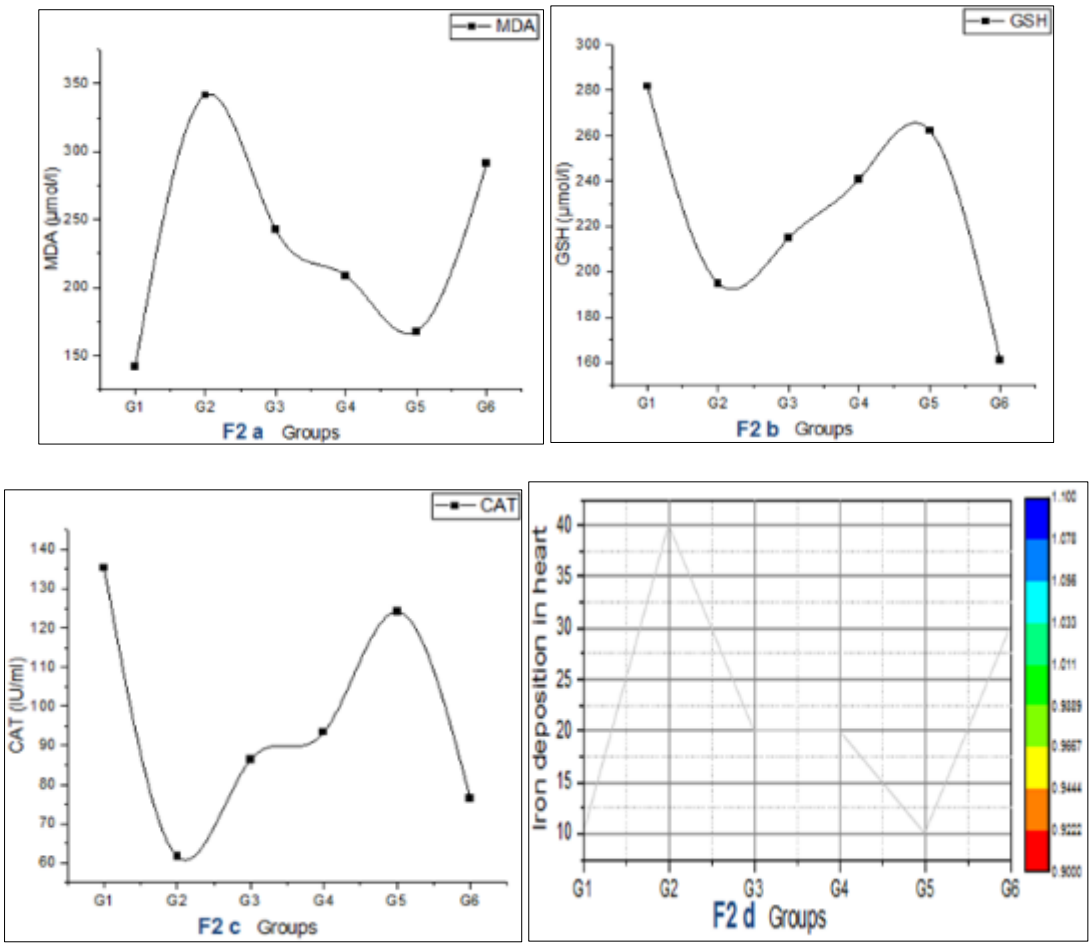


**Figure 2** (a) MDA concentration, (b) GSH concentration, (c) CAT enzyme activity, and (d) iron deposition in heard tissue

**Table 2** Impact of Olive Oil (OO), Hydroxytyrosol (HXT) and Atorvastatin (ATOR) on Concentration of MDA, GSH and CAT Enzyme Activity with Iron deposition (Id) in Heart Tissue of experiment rats.

| Parameters/Groups | MDA (µmol/l) | GSH (µmol/l) | CAT (IU/ml) | Iron deposition in heart |
|---|---|---|---|---|
| Control | 141.600±9.76$^{f}$ | 281.600±10.02$^{a}$ | 135.400±9.61$^{a}$ | Trace |
| HFD | 341.600±9.24$^{a}$ | 195.000±11.18$^{e}$ | 61.600±5.94$^{e}$ | +++ |
| OO +HFD | 242.600±11.35$^{c}$ | 214.800±5.45$^{d}$ | 86.400±5.18$^{c}$ | + |
| HTX +HFD | 208.600±9.71$^{d}$ | 240.800±7.85$^{c}$ | 93.400±4.67$^{c}$ | + |
| (HTX + OO) + HFD | 167.600±5.59$^{e}$ | 262.200±5.63$^{b}$ | 124.200±6.83$^{b}$ | Trace |
| ATOR +HFD | 291.200±7.60$^{b}$ | 161.000±9.62$^{f}$ | 76.400±6.12$^{d}$ | ++ |

The values represent the arithmetic average ± standard deviation (SD) • Numbers with various letters following them vertically denote statistically significant differences (P≤0.05); The symbols (+) represent a low degree, (++) represent a medium degree, and (+++) represent a high degree.

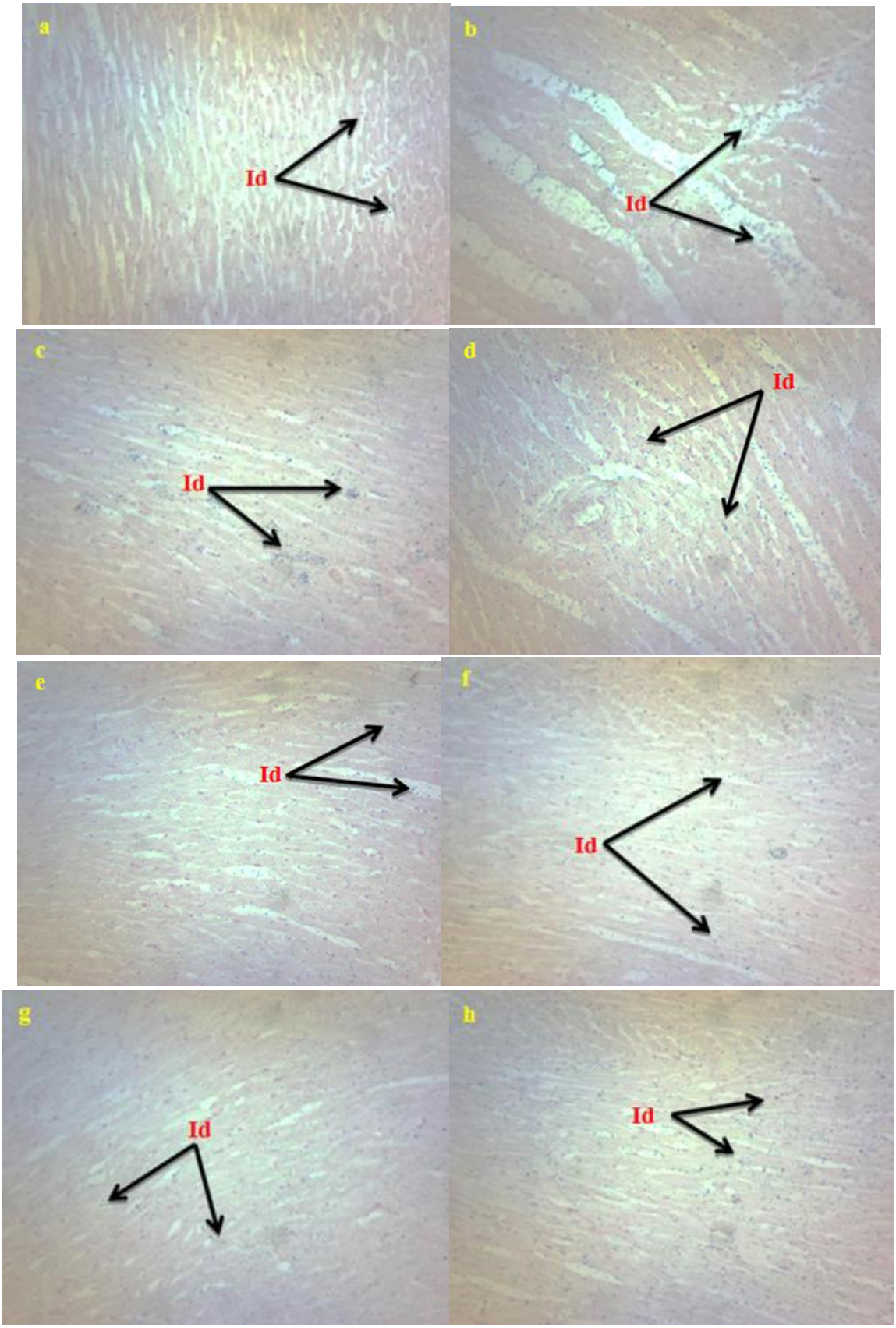


**Figure 3 (a)** Heart section of the healthy control group. (b, c, d) Heart section of (HFD) group treated with cholesterol. (e) Heart section of (HFD) group gavage with (OO) only. (f) Heart section of (HFD) group gavage with (HXT) only. (g): Heart section of (HFD) group gavage with (OO) + (HXT). (h) Heart section of (HFD) group gavage with (ATOR), (blue Iron stain 200X).

The results of our study showed in In Figure 2 (a,b,c,d). Figure 3 (b,c,d) and Table (2) a significant rise in the concentration of MDA and iron deposition and a significantly lower n concentration of GSH and the activity of the CAT enzyme in the heart tissue of the hyperlipidemia group comparison to the standard group, and this supports the research (Phachonpai *et al*., 2023) that showed the use of (HFD) in rats fed four weeks showed dyslipidemia, an increase in glycemic index, and the accumulation of oxidative stress through an increase in the concentration of MDA and lipid peroxidation, and a decrease in the activity of the antioxidant enzyme SOD and glutathione peroxidase (GPx) in the blood. Excessive fat leads to a rise in oxidative stress and a reduction in the concentration of antioxidants, which play an essential role in causing insulin resistance or reducing insulin secretion (Burgos-Morón *et al*., 2019). Hyperlipidemia is the primary factor of the formation of oxidative stress, which may lead to more oxidation of A discrepancy within antioxidant capacity lipids and ineffective antioxidant protection in biological structures, and an imbalance between antioxidant status and oxidative stress plays a pivotal role in histopathological disorders (Singh *et al.*, 2022).

Our results showed that the use of olive oil and hydroxytyrosol leads to an improvement in the level of oxidation balance and a reduction of iron deposition in the heart tissue, as the use of plant products has received increasing attention due to its biological and pharmacological properties that can help in the prevention and treatment of many diseases, as confirmed by (Usha *et al*., 2017) oxidative stress can be decreased by several antioxidants, including flavonoids, carotenoids, polyphenols, and phytosterols. The phenolic compounds in olive oil contribute to wound healing by affecting tissue regeneration (González-Acedo *et al*., 2023). The phenolic substances in olive oil are known for their antioxidant capabilities due to their ability to break the fat oxidation cycle (Bucciantini *et al*., 2021). In addition, olive oil has significant health benefits as it acts as an anti-inflammatory (Santangelo *et al*., 2018). Phenolic substances derived from plants used as medicinal herbs and found in fruits and vegetables positively affect tissue regeneration (Melguizo-Rodríguez *et al*., 2022). The ability of phenolic compounds presents in olive oil to promote the development and distinction of cells has also been demonstrated (Abate *et al*., 2021).

As for the role of atorvastatin in the level of oxidation balance and iron deposition, the results of our research agree with the research (Karami *et al*., 2023), which showed that statins possess antioxidant effects by decreasing the level of malondialdehyde and lipid peroxidation, creatinine and urea, and increasing the levels of superoxide dismutase, and glutathione with reducing histological disorders when atorvastatin was used in rats exposed to oxidative stress. Possessing anti-inflammatory and antioxidant activities, atorvastatin may improve the progression of atherosclerosis and reduce levels of Total cholesterol, triglyceride, MDA, IL-6, and TNF-α by lowering lipids, inhibiting inflammation and suppressing oxidative stress compared to hyperlipidemia group (Yao *et al*., 2023).

### 3.3. Lung histological examination

The findings of the current research for the group of healthy controls showed the normal shape of the lung section, as it showed the alveolar alveoli (A) and the alveolar sacs (AS) as shown in Figure 4, Figure 5(a) and Table (3), respectively. While there were several histological changes in (HFD) group nourished a hyperlipidemia diet, including Damage Alveolar Sacs (DAS), Damage Alveolar Sacs (DAS), Thickening Vessels (TV) and Thickening Bronchioles (TB). Fibrosis (Fi), Inflammatory Infiltration (II), Hemorrhage (H), Congestions (CON), Degeneration (D) to a large degree (+++), Necrosis (N) and Karyolysis (KL) to a medium level (++) Like in Figure 4 and Figure 5 (b,c,d) and Table (3).

As pleasingly as (HFD) group that was gavage with (OO) extract only, an improvement in Alveolar Damage (DA), Thickening Vessels (TV) and Fibrosis (Fi) to a medium degree (++) was observed, and Damage alveolar sacs (DAS), thickening bronchioles (TB), hemorrhage (H) and necrosis (N) to a weak degree (+), congestion (CON) and karyolysis (KL) to a rare degree (Trace). At the same time, there was no improvement in the degree of Karyolysis (KL) as shown in Figure 4, Figure 5 (e) and Table (3).

As for the (HFD) group, which was gavage (HXT) only, it managed to show advancement in alveolar damage (DA), fibrosis (Fi), inflammatory infiltration (II), hemorrhage (H), congestions (CON) and Karyolysis (KL) to a weak degree (+), damage to the alveolar sacs (DAS), thickening vessels (TV), thickening bronchioles (TB), degeneration (D) and necrosis (N) to a rare level (Trace) Like in Figure 4, Figure 5 (f) and Table (3).

As well as the high-fat diet (HFD) group, which was gavage (OO) extract + (HXT), managed to show a notable development contrasted to all treatments. Damage Alveolar (DA), hemorrhage (H), and Karyolysis (KL) decreased to a rare degree (Trace); it was fully recovered from Damage Alveolar Sacs (DAS), Thickening Vessels (TV), Thickening Bronchioles (TB), Fibrosis (Fi), Inflammatory Infiltration (II), Degeneration (D) and Necrosis (N) As in Figure 4, Figure 5 (g) and Table (3).

While the high-fat diet (HFD) group receiving an ATOR medication by gavage showed only a tiny improvement over the high-fat diet (HFD), as it improved the damage Alveolar (DA), thickening vessels (TV) and thickening bronchioles(TB), Fibrosis (Fi), inflammatory infiltration (II), hemorrhage (H), congestion (CON), degeneration (D), karyolysis (KL) to a moderate degree (++), and damaged alveolar sacs (DAS), congestions (CON) and necrosis (N) to a rare skill (Trace), as shown in Figure 4, Figure 5 (h) and Table (3).

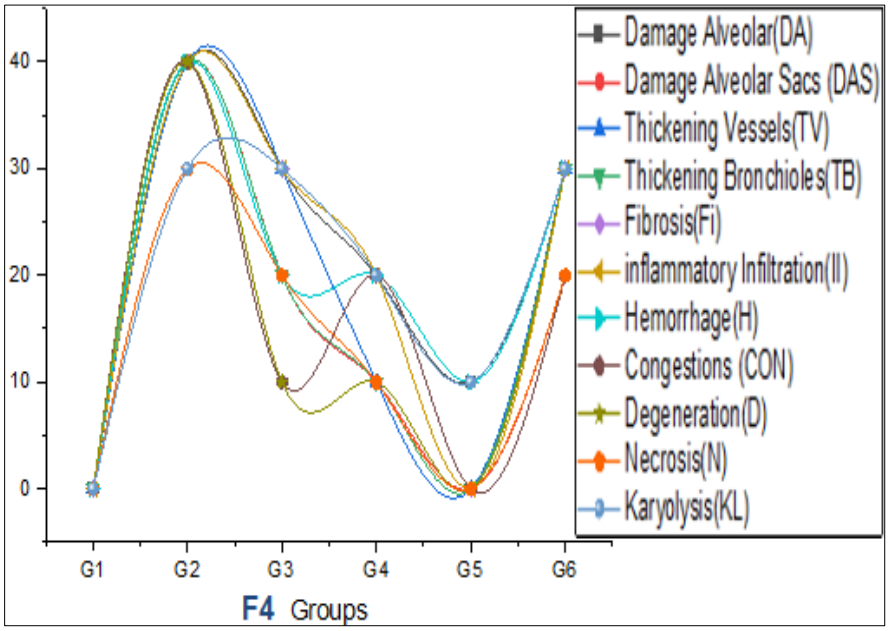


**Figure 4** Curve showing the percentage of histological damage to the lung

Our results in the lung of (HFD) group in Figure 4 and Figure 5 (b,c,d) and Table (3), which showed histological disorders, agree with the findings of the study (Saadat *et al*., 2020) that demonstrated pathological changes such as inflammation, muscle hypertrophy, and emphysema when hyperlipidemia is induced in rats. These pathological changes included infiltration of inflammatory cells in the surrounding areas of the trachea and neovascular, airway epithelium thickening, bronchial dilation, and air space hypertrophy. Moreover, a study (Saadat *et al*., 2019) showed that induced hyperlipidemia results in pathological alterations such as inflammation, muscular hypertrophy, emphysema, and an essential rise in serum scale of total cholesterol, triglyceride, and LDL-c comparison to the standard group. Because interleukins contribute to lipid metabolism by enhancing hormone-sensitive lipase expression, which breaks down lipids, lipid profile changes significantly impact immune response and inflammatory responses (Braun *et al*., 2018).

As with the function of (OO) with (HXT) in normalizing lung tissue problems, the reason is because of lowering the level of negative cholesterol and free radicals and raising the level of positive cholesterol and antioxidants, as the high level of positive cholesterol reduces the deposition of fat in organs (Ahmed *et al*.,2021; Ahmed *et al*., 2022[b]). Eliminating stored fat in the body reduces blood pressure and cumulative sugar, increases positive cholesterol and insulin sensitivity, and thus improves the tissue imbalances caused by Hyperlipidemia (Ahmed *et al*.,2022[c]).

The function of Atorvastatin (ATOR) in relieving problems of histopathological problems is because of the role of statins in reducing cholesterol, triglyceride, and LDL-C levels and increasing HDL-C levels (Ravi,2021). Atorvastatin also reduced the levels of cytokines in the current study, which is consistent with the study (YILDIZELI *et al*., 2017). The anti-inflammatory effects of statins have also been demonstrated by suppressing inflammatory cytokine production and airway inflammation by inhibiting the production of interleukins (Imamura *et al*., 2009).

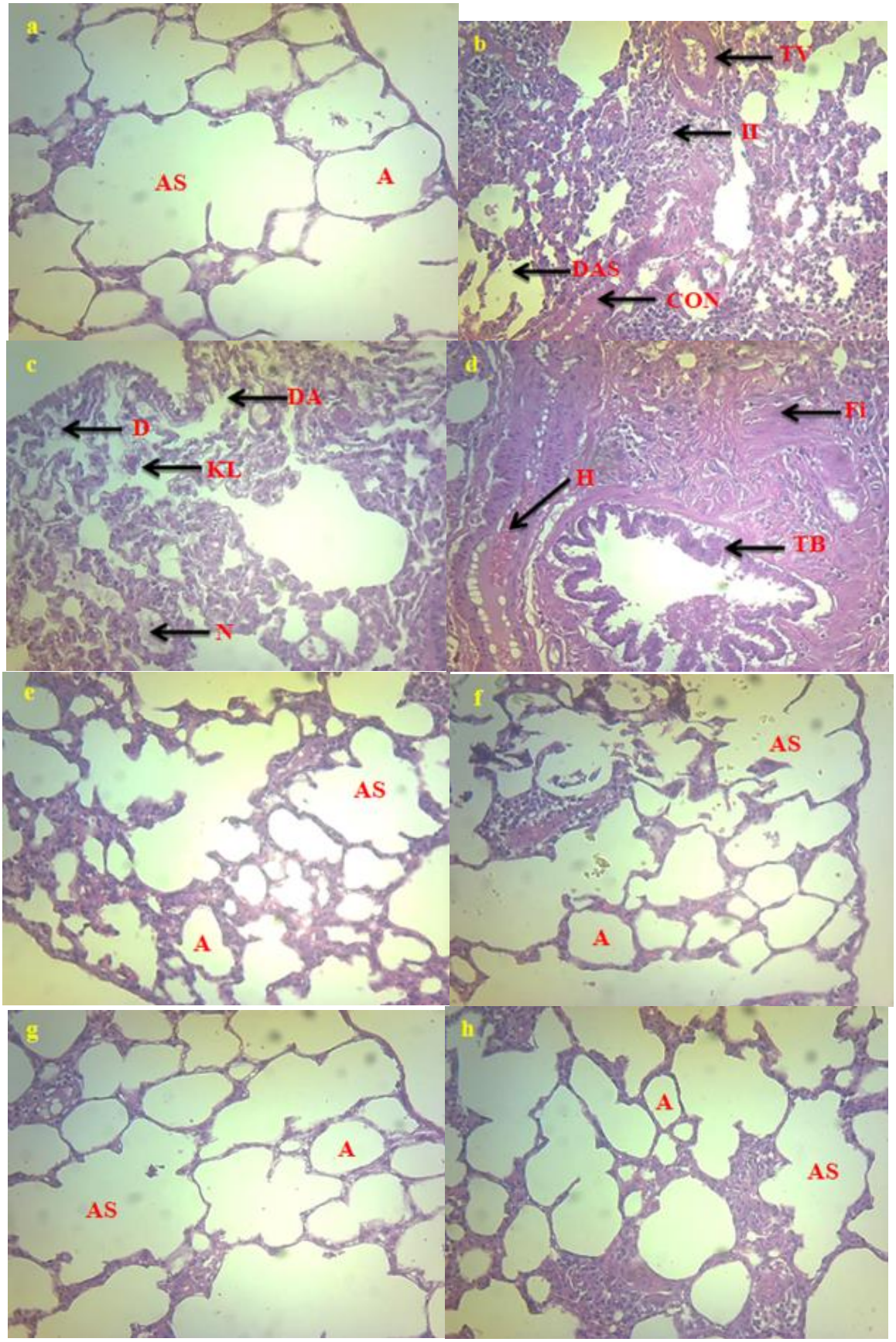


**Figure 5 (a)** Lung section of the healthy control group. Figures (b, c, d) Lung section of the (HFD) group treated with cholesterol. Figure (e) Lung section of (HFD) group gavage with Olive Oil (OO) only. Figure (f) Lung section of (HFD) group gavage with Hydroxytyrosol (HXT) only. Figure (g) Lung section of (HFD) group gavage with Olive Oil (OO) +

Hydroxytyrosol (HXT). Figure (h) Lung section of (HFD) group gavage with Atorvastatin (ATOR), (Hematoxylin and eosin 200X).

**Table 3** Impact of Hydroxytyrosol (HXT), Olive Oil (OO) and Atorvastatin (ATOR) on histopathological disorders in the lung of experiment rats

| Parameters/ Groups | Damage Alveolar (DA) | Damage Alveolar Sacs (DAS) | Thickening Vessels (TV) | Thickening Bronchioles (TB) | Fibrosis (Fi) | inflammatory Infiltration (II) | Haemorrhage(H) | Congestions (CON) | Degeneration(D) | Necrosis(N) | Karyolysis(KL) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Control | Nil | Nil | Nil | Nil | Nil | Nil | Nil | Nil | Nil | Nil | Nil |
| HFD | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | ++ | ++ |
| HFD + OO | ++ | + | ++ | + | ++ | ++ | + | Trace | Trace | + | ++ |
| HFD + HXT | + | Trace | Trace | Trace | + | + | + | + | Trace | Trace | + |
| HFD + (HTX + OO) | Trace | Nil | Nil | Nil | Nil | Nil | Trace | Nil | Nil | Nil | Trace |
| HFD + ATOR | ++ | + | ++ | ++ | ++ | ++ | ++ | + | ++ | + | ++ |

• The symbols (+) represent a low degree, (++) represent a medium degree, and (+++) represent a high degree.

## 4. Conclusions

The outcomes of the latest study demonstrate that the high-fat diet (HFD) prompts to lower in the concentration of adiponectin, a rise in the concentrations of TNF-α and IL-6 in the serum, in addition to high MDA concentration, iron deposition, low GSH level, CAT enzyme activity in heart tissue, and the appearance of histological abnormalities in the lung.

The results also showed that Olive Oil(OO) and Hydroxytyrosol(HXT) Played a significant role in raising the concentration of Adiponectin, lowering the concentrations of TNF-α and IL-6 in the serum, in addition to enhancing the scale of enhancing and reducing iron deposition in the heart tissue and alleviating histopathological disorders in the lung contrasted to (HFD) group, and the reason for this is due to the active substances Existing in olive oil and the importance of Hydroxytyrosol being a polyphenol; finally, the results showed that mixing (OO) and (HXT) together have better efficacy than using them alone, as well as Atorvastatin(ATOR) in curbing the harmful effects of hyperlipidemia.

## Compliance with ethical standards

### *Statement of ethical approval*

This study was conducted in accordance with the ethical standards of the European and German Animal Welfare legislation, declaration principles set out by Helsinki, and the National Institutes of Health guidelines for the care and use of animals in research. All protocols were approved by the local ethics committee of the University of Kirkuk, Iraq.

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