Research article

Comparison of the effect of olive oil and its derivatives with atorvastatin in mitigating physiological and histological disorders due to hyperlipidemia in male rats

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(Received: April 2023 Revised: May 2023 Accepted: June 2023)

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ABSTRACT

Introduction: Hyperlipidemia destroys liver tissue, increasing liver enzymes. Increases liver extract ferritin, kidney and liver iron, while decreasing Hepcidin hormonal levels. Olive oil, hydroxytyrosol, and Atorvastatin were compared on liver enzymes, HMG-reductase activity, ferritin, hepcidin hormone, and iron deposition in liver and kidney tissues in experimental hyperlipidemic albino male rats.

Methodology: The experiment involved 50 albino rats grouped into two (control and test) of 25 rats each, then divided into 5 groups of 5 rats each. Animals in the first control group (blank) were fed the standard diet and gavaged distilled water for eight weeks, whereas the second to fifth groups of the first subgroup were fed the same standard diet for eight weeks followed by oral gavage with olive oil, hydroxytyrosol, olive oil + hydroxytyrosol, and atorvastatin, respectively, from the third week. Animals in the first test group were fed a diet containing 2% cholesterol for the duration of the experiment, whereas rats in the seventh, eighth, ninth, and tenth test groups were fed a high-cholesterol diet 2% for two weeks, followed by oral gavage with olive oil extract for the seventh group, hydroxytyrosol for the eighth group, olive oil extract + hydroxytyrosol for the ninth group, and a drug Atorvastatin for the tenth.

Results: Hyperlipidemia significantly increased the concentrations of liver enzyme in serum, ferritin and iron deposition in liver and kidney tissues, while decreasing the HMG-reductase activity and hepcidin hormonal levels in liver extract was seen in hyperlipidemia group in comparison to healthy controls. While the groups of treated animals showed a significant enhancement in each of the aforementioned factors, as olive oil extract + hydroxytyrosol outperformed all treatments.

Conclusion: Hypercholesterolemia increases liver enzymes, ferritin, and iron deposition while lowering hepcidin hormone and HMG-reductase activity. Hypercholesterolemia was mitigated by olive oil extract and hydroxytyrosol.

Keywords: Hyperlipidemia; liver enzymes; HMG-reductase; ferritin; hepcidin hormone; iron deposition.

INTRODUCTION

Hyperlipidemia responsible for cardiovascular disease is the leading cause of approximately 2.6 million deaths worldwide each year (1). Hyperlipidemia leads to metabolic changes that cause lipid disorders by increasing the levels of total cholesterol (TC), low-density lipoprotein-cholesterol (LDL-C) and triglycerides (TG) while decreasing the level of high-density lipoprotein-cholesterol (HDL-C). These changes occur as a result of an increase in the flow of excess non-esterified fatty acids to the liver, resulting in a decrease in the efficacy of HMG-reductase that enters the process of internal cholesterol synthesis. Hypercholesterolemia also leads to a raise in the concentrations of hepatic enzymes such as ALT, AST and ALP, and this boost in liver enzyme values indicates damage of some liver cells (2). Furthermore, previous epidemiological studies confirmed that oxidative stress resulting from destructive fat accumulation is the outcome of iron metabolic changes, such as stored iron (ferritin) level or serum iron level which indicate iron storage (3).

Statins are considered the standard drug in the treatment of hypercholesterolemia and in patients at increased risk of cardiovascular disease. Statins are very reliable, but they are associated with many side effects (4). The mechanism of the action of statins in lowering blood lipids can be understood through reversible competitive inhibition of HMG-reductase enzyme, which is a step in determining the rate of cholesterol biosynthesis (5). The beneficial role of olive oil consumption is widely acknowledged, and the metabolic effects of olive oil in humans shows it to improve the antioxidant levels and reduce liver inflammation and damage caused by increased concentration of liver enzymes such as AST, ALT, ALP (6). Hydroxytyrosol, the major polyphenol component of olive oil, is increasingly being studied due to its diverse therapeutic activities, such as antioxidant, anti-inflammatory activities (7).

MATERIALS AND METHODS

Materials

Olive oil (OO) was obtained by thermal pressing method. Male rats were treated with olive oil at a dose of 0.5 ml per 1 kg of body mass. Obtaining hydroxytyrosol (HXT) from Shaanxi Bolin Biotechnology-Shaanxi and male rats were treated with hydroxytyrosol with a dose of 50 µl /kg of body mass. Male rats were treated with Atorvastatin (ATOR) at a concentration of 2.06 mg/kg (8).

Animals

Male Sprague Dawley rats weighing between 200-260 g and aged 16 to 18 weeks were used in the experiment. The animals were housed in special cages that incorporated a stabilized temperature (22±2°C) and a 12-hour light/dark cycle. The animals were allowed unlimited access to food and water for two weeks while the cages were cleaned and sterilized daily, allowing them to acclimatize to the habitat and ensuring they were disease-free.

Experimental design

In this experiment 50 male adult albino rats of similar weights were randomly chosen, and assigned to ten groups (G1-G10) of five rats each. The groups were further subdivided as control (G1-G5) and test (G6-G10) groups. Animals in the control group (G1-G5) were fed with different diets which were as follows:

G1 group: (control blank) fed with a typical diet containing no cholesterol and administered filtered water for eight weeks.

G2 group: A cholesterol-free diet for eight weeks and gavaged with 0.5 mL/kg body weight olive oil for six weeks from the third week onwards.

G3 group: A standard cholesterol-free diet for eight weeks and then administered with HXT (50 µl/kg/bw) for six weeks beginning with the third week.

G4 group: A standard cholesterol-free diet for eight weeks and administered (olive oil 0.5 ml/kg/bw + HXT 50µl/kg/bw) for six weeks from the beginning of the third week.

G5 group: A standard cholesterol-free diet for eight weeks and administered with atorvastatin (2.06 mg/ kg/bw) for six weeks since the third week.

Similarly, animals in the test groups (G6-G10) were fed with the following diet:

G6 group: Cholesterol diet (2% w/w) of the standard diet and administered with distilled water for eight weeks.

G7 group: Cholesterol diet (2% w/w) of the standard diet for eight weeks and olive oil (0.5 ml/ kg/bw) for six weeks from the third week.

G8 group: A cholesterol diet (2% w/w) of the standard diet for eight weeks and HXT (50 µl/ kg/bw) for six weeks from the third week.

G9 group: A cholesterol diet (2% w/w of the standard diet) for eight weeks and administered (olive oil 0.5 ml/kg/bw + HXT:50 µl/ kg/bw) for six weeks from the third week onwards.

G10 group: A cholesterol diet (2% w/w of the standard diet) for eight weeks and administered atorvastatin (2.06 mg/kg/bw) for six weeks from the third week onwards.

Sample collection

Blood samples were collected at the end of the experiment. After 12 hours of starvation, the animals were anesthetized by intramuscular injection with Xylazine and ketamine 5-35 mg/kg/b.w. Heart blood was drawn and transferred to sterile tubes without anticoagulants, left at room temperature for 15 minutes to induce blood coagulation. The tubes containing blood were centrifuged at 3000 rpm for 15 minutes to obtain serum. Serum was used in biochemical analysis and stored at -20 °C until use. Liver and kidney were removed and their tissue sections examined for the percentage of iron deposition.

Preparation of liver tissue extract

Liver (1 g) was taken and placed in a homogenization medium consisting of 20 mM of EDTA and 0.25 M Tris-HCL buffer (pH 7.4). This was followed by crushing the liver using a tissue crushing device at a speed of 16,000 rpm. The resulting extract was transferred to a refrigerated centrifuge to maintain the vitality of the crushed tissue at a speed of 3000 rpm in 15 minutes, then the filtrate was taken and placed at a temperature of -80 ºC until the required tests were performed.

Biochemical tests for blood serum and liver tissue extract

The concentration of ALT, AST and ALP enzymes in the blood serum was determined according to the method outlined by the French company (Biolabo) based on the colorimetric method. ELISA technique-based kits were used to estimate HMG-CoA reductase activity and ferritin concentration, hepcidin hormone concentration in liver extract (Mybiosource, USA).

Histological preparations

The livers and kidneys removed were cleaned with physiological solution. The Prussian blue iron stain was used to prepare microscopic tissue sections according to the directions provided by the Chinese SunLong Biotech Company (9). The microscopic tissue sections were examined and photographed using a compound optical microscope.

Data analysis

Data was analyzed based on the examination of Variance (ANOVA) approach. Duncan's multiple range test was performed to see whether there were significant variations, at a significance level of ($P \leq 0.05$).

RESULTS

Concentration of ALT, AST and ALP Enzymes

The results exhibited in Table 1, show a noticeable rise in the level of liver enzymes (ALT, AST, and ALP) in animals in the cholesterol-treated groups (G6-10) compared with those of control groups (G1-G5). However, the level of these enzymes was observed to significantly decrease in test groups (G7-G10) animals compared to the cholesterol-treated control group (G6). The level of liver enzymes also decreased in the healthy control groups (G2-G5) in comparison to the healthy control group (G1). Olive oil and hydroxytyrosol together outperformed all treatments.

HMG-reductase activity, ferritin concentration, hepcidin hormone and iron deposition in liver and kidney tissues

The current study's findings revealed a significant decrease in the HMG-reductase activity and hepcidin hormone level, as well as a significant increase in ferritin and iron deposition in the liver tissue of the cholesterol-treated group (G6) compared to the healthy control (G1) group (Table 2, Figs 1 and 2).

Table 1: Effect of hydroxytyrosol, olive oil and atorvastatin on the concentration of ALT, AST and ALP enzymes in the blood serum of cholesterol-treated male albino rats

Groups	Parameters		
	ALT (IU/L)	**AST (IU/L)**	**ALP (IU/L)**
G1, Control (blank)	42.40±2.41^{e}	37.20±2.86^{e}	42.60±2.07^{e}
G2, Olive oil (OO)	35.00±1.58 ^{f}	32.00±1.58^{e}	38.40±2.40^{e}
G3, Hydroxytyrosol (HXT)	36.00±2.24^{ef}	30.60±2.07^{e}	37.00±1.58^{fe}
G4, (HXT)+ (OO)	34.60±3.13^{f}	33.80±1.92^{e}	34.20±2.28^{e}
G5, Atorvastatin	40.20±1.92^{ef}	33.60±3.58^{e}	41.00±3.16^{de}
G6, Hyperlipidemic diet (HLD)	160.00±7.91^{a}	175.00±7.91^{a}	185.60±8.02^{a}
G7, (OO)+HLD	129.40±7.02^{b}	141.60±5.94^{b}	139.00±7.14^{b}
G8, (HXT) +HLD	105.20±7.60^{c}	116.40±6.11^{c}	107.00±9.85^{c}
G9, (HXT) + (OO)) +HLD	63.20±5.81^{d}	62.40±7.99^{d}	61.60±8.29^{d}
G10, Atorvastatin +HLD	131.80±6.10^{b}	147.20±5.63^{b}	139.40±7.09^{b}

Values are represented as arithmetic mean ± standard deviation **.** Superscript letters indicate significant differences (P≤0.05).

Table 2: Effect of hydroxytyrosol, olive oil and atorvastatin on HMG-reductase, ferritin, hepcidin hormone concentration in liver extract and iron deposition in the liver and kidney tissues of animals on cholesterol treatment

Groups	Parameters				
	HMG (ng/ml)	**Ferritin (ng/dL)**	**Hepcidin (ng/dL)**	**Iron deposition in liver**	**Iron deposition in kidney**
G1, Control	1875.00±19.36^{a}	5.42±0.28^{f}	460.40±17.47^{d}	trace	trace
G2, Olive oil (OO)	1726.00±20.74^{b}	4.40±0.32^{g}	521.80±16.78^{c}	trace	trace
G3, Hydroxytyrosol (HXT)	1575.80±19.12^{c}	4.00±0.16^{h}	571.00±15.41^{b}	trace	trace
G4, (HXT) + (OO)	1376.40±19.27^{d}	2.96±0.27^{i}	666.60±15.52^{a}	trace	trace
G5, Atorvastatin	1576.80±17.73^{c}	4.36±0.30^{g}	523.40±18.68^{c}	trace	trace
G6, Hyperlipidemic diet (HLD)	877.00±19.26^{h}	10.46±0.36^{a}	272.00±16.00^{g}	+++	+++
G7, (OO)+HLD	977.20±16.35^{g}	8.34±0.27^{c}	321.80±16.66^{f}	++	++
G8, (HXT) +HLD	1080.00±17.33^{f}	7.26±0.24^{d}	370.40±15.24^{e}	++	+
G9, (HXT) + (OO)) +HLD	1281.60±17.31^{e}	6.280±0.24^{e}	449.00±20.74^{d}	trace	trace
G10, Atorvastatin +HLD	1054.00±33.62^{f}	9.26±0.21^{b}	334.00±18.47^{f}	++	++

The values are represented as arithmetic mean ± standard deviation **.**Superscript letters indicate significant differences (P≤0.05) **.**The iron deposition in the liver and kidney is represented as low (+), medium (++), and high (+++).

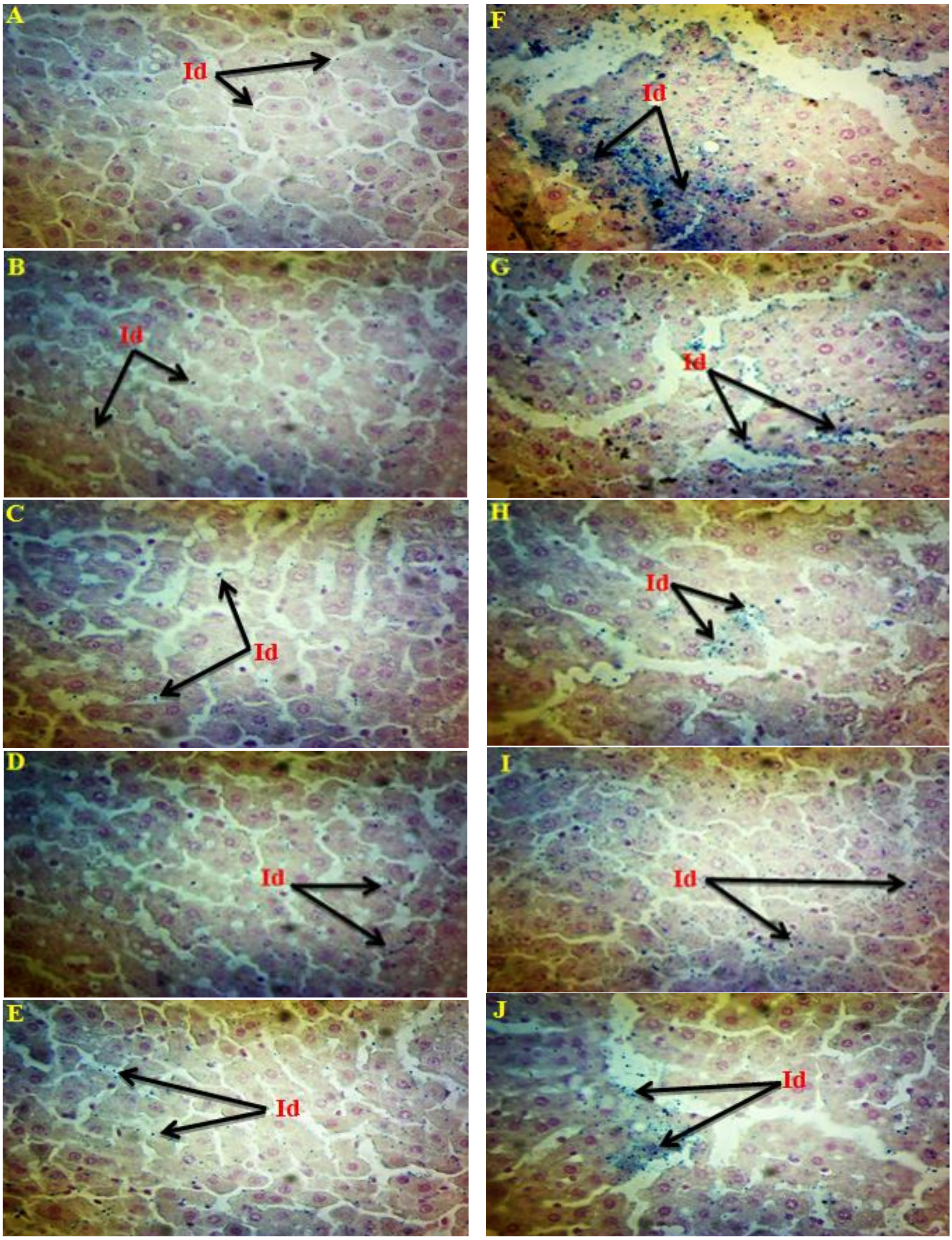

Fig. 1: Histopathological sections of rat liver showing iron deposition. Figs. A-E show control group (G1-G5) liver sections, while Fig. F-J show test group (G6-G10) sections. Highest iron deposition (+++) in liver is seen in Fig.1F, for animals fed with hyperlipedimic diet (G6). Medium (++) level of iron deposition is seen in groups G7, G8, and G10 corresponding to sections Figs. G, H and J respectively. All other liver sections had traces of iron deposition. Sections were stained with Prussian Blue and viewed under 200X.

Among the test groups (G7-G10), in groups treated with cholesterol and administered with olive oil (G7), hydroxytyrosol (G8), (olive oil + hydroxytyrosol (G9) and atorvastatin (G10), the HMG-reductase activity and the level of hepcidin hormone increased, while and the level of ferritin and iron deposition reduced compared to the cholesterol-treated control group (G6). As such the level of HMG-reductase activity, ferritin, and iron deposition lowered, and the level of hepcidin hormone increased in the healthy control group administered with olive oil (G2), hydroxytyrozole (G3), (olive oil + hydroxytyrosol) (G4) and atorvastatin (G5). With regard to the degree of iron deposition in liver and kidney tissue sections, our investigation found that the cholesterol-treated group (G6) had a higher proportion of iron (+++)

than the healthy control group (G1), Within the test groups, results for the level of iron deposition in rat liver and kidney tissues showed the same results with the highest deposition in rats fed with hyperlipidemic diet +HLD (G6), followed by medium levels in G7, G8, G10 groups (Table 2). Traces of iron deposition was seen in the healthy control groups (G1-G5), as well as in the test group G9, where the animals were administered a diet consisting of olive oil + HLD (Table 2, Figs.1 and 2).

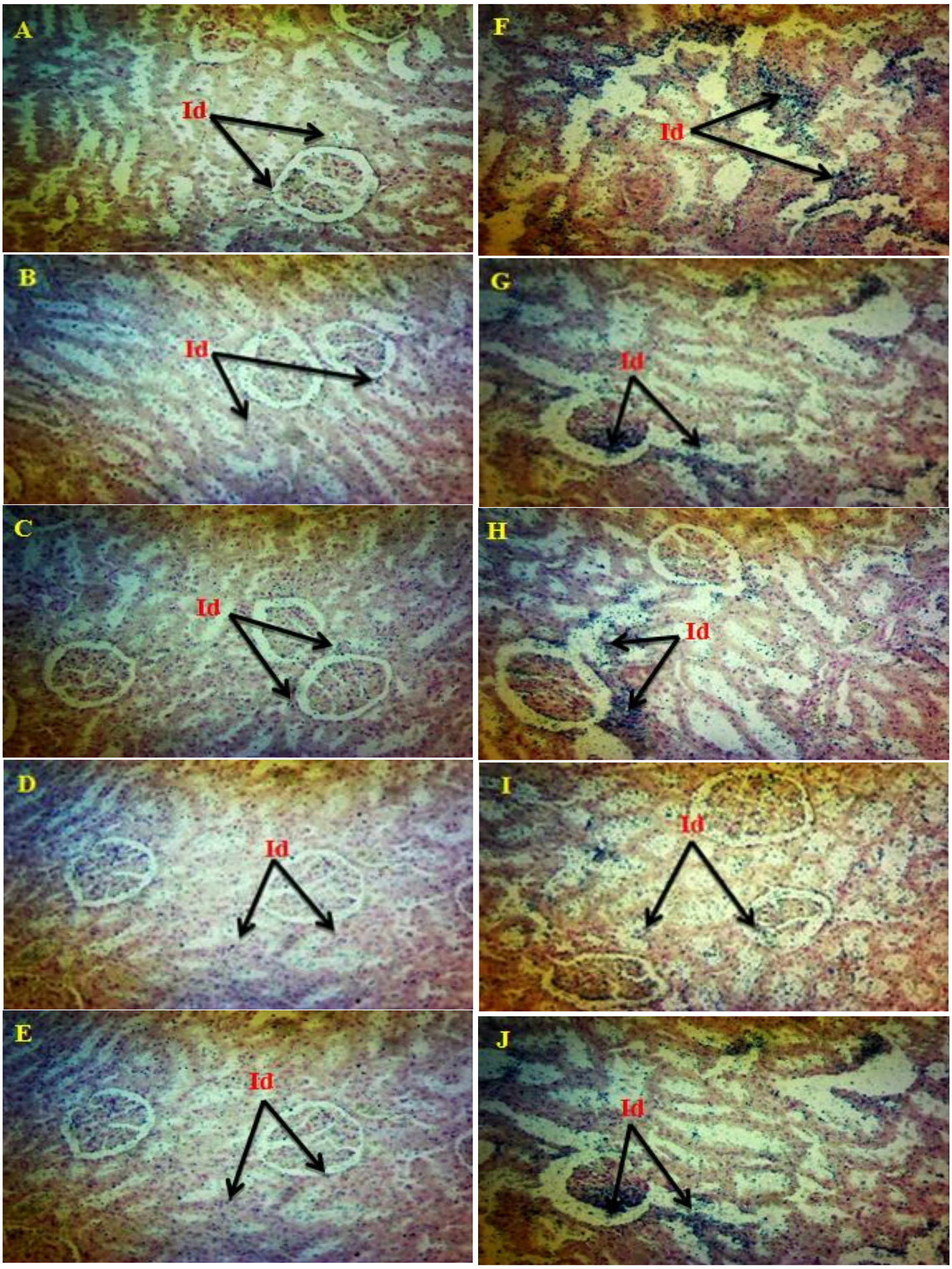

Fig. 2: Histopathological sections of rat kidney showing iron deposition. Figs. A-E are control group (G1-G5) kidney sections, while Figs. F-J are test group (G6-G10) sections. Highest iron deposition (+++) in the kidney is seen in Fig.1F, for animals fed with hyperlipedimic diet (G6). Medium (++) level of iron deposition is seen in groups G7, and G10 corresponding to sections Figs. G and J respectively. Low (++) level of iron deposition is seen in groups G8, Figs H. All other kidney sections had traces of iron deposition. Sections were stained with Prussian Blue and viewed under 200X.

DISCUSSION

Our findings showed that the cholesterol-treated groups (G6–G10) had significantly higher levels of ALT, AST, and ALP enzymes than the healthy controls (G1–G5), which is consistent with the findings of a previous similar study (10). The high levels of these enzymes have been attributed to hepatocyte injury and cytosol enzymes escape into blood serum (11). In the animal group administered together with olive oil and hydroxytyrosal, the liver enzyme levels were observed to be reduced which could be attributed to the effectiveness of hydroxytyrosal which has been shown to in elevate the levels of catalase, superoxide dismutase, and glutathione reductase enzymes, thereby reducing the histological disorders of liver (12). Our findings were also consistent with a previous study (13), which revealed a significant reduction in GGT and MDA levels, and a significant increase in GSH and CAT levels, bringing about an improvement in liver disorders when treated with a combination of olive oil and hydroxytyrosol. The study attributed the improvement to the active ingredients present in olive oil and hydroxytyrosol, having the capacity to increase the antioxidant levels and inhibiting the formation of free radicals.

Concerning the effectiveness of atorvastatin in reducing concentrate of ALT, AST and ALP enzymes, our results agree with the study of (14) which manifested a reduction in the concentration of ALT, AST and ALP enzymes in male rats of a group treated with Atorvastatin compared to the hyperlipidemia group for a period of eight weeks. This result is explained on the basis of the drug's role in reducing the level of free radicals and increasing the level of antioxidants, thus reducing cell damage and leakage of liver enzymes, as confirmed (15) that the use of Atorvastatin leads to a decrease in the level of ONOO- and an increase in the scale of SOD and GPx When hyperlipidemia was induced in male rats compared with the hyperlipidemic group.

Our findings also revealed a significant decrease in HMG-reductase activity and the hepcidin hormonal levels, as well as a significant increase in ferritin level and iron deposition in the liver and kidney tissues of the cholesterol-treated group (G6) when compared to healthy controls (Table 2). This finding is consistent with a previous study (16), which showed that weight gain and lipid disorders cause an increase in ferritin concentration, due to decreased hepcidin hormone levels. The increased ferritin concentrations can lead to increased deposition of iron in liver and kidney (17). A study conducted by (18) confirmed that the increase in body weight as a result of the accumulation of fat causes an increase in the level of ferritin. On the other hand, the results of a study carried out by (19) exposed a decrease in HMG-reductase and HDL and an increase in LDL, TC and TG when hyperlipidemia was induced in male rats. The decrease in HMG-reductase was ascribed to the escalating cholesterol concentration in the plasma because of its addition to the diet. In a study published by (20) it was confirmed that the level of HMG-reductase is inversely proportional to HDL and directly to LDL, since transferring of cholesterol to tissues decreases the need for internal manufacture of cholesterol.

The health benefits of olive oil as a component of the Mediterranean diet have been extensively researched and linked to its polyphenol content. The polyphenols in olive oil have been shown to possess antioxidant and anti-inflammatory properties, leading to a reduction in endothelial dysfunction and lipid buildup (21). In addition, these polyphenols help protect LDL and DNA from oxidation (22). Use of olive oil in combination with hydroxytyrosol was shown to lead to a better modification of the lipid profile, iron, and hepcidin hormone levels in the blood serum than when used separately. The results in this study were in line with the findings of (23).

The atorvastatin drug used showed to normalize the HMG-reductase activity, ferritin and Hepcidin levels, as well as lowering LDL, TC, and TG levels, and increasing HDL cholesterol levels. The impact of Atorvastatin in lowering MDA and increasing GSH in hypercholesterolemia induction in rats has been also attributed to increasing hepcidin levels and decreasing ferritin and iron deposition (24). A study also confirmed that the use of atorvastatin leads to a decrease in the level of total cholesterol, triglycerides, LDL-c and VLDL-c, and an increase in the level of HDL-c in the liver when induced with hyperlipidemia in male rats (25).

CONCLUSION

The current investigation found that hyperlipidemia causes an increase in the levels of hepatic enzymes (ALT- AST- ALP) in blood serum. Hyperlipidemia reduces HMG-reductase activity and hepcidin hormone levels while increasing ferritin levels and iron deposition in liver and kidney tissues. The combined use of olive oil and hydroxytyrosol, can help reduce the negative effects of hyperlipidemia.

CONFLICT OF INTEREST

The authors have no conflicts of interest.

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