Protective Effect of Nettle and Olive Leaves on Hyperlipidemia in Experimental Rats
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ABSTRACT

Dyslipidemia is a major contributor to atherosclerotic cardiovascular disease. Recently, World Health Organization (WHO) reported that cardiovascular diseases (CVD) account for 46% of ever all mortality in Egypt. So that; the present study investigated, the protective effect of nettle and olive leaves on hyperlipidemia in experimental rats. Thirty six male albino rats weighing 130 ± 20 g used in this study and divided into equal six groups ( 6 rats each), the first kept as anegative control group (-ve) received basal diet throughout the experiment period, while the second was the (+ve) control group which fed on hyperlipidemic diet for four weeks, while the four others groups given hyperlipidemic diet supplemented with nettle leaves (5%) , (10 %) and olive leaves(5 %), (10%) respectively for four weeks (astreatment groups ) .The chemical composition and phenolic compounds of both leaves were done. At the end of the experiment, biological data were calculated; blood samples were taken to biochemical analysis. In addition, histopathological examination was done. The results revealed that hyperlipidemic diet in the (+ve) control group increased body weight gain ,relative organ weight, serum lipid profile, Malondialdehyde (MDA), liver enzymes and serum glucose, decreased in serum HDL-C, serum Superoxide Dismutase (SOD), and Glutathione Peroxidase (GPx) . All treated groups with two leaves showed improvement previously parameters compared with positive control group. In conclusion, the consumption of nettle and olive leaves could be used for improving lipid profile, liver function and protect from hyperlipidemia in experimental rats.

Key words: lipid profile- nettle - olive - leaves – antioxidant enzymes- liver function
INTRODUCTION

Hyperlipidemia (HLD) is a condition that incorporates various genetic and acquired disorders that describe elevated lipid levels within the human body (Hill and Bordoni, 2021). HLD is characterized by high levels of low-density lipoprotein cholesterol (LDL-C) or high triglycerides (TG), and plays an important role in the pathogenesis of cardiovascular diseases (Li et al., 2021).

From ages, human diseases are treated by herbal medicines in almost every infection. In modish age, it is choice to make use of them instead of the synthetic ones as there are fewer side effects of traditional medicines. Plants are natural source of treatment and are used from ages for food and medicine (Shah et al., 2021). Herbal plants have also been found to nourish the body and provide vitamins, minerals, and many trace elements that are easy to absorb (Melkegna and Jonah, 2021).

Stinging nettle (Urticadioica L.) is a perennial herb with a long history of traditional medicinal uses in many countries. Nettle leaves are rich in chlorophyll, carotenoids, vitamins, proteins, fats, carbohydrates, organic acids, minerals, and trace elements. The leaves of stinging nettle contain abundant amounts of natural phenolic compounds, which may function as effective natural antioxidants (Paulauskienė et al., 2021). This herb has a history of use in traditional medicine to treat diverse conditions including lipid disturbances. Positive effect of Urticadioica L. against lipid derangements has been demonstrated in vivo studies (Namazi et al., 2018).

Olive tree leaves have been widely used as traditional remedies to cure several diseases. Chemical characterization analysis revealed that olive tree leaves contain several bioactive compounds that may exert beneficial effects in certain morbidities such as metabolic syndrome, and hypertension (Ranieri et al., 2021).

Since little information is available regarding the preventive influences of Urticadioica L. and Oleaeuropaea L. leaves, on hyperlipidemia in vivo, this study was carried out.

MATERIALS & METHODS

Plant materials:
Dry *Urtica dioica* L. and *Olea europaea* L. leaves were purchased from The Local Company for Herbs and Medicinal Plants, Cairo Governorate, Egypt.

**Preparation of plant:**
Dry *Urtica dioica* L. and *Olea europaea* L. leaves were homogenized in the blender then stored at room temperature in closed glass bottles in the dark until using.

**Animals:**
Thirty-six adult male albino rats *Sprague Dawley* strain weighing (130 ± 20 g) were housed in well-aerated cages under hygienic condition and were fed on basal diet for one week to adapt.

**Methods:**
**Chemical analysis**
- Chemical composition (protein, fat, moisture, ash and carbohydrates calculated by difference) determined according to (A.O.A.C, 2010).
- Phenolic compounds and flavonoids of both powder evaluated by method of Tarola et al., (2013).

**Experimental design:**
The Animals were divided into equal six groups. The first group (6 rats) fed on basal diet according to Reeves et al., (1993), and served as a negative control group (-ve) control, while the second was the (+ve) control group which fed on hyperlipidemic diet for four weeks according to Rashwan (1994), the third and fourth groups fed on hyperlipidemic diet supplemented with nettle leaves powders (5%) and (10 %) respectively. The fifth and sixth groups fed on hyperlipidemic diet supplemented with olive leaves powders (5%) and (10 %) respectively for four weeks (astreatment groups). At the end, animals were weighed, fasted overnight, and then sacrificed after anesthesia ones this. Blood samples will be was collected from hepatic portal vein of each rat into dry clean centrifuge tubes. Serum was carefully separated by centrifugation of blood samples at 3500 rpm (round per minute) for 15 minutes at room temperature, transferred into dry clean Eppendorf tubes, then kept frozen at - 20°C for latter determinations. Liver, kidney and heart has been removed from rats by careful dissection and washed in saline solution (0.9%), dried using
filter paper and independently weighed.

**Biological evaluation**

During the experiment (28 days), feed intake was recorded every day and body weight was recorded every week. Biological evaluation of the different diets was carried out by calculating of body weight gain % (BWG %) and feed efficiency ratio (FER) according to Chapman *et al.*, (1959).

**Biochemical analysis of serum**

Serum was analyzed for various biochemical parameters like lipid profile, total cholesterol, triglycerides and HDL-C were evaluated on the authority of Allain *et al.*, (1974); Trinder and Ann, (1969) and Lopes –Virella *et al.*, (1977) but LDL-C and VLDL-C calculated as claimed by Friedwald *et al.*, (1972). The atherogenic indexe was calculated as stated by Tilvis and Miettinen (1986). Antioxidant enzymes super oxide dismutase (SOD), glutathione peroxidase (GPx) and Malondialdehyde (MDA) level as a parameter for the lipid peroxidation were evaluated in the opinion of Kakkar *et al.*, (1984), Ellman (1959) and Draper *et al .*, (1993).

Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Alkalinephosphatase (ALP) were measured on the report of Bergmeyer *et al.*, (1986) and Roy, (1970). Glucose was estimated in the serum as maintained by Trinder (1959).

**Histopathology investigation**

The heart and liver were fixed in 10% buffered neutral formalin immediately following excision from animals. Fixed tissues were subsequently processed for histopathology examinations as previously described by Carleton (1979).

**Statistical analysis**

Results are expressed as mean ± standard deviation (SD). Differences between means indifferent groups were tested for significance using a one-way analysis of variance (ANOVA) followed by Duncan's test and probability value of 0.05 or less was considered significant. Comparative of means were performed according to least significant differences test (LSD) according to (Snedecor, 1969) using SPSS (version 20).
RESULTS & DISCUSSION

Table (1) showed the averages (g) of moisture, protein, fat, carbohydrate and ash (g/100gm) in nettle and olive leaves powder. The results of chemical composition for nettle revealed that ash recorded the highest average followed by carbohydrate, crude protein, fiber, moisture and crude fat respectively. While, the results of chemical composition for olive leaves powder revealed that carbohydrate recorded the highest average followed by fiber, crude protein, ash, moisture and crude fat respectively. These results agree with Maria et al., (2019) who revealed that carbohydrates, protein and minerals of nettle leaves powder were high. While, moisture and crude fat contents were relatively low. Salama et al., (2020) who reported that the olive leaves powder have high carbohydrate, Crude protein contents while moisture and crude fat contents were relatively low.

Data in table (2) clarified the nettle leaves powder recorded the higher content from Benzoic acid, rosemarinic, Neringein, Myricetin, 3-Hydroxytyrosol, Quercitin, Resvertol, p- Hydroxy benzoic acid, Catechol, Ellagic, Kampherol and Quinol , o-Coumaric acid, Catechin, Syringic acid ,Gallic acid, Ferulic acid, Cinnamic acid as shown in fig.(1). This is accordance with Repajić et al., (2021) who reported that nettle leaves accumulate higher amounts of polyphenols and chlorophylls. In general, leaves are the richest part of a nettle in bioactive compounds; therefore, they are mostly used in processing.

Statistical data in table (3) observed the olive leaves powder recorded the higher content from rosemarinic, Ellagic, Quercitin ,Myricetin, Benzoic acid, Rutin, Kampherol, Resvertol, 3-, Catechol, p- Hydroxybenzoic acids, Caffeic acid, Vanillic acid , Chlorogenic ,Ferulic acid ,cinnamic acid as shown in fig. (2). This is accordance with Ghanem et al ., (2019) who reported that Chemically, leaves of olive contain considerable biophenols such as the other parts of the olive tree Oleuropein and its metabolites, including tyrosol and hydroxytyrosol, are the most abundant phenolic compounds known in the olive leaf. Oleuropein has antibacterial, antiviral, antitumor, blood pressure, and blood lipid-reducing factor, anticancer, and
cardio-protective activities. In table (4) hyperlipidemic control (+ve) group revealed more BWG %, FI and FER compared with the control (-ve). All hyperlipidemic rats fed on olive and nettle leaves showed significant decreases in mean values as compared to control (+ve) group. The mean value of (FI) control (-ve) group was lower than control (+ve) group, being 20.95±2.38 and 23.23±2.94 respectively, the best results for FI (g/day) recorded by group fed on olive leaves 10% which closed to normal group. It could be noticed that the mean value of FER of control (-ve) group was higher than control (+ve) group, being .087 ±.02 and 0.15±.02 respectively, the best results for FER recorded by hyperlipidemic rats fed on Nettle 5%being.0.14 ±.015. This result is harmony with Fan et al., (2020) who reported that the high fat (HF) diet supplemented with U. dioica (HFUT) had significantly reduced weight gain compared to the HF diet over the 12 weeks. U. dioica protects against diet-induced obesity through mechanisms involving lipid accumulation and glucose metabolism in skeletal muscle, liver, and adipose tissue. It may be due to the U. dioica are likely attributed to the high fiber content, phytochemical components, high protein content.

The finding in table (5) suggest that, significant (p ≤ 0.05) increased in (cholesterol and triglyceride ) in (+ve) control group compared with (-ve) control group. While, these parameters decreased in all treated groups especially Olive 10% and Nettle 10%. On the other hand, HDL parameter recorded high increase in Olive 10%followed by Nettle 10% group. These results agree with Eldamaty, (2018) who observed that the Urticadioica leaves powder has lowered the levels oflipids and lipoproteins in blood. The significant decrease was found in lipid profile as total cholesterol, cholesterol fractions and LDL/HDL ratios via lower concentrations of LDL. On the other hand, these results agree with Fki et al ., (2020) who indicated the hypolipidemic effect of the olive leaf phenolic in high-fat and high-cholesterol diet rats It is mainly due to the typical phenolic composition linked to potent biological activities . The major active component in olive leaves is oleuropein.

Results in table (6) revealed that, significant (p ≤ 0.05) increased in (LDL, VLDL and AI)
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in (+ve) control group compared with (-ve) control group. While, these parameters decreased in all treated groups especially Olive 10% and Nettle 10%. On the other hand, HDL parameter recorded high increase in Olive 10% followed by Nettle 10% group. Leaves of olive might be an excellent and promising source of bioactive-compounds, which in turn have high antioxidative properties as reported by Elmaadawy and Alsadeq (2015). Also, these results agree with Eldamaty, (2018) observed that the Urticadioica leaves powder has lowered the levels of lipids and lipoproteins in blood; this is due to that the uniquely high level of polyphenols in Urticadioica leaves may play an important role in contributing to the health benefit such as lowering cholesterol and hyperlipidemia level. The protective effect of U. dioica leaf hydroalcoholic extract against atherosclerosis in rats. Atherosclerosis was experimentally induced in the laboratory by high-fat diet atherosclerotic rats (UD) that received 100 mg/kg/day of ethanolic extract of UD orally. Authors showed, through histopathological evaluations of the aortic arch, that UD group had an improvement of atherosclerotic. UD significantly reduced medial (p < 0.05) but not intimal thickness. The LDL-C/HDL-C ratio significantly decreased in UD group as reported by (Grauso et al., 2020). More than hypolipidemic effects of olive leaf extracts have been proved in a number of studies. It was found that 50 and 100 mg/kg/day doses of olive leaf extract may positively affect atherosclerosis by decreasing total cholesterol and low-density lipoprotein (LDL) cholesterol levels in rats (Tek and Ağagündüz 2020).

Biomarker data in table (7): showed that, significant (p ≤ 0.05) decreased in (SOD and GPx) in (+ve) control group compared with (-ve) control group. While, MDA was increased in (+ve) control group. However, the reverse recorded for treated groups especially Olive 10% and Nettle 10%. This significant observation could be explained by the capacity of oleuropein to reduce free radical accumulation, generated after the lipid per-oxidation (Jemai et al., 2020). These results agree with Telo et al. (2017) who administrated that the group fed
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with nettle showed a slight increase in SOD and GPx activities and decreased in the lipid peroxidation.

Liver function enzymes in table (8): showed that, significant (p ≤ 0.05) increase in (AST, ALT and ALP) in (+ve) control group compared with (-ve) control group. While, these parameters decreased in all treated groups especially Olive 10% and Nettle 10%. Similar results were obtained by Shaheen and Elkersh (2019) who revealed that, olive leaf extract had very high phenol content and possess strong antioxidant activity administration, which resulted in improved serum ALT, AST and ALP activities and increased serum total antioxidant capacity. So, the improving effect of olive may related to its antioxidant activity. Hepato-protection is the ability to prevent damage to the liver, prevent the liver affections prophylactically and maintains balance in liver enzymes as reported by Eldamaty, (2018) who showed that diabetics rat fed on Urticadioica leaves in basal diet were decreased in AST and ALT compared with control positive.

Data of glucose analysis in table (9) revealed that, significant (p ≤ 0.05) increase in Serum Glucose in (+ve) control group compared with (-ve) control group. While, this parameter decreased in treated groups especially Olive 10% and Nettle 10%. These results agree with Fan et al., (2020) revealed that U. dioica reduced fasting glucose determined by glucometer. Also Eldamaty, (2018) reported that Stinging nettle (Urticadioica) has a great medicinal value such as relieve of lowering glucose in blood. In the other study revealed that olive leaf extracts ameliorated the level of glucose as reported by Sakr et al., (2016) who concluded that the ameliorative effect of olive leave extracts against toxicity of diabetes in rats may be attributed to the presence of its phenolic compounds.

Histopathological examination of liver tissue:
This study examined the effect of high fat high on liver tissues using histological examination. Present results indicated that, Microscopic pictures of H&E stained liver sections showing normal hepatic architecture with radially arranged hepatic cords around central veins (CV) with normal portal areas (PA) and sinusoids in control normal group as...
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shown in photo (1). Liver sections of (+ve) group showing highly disrupted hepatic architecture due to diffuse ballooning degeneration (black arrows) with multifocal necrosis (blue arrows) of hepatocytes, portal inflammation (yellow arrows) Low magnification X:100 bar 100 and high magnification X:400 bar 50 as shown in photo (2). These results agree with Giammanco et al., (2016) examined the histological sections of liver tissue from high fat diet rats. Note the widespread intracellular vacuolization of hepatocytes and the resulting relocation of cell nuclei in a peripheral position. This results agree with Lasker et al., (2019) who showed that the hepatic tissue from the control group revealed a normal architecture of hepatocytes, with no appearance of lipid/fat deposition and inflammatory cell infiltration, whereas HF diet-fed groups showed degenerative changes in hepatocyte along with lipid/fat droplet deposition and inflammatory cell infiltration.

While the group which received olive 5% showing mildly disrupted hepatic architecture due to moderate per portal hydropic degeneration (black arrows) of hepatocytes and mild lobular inflammation (yellow arrows) as shown in photo (3). Liver sections from group received 10% olive showing greatly restored hepatic architecture with few cytoplasmic vacuoles (black arrows) in hepatocytes around PAas shown in photo (4). Liver sections from group received nettle 5% showing moderately disrupted hepatic architecture due to hydropic degeneration (black arrows) and large cytoplasmic vacuoles in hepatocytes (red arrows) around CV and PAas shown in photo (5). Liver sections from group received nettle 10% showing partially restored hepatic architecture with few minute cytoplasmic vacuoles in hepatocytes around CV (red arrows) and mild hydropic degeneration in hepatocytes (black arrows) around PA. Low magnification X: 100 bar 100 and high magnification X: 400 bar 50. As shown in photo (6). This results agree with Omagari et al., (2021) reported that Oleuropein, an active constituent of olive leaf, have protective effects against non-alcoholic fatty liver disease (NAFLD) in vivo. The oleuropein with which the HFD was supplemented reduced the hepatic mRNA level of the genes that
encoded the key regulators of the hepatic fatty acid uptake and transport. In addition, the oleuropein reduced the expression of a number of hepatic genes involved in the oxidative stress responses and detoxification of lipid peroxidation products and pro-inflammatory cytokine genes. These results agree with Fan et al., (2020) who concluded that *U. dioica* protects against diet induced obesity through mechanisms involving lipid accumulation and glucose metabolism in skeletal muscle, liver, and adipose.

**Histopathological examination of Heart tissue:**
This study examined the Effect of high fat high on Heart tissues using histological examination. Our results indicate that.: Microscopic pictures of H&E stained heart sections showing normal longitudinally and crossly sectioned cardiac muscle fibers with normal blood vessels and interstitial tissue in control normal group as shown in **photo (1)**. Heart sections of +ve group showed congested blood vessels (red arrows), marked perivascular edema (blue arrows) and macro-vesicular vacillations of crossly sectioned cardiac muscle fibers (black arrows) as shown in **photo (2)**. These results agree with Sahraoui et al., (2016) revealed that High fat diet induced structural disorganization and interstitial edema associated with the accumulation of infiltrating cells and lipids within the myocardium, suggesting cardiomyopathy. High fat diet exacerbated myocardial interstitial and perivascular fibrosis.

Microscopic pictures of H&E stained heart sections showing from group received olive 5% showing mildly congested blood vessels (red arrows), perivascular and interstitial edema (blue arrows) and few macro vesicular vacillations of crossly sectioned cardiac muscle fibers (black arrows) as shown in **photo (3)**. Heart sections from group received olive 10% showing greatly restored histological appearance as shown in **photo (4)**. Heart sections from group received nettle 5% showing congestion (red arrows), perivascular and interstitial edema edema (blue arrows) with some macro vesicular vacillations of crossly sectioned cardiac muscle fibers (black arrows) as shown in **photo (5)**. Heart sections from group received nettle 10% showing mild congestion (red arrows) as shown in **photo (6)**. These results agree with Namazi et al., (2018) who showed there was a significant decrease in The aortic arch thickness of...
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In histopathological evaluations of the aortic arch, which Conclusion Ethanolic extract of UD prevents establishment of atherosclerotic lesions in rat aorta, which is associated with positive effects on serum lipid profile without significantly affecting antioxidant status. Also, Tesfaye et al., (2021) reported The cardio protective activity of the crude extract and solvent fractions of Urticasimensis leaves were confirmed by the histopathologic examination of the cardiac tissues. The cardioprotective effect could be attributed to the antioxidant activity of the plant extracts. The cardioprotective activity of solvent fractions of Urticasimensis leaves were confirmed by the histopathologic examination of the cardiac tissues of treated animals. The cardiac tissues in the normal control group showed normal morphological architecture with no cellular necrosis, interstitial space edema and hemorrhage.

CONCLUSION:
This is study evaluated the protective effect of the nettle and olive leaves on hyperlipidemia in rats. Which in the leaves of nettle and olive have contain abundant amounts of natural phenolic compounds, flavonoids, phenolic acids, anthocyanins, and other phenols, which may function as effective natural antioxidants. The consumption of nettle and olive leaves could be used for improving lipid profile, liver function and protect from hyperlipidemia in experimental rats.

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Table 1: The averages of moisture, protein, fat, carbohydrate and ash (g/100g) dryin nettle and olive leave powder

| Proximate composition(g/100g) | nettle leaves | olive leaves |
|-------------------------------|--------------|-------------|
| Moisture                      | 9.4          | 7.1         |
| Crude protein                 | 18.60        | 10.36       |
| Crude fat                     | 1.15         | 3.42        |
| Ash                           | 41.61        | 8.39        |
| Carbohydrate                  | 29.24        | 69.73       |
| Fiber                         | 17.16        | 22.60       |

Table 2: The phenolic and flavonoids profiles in *Urticadioica*

| Flavonoids                  | mg / g leaves | Penolic acids   | mg / g leaves |
|-----------------------------|---------------|-----------------|---------------|
| Pyrogallol                  | -             | Gallic acid     | 6.74126       |
| Quinol                      | 18.75611      | p- Hydroxy benzoic acid | 31.97594     |
| 3-Hydroxytyrosol            | 148.37622     | Vanilllic acid  | -             |
| Catechol                    | 29.86980      | Caffeic acid    | 4.41400       |
| Catechin                    | 8.31606       | Syringic acid   | 6.89341       |
| Chlorogenic                 | -             | p- Coumaric acid | 3.78981e-1   |
| Rutin                       | -             | Benzoic acid    | 1137.97205    |
| Ellagic                     | 25.24869      | Ferulic acid    | 4.77484       |
| Resvertol                   | 53.28780      | o- Coumaric acid | 11.61914  |
| Quercitin                   | 102.62865     | Cinnamic acid   | 1.00435       |
| rosemarinic                 | 420.34409     |                 |               |
| Neringein                   | 261.62098     |                 |               |
| Myricetin                   | 189.87756     |                 |               |
| Kampherol                   | 22.11440      |                 |               |
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Figure 1. Phenols & flavonoids of nettle leaves

Table 3: The phenolic and flavonoids profiles in olive leaves

| Flavonoids          | mg / g leaves | Penolic acids       | mg / g leaves |
|---------------------|---------------|---------------------|---------------|
| Pyrogallol          | -             | Gallic acid         | -             |
| Quinol              | -             | p-Hydroxy benzoic acid | 182.71399    |
| 3-Hydroxytyrosol    | 430.31062     | Vanillic acid       | 102.01740     |
| Catechol            | 280.73027     | Caffeic acid        | 157.46667     |
| Catechin            | -             | Syringic acid       | -             |
| Chlorogenic         | 61.71219      | p-Coumaric acid     | -             |
| Rutin               | 809.39698     | Benzoic acid        | 1153.61157    |
| Ellagic             | 1916.31634    | Ferulic acid        | 40.43528      |
| Resvortol           | 609.78548     | o-Coumaric acid     | 111.57746     |
| Quercitin           | 1288.72774    | Cinnamic acid       | 29.61781      |
| rosemarinic         | 3181.04967    |                    |               |
| Neringein           | -             |                    |               |
| Myricetin           | 1232.29864    |                    |               |
| Kampherol           | 621.19737     |                    |               |
Table 4: Protective effect of nettle and olive leaves on body weight gain (BWG %) and feed efficiency ratio (FER) of hyperlipidemia rats (n= 6 rats)

| Parameters                | FL (g)    | BWG (%)    | FER        |
|---------------------------|-----------|------------|------------|
| Control – ve              | 20.95±2.38f | 42.93±12.35c | .087±.02c |
| Control + ve              | 23.23±2.94b | 78.23±19.66a | 0.15±.02a |
| Nettle 5%                 | 22.8 ±4.26c | 73.03±12.78a | 0.14 ±.015ab |
| Nettle 10%                | 27.6±6.44a  | 53.97±9.50bc | 0.09±.012c |
| Olive 5%                  | 22.4 ±7.49d | 63.25±16.24ab | 0.12±.02b |
| Olive 10%                 | 21.23 ±5.93e | 46.70±14.12bc | .09±.02c |
| LSD                       | 6.19       | 17.06      | 0.02       |

Values denote arithmetic means ± SD. Means with different letters (in the same column are significantly at (p ≤ 0.05) using one-way ANOVA test, while those with similar letters are non-significant.
Table 5: Protective effect of nettle and olive leaves on Total cholesterol (T.C) and Triglycerides (T.G) of hyperlipidemic rats (n= 6 rats)

| Groups     | Total cholesterol (mg/dl) | Triglycerides (mg/dl) |
|------------|---------------------------|-----------------------|
| Control – ve | 68.0 ±7.87d               | 86.16 ± 12.38d        |
| Control + ve | 150.8 ± 11.90a           | 200.5 ± 22.58a        |
| Nettle 5%   | 116.3 ±15.90b             | 153.6 ± 22.43b        |
| Nettle 10%  | 89.0 ± 9.31c              | 118.3 ± 17.61c        |
| Olive 5%    | 112.0 ± 10.80b            | 145.83 ± 16.30b       |
| Olive 10%   | 75.3 ± 5.78d              | 106.66 ± 14.19d       |
| LSD         | 12.6                      | 21.23                 |

Values denote arithmetic means ± SD. Means with different letters (in the same column are significantly at (p ≤ 0.05) using one-way ANOVA test, while those with similar letters are non-significant.

Table 6: Protective effect of nettle and olive leaves on lipoprotein fractions (HDL-C, LDL-C, VLDL-C) and atherogenic index (AI) of hyperlipidemia

| Parameters Groups | HDL-C mg/ dl | LDL-C mg/ dl | VLDL-C mg/ dl | AI          |
|-------------------|--------------|--------------|---------------|-------------|
| Control – ve      | 47.33 ± 4.41a| 7.36 ± 4.12d | 17.23 ± 2.47d | .445 ± .163c|
| Control + ve      | 32.00 ± 5.79c| 78.73 ± 10.52a| 40.10 ± 4.51a | 3.82 ± .781a |
| Nettle 5%         | 34.16 ± 4.26bc | 51.43 ± 10.98b | 30.73 ± 4.48b | 2.43 ± .552b  |
| Nettle 10%        | 45.00 ± 4.93a| 20.33 ± 7.22c | 23.67± 3.52c | .995 ± .277d  |
| Olive 5%          | 38.83 ± 4.26b | 44.0 ± 4.21b  | 29.17± 3.26b | 1.89 ±157c   |
| Olive 10%         | 45.33 ± 4.41a| 8.66 ± 2.93d  | 21.33±2.83d  | .671 ± .154de |
| LSD               | 5.56          | 8.5          | 4.24          | 0.49         |

Values denote arithmetic means ± SD. Means with different letters (in the same column are significantly at (p ≤ 0.05) using one-way ANOVA test, while those with similar letters are non-significant.
Table 7: Protective effect of nettle and olive leaves on SOD, GPX and MDA of hyperlipidemic rats (n= 6 rats)

| Parameters Groups | SOD U/L       | GPXng/ml   | MDA m mol/gm |
|-------------------|---------------|------------|--------------|
| Control – ve      | 52.06±3.826a  | 91.4±6.82a | 10.5±2.41d   |
| Control + ve      | 27.46±3.75d   | 54.03±7.11d| 26.4±3.66a   |
| Nettle 5%         | 36.23±6.13c   | 64.3±8.24c | 21.3±2.71b   |
| Nettle 10%        | 42.43±5.52bc  | 82.3±3.74b | 12.4±2.07d   |
| Olive 5%          | 39.40±5.88bc  | 70.3±5.78c | 17.2±1.55c   |
| Olive 10%         | 45.63±5.25b   | 83.7±7.97ab| 13.1±1.32d   |
| LSD               | 6.07          | 8          | 2.8          |

Values denote arithmetic means ± SD. Means with different letters (in the same column are significantly at \( p \leq 0.05 \) using one-way ANOVA test, while those with similar letters are non-significant.

Table 8: Protective effect of nettle and olive leaves on serum liver function enzymes of hyperlipidemic rats (n= 6 rats)

| Parameters Groups | AST U/L       | ALT U/L       | ALP U/L       |
|-------------------|---------------|---------------|---------------|
| Control – ve      | 70.67±12.92d  | 26.6 ± 6.86d  | 126.0±23.7d   |
| Control + ve      | 185.33±31.65a | 61.5 ±12.21a  | 258.1±38.9a   |
| Nettle 5%         | 142.33±20.22b | 50.1 ±9.45b   | 216.1±31.5b   |
| Nettle 10%        | 105.83±14.83c | 39.1 ±9.10bc  | 169.3±19.9c   |
| Olive 5%          | 123.50±19.72b | 44.6 ±9.77bc  | 190.3±32.2bc  |
| Olive 10%         | 98.83±17.03c  | 35.6 ±5.24cd  | 160.5±19.7c   |
| LSD               | 23.9          | 10.67         | 33.7          |

Values denote arithmetic means ± SD. Means with different letters (in the same column are significantly at \( p \leq 0.05 \) using one-way ANOVA test, while those with similar letters are non-significant.
Table 9: Protective effect of nettle and olive leaves on serum glucose of hyperlipidemic rats (n= 6 rats)

| Groups            | Serum Glucose (mg/dl) |
|-------------------|-----------------------|
| Control – ve      | 78.1±10.79d           |
| Control + ve      | 136.5±22.1a           |
| Nettle 5%         | 116.0±14.02b          |
| Nettle 10%        | 94.6±13.17cd          |
| Olive 5%          | 108.5±17.07bc         |
| Olive 10%         | 93.3±8.80cd           |
| LSD               | 17.6                  |

Values denote arithmetic means ± SD. Means with different letters (in the same column are significantly at (p ≤ 0.05) using one-way ANOVA test, while those with similar letters are non-significant.
- Liver tissue

**Photo 1:** Microscopic pictures of H&E stained liver sections showing normal hepatic architecture with radially arranged hepatic cords around central veins (CV) with normal portal areas (PA) and sinusoids in control normal group.

**Photo 2:** Liver sections from untreated rat group kept on Liver sections of +ve group showing highly disrupted hepatic architecture due to diffuse ballooning degeneration (black arrows) with multifocal necrosis (blue arrows) of hepatocytes, portal inflammation (yellow arrows).
**Photo 3:** Liver sections from group treated with olive 5% showing mildly disrupted hepatic architecture due to moderate periportal hydropic degeneration (black arrows) of hepatocytes and mild lobular inflammation (yellow arrows).

**Photo 4:** Liver sections from group treated with olive 10% showing greatly restored hepatic architecture with few cytoplasmic vacuoles (red arrows) in hepatocytes around PA.
Photo 5: Liver sections from group treated with nettle 5% moderately disrupted hepatic architecture due to hydropic degeneration (black arrows) and large cytoplasmic vacuoles in hepatocytes (red arrows) around CV and PA.

Photo 6: Liver sections from group treated with nettle 10% showing partially restored hepatic architecture with few minute cytoplasmic vacuoles in hepatocytes around CV (red arrows) and mild hydropic degeneration in hepatocytes (black arrows) around PA.
• **Heart tissue**

**Photo 1:** Microscopic pictures of H&E stained heart sections showing normal longitudinally and crossly sectioned cardiac muscle fibers with normal blood vessels and interstitial tissue in control normal group.

**Photo 2:** Heart sections of +ve group showing congested blood vessels (red arrows), marked perivascular edema (blue arrows) and macro vesicular vacillations of crossly sectioned cardiac muscle fibers (black arrows).
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Photo 3: Heart sections from group treated with olive 5% showing mildly congested blood vessels (red arrows), perivascular & interstitial edema (blue arrows) and few macrovesicular vacuolations of crossly sectioned cardiac muscle fibers (black arrows).

Photo 4: Heart sections from group treated with olive 10% showing greatly restored histological appearance.
Photo 5: Microscopic pictures of H&E stained heart sections showing from group treated with nettle 5% congestion (red arrows), perivascular & interstitial edema (blue arrows) with some macrovesicular vacuolations of crossly sectioned cardiac muscle fibers (black arrows).

Photo 6: Heart sections from group treated with nettle 10% showing mild congestion (red arrows).
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The protective effect of Nettle and Olive leaves on hyperlipidemia in experimental rats

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Abstract

Rising blood fat is the main cause of vascular disease and cardiovascular diseases. The World Health Organization has reported that cardiovascular diseases account for 46% of total deaths in Egypt and for this reason; the study currently examines the protective effect of Nettle and Olive leaves on rising blood fat.

Sixty and thirty-two male rats weighing 130 ± 20g were divided into six equal groups (6 rats per group). The first group served as a negative control (v−), while the second group served as a positive control (v+) that was fed a high-fat diet for four weeks. The other four groups received a high-fat diet supplemented with Nettle leaves (5%) or Olive leaves (5%) or (10%) on a weekly basis for four weeks (as treated groups). Chemical and phenolic analysis was performed on both Nettle and Olive leaves. At the end of the experiment, biological data were calculated and blood samples were taken for biochemical analysis. In addition, tissue examination was performed. The results showed that the high-fat diet group in the positive control group increased the weight of the rats, the weight of organs, the blood fat percentage, and the liver enzymes (a marker of oxidative damage) and enzymes of the liver and blood glucose. While the protein high density (HDL) and the superoxide dismutase enzyme (a key enzyme in antioxidants) and glutathione peroxidase decreased. All treated groups in both plants showed improvement in the previous symptoms compared to the positive control group. Finally, it can be used Nettle and Olive leaves to improve the fat level and liver functions in rats.

Keywords: Blood fat – Nettle leaves – Olive leaves – Antioxidant enzymes – Liver functions.