Histological Study of the Effect of Aqueous Extract of the Petroseliunm Sativum on Some Biochemical Parameters of Female white Rats Exposed to Oxidative Stress

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Abstract
This experiment was conducted to investigate the protective effect of the aqueous extract of the petroseliunm sativum and its effect as a physiological and histological antioxidant for some blood parameters in white female rats exposed to oxidative stress induced by cholesterol. The study included the use of 28 female rats aged (4-3 months) distributed into 4 groups of (7) rats for each group. WBCs), measuring the level of the enzyme alanine aminotransferase (ALT), then explained and took liver samples that were preserved with 10% formalin, and then prepared microscopic slides from it to conduct the histological study.
Keywords: Histological study; petroseliunm sativum; biochemical parameters

1. Introduction

The liver occupies a central position in the metabolism process, as all nutrients are transported directly to the liver via the hepatic portal vein, and iron-rich blood is directed from the spleen to the hepatic portal vein as well, and much of the nutrients delivered to the liver are diverted the route of hepatocytes to stored products, such as glycogen, which provides the glucose needed by the body. The liver cells are organized into hexagonal lobes about 2 mm in length and 700 µm in diameter. The lobules are surrounded by loose connective tissue. The liver is susceptible to several diseases. Viral hepatitis is more Common causes of chronic liver disease, in addition to the imbalances that occur in the delicate balance between oxidants and antioxidants, leading to oxidative stress (Horvatits et al., 2022), the liver is the largest gland in the body and weighs about 1500 grams. It is in the right upper quadrant of the abdominal cavity, below the diaphragm. The liver is divided into four lobes - right, left, square, and caudal - the first of which constitutes the largest part of it. The liver consists of complex metabolic cells that contain high levels of enzymes important to carry out metabolic reactions, and in the event of damage to the cell membrane or its organelles, these enzymes leak to release outside the cells and then released to the blood and this process occurs very quickly, where the activity of enzymes in the blood increases, and this activity can be measured within hours of the injury (Chhabra et al., 2022). Alanine (ALT) and lactate dehydrogenase (LDH). ALT is distributed in many tissues, but it is present in high concentrations in the liver (Kresnamurti et al., 2021) Most cases of acute liver injury often led to high levels of ALT enzyme, and that is 48-24 hours after the injury, and the effectiveness of ALT remains high in the case of chronic liver injury. Chronic hepatitis is usually diagnosed with an increase in the level of cytoplasmic enzymes, especially ALT enzyme (Vujkovic et al., 2021), and oxidative stress is the common cause of chronic liver injury. In this case, an increase in the activity of ALT enzyme is observed. The oxidative stress produces free radicals, which can be defined as very effective chemical units that possess one of the electrons. It is not paired in the outer envelope, and it can interact with biomolecules, causing the tissues containing them to break down (Li et al., 2015) There are many plants that have been used in the treatment of oxidative stress resulting from the formation of free radicals inside the body and the resulting diseases, including Petroselinum, a biennial herbaceous plant that follows the tentacle family. Its height ranges from 6 to 20 cm. It has many stems, all of which grow from one root, and the stems are upright, rounded and branched. Compound papers. The flowers are in white compound groups and the inflorescences are tent complexes, and the parsley is distinguished by its pungent aromatic smell and its bright green leaves. Among its types are smooth municipal and curly syphilis. Parsley oil can be obtained from the seeds (Abou Khalil et al., 2016) Therefore, the study was designed to investigate the effect of the aqueous extract of the petroseliunm sativum on some biochemical variables related to liver function in female white rats exposed to oxidative stress with cholesterol.

2. Material and Methods

Preparation of the aqueous extract of Petroselinum sativum

The dry leaves of the metallic plant were crushed and turned into a fine powder using the blender. 25 grams of dry, crushed leaves were taken and placed in a glass beaker with a capacity of 1000 and 500 ml

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of water was added to it. Then the substance was mixed with the electric mixer for 15 minutes, then the mixture was left for 18 hours. The mixture was filtered by Several layers of gauze and separated by a centrifuge to get rid of insoluble substances. Then the extract was placed in 250 ml glass dishes and placed in the electric oven at 40 ° C to obtain the crude aqueous extract (Aiyegoro & Okoh, 2010)

Experimental animals

In this experiment, 28 adult female white rats aged 3-4 months, with weights ranging between (225-300) g, were used. The animals were raised in a special room with the correct conditions for animal husbandry such as feeding, temperature, lighting and ventilation in the laboratories of the Pathological Analysis Department. College of Applied Sciences

The animals were distributed into 4 groups of (7) animals for each group and were treated for 30 days as follows.

Control group: normal drinking water was given for the duration of the experiment.

Cholesterol group: 100 mg/kg of cholesterol was given in the diet during the trial period.

Petroselinum sativum group: the aqueous extract of the petroseliunm sativum was dosed at a concentration of 100 mg / kg of body weight and at a dose of 1 ml.

Cholesterol and Petroselinum sativum group: The diet containing cholesterol was given at a concentration of 100 mg/kg for two weeks, then it was treated with the aqueous extract of the petroseliunm sativum for two weeks.

Collected blood samples:

After the end of the specified period of the experiment (30) days, the animals were starved for 12 hours and then weighed, then blood samples were drawn from the heart directly by the cardiac puncture method using a medical syringe with a capacity of (5) cm 3, as approximately (4-5) ml of the blood. The blood was divided into two parts according to the type of examination, as (1)) cm3 of blood was placed in plastic tubes with tight lids containing Ethylene Diamine Tetra Acetic Acid (EDTA), an anticoagulant, for the purpose of conducting a WBCs examination), which was carried out in the same year. On the day of the autopsy, the rest was placed in test tubes free of anticoagulant and left at room temperature for 20 minutes until the blood clotted, and then it was centrifuged at 3000 revolutions per minute for 15 minutes for the purpose of obtaining the blood serum, and preserving the serum by freezing At a temperature of -20 ° C until the biochemical examination is performed, which includes measuring the level of the enzyme alanine aminotransferase (ALT), a test tube was taken and (0.5) cm^3 of reagent solution (R1) was placed in it, then the tube was placed in the incubator at a temperature of 37 ° C for 5 minutes after that. It was extracted from the incubator and 0.1 cm^3 of serum was added to it, the tube mixed well and returned to the incubator for 30 minutes, then it was extracted a second time from the incubator and (0.5) cm^3 of reagent solution (R2) was added. The contents of the tube were mixed well and left for 20 minutes. at room temperature Then 5 cm^3 of sodium hydroxide was added to it and left for 5 minutes, then the absorbance was read at a wavelength of (505 nm). U/L

Obtaining tissue samples

After the end of the experiment, the rats were killed, and then liver samples with a size of 0.5 cm 3 were taken, they were placed in formalin at a concentration of 10% for (12) hours, and after washing with water for (10) minutes, a series of tissue passes was conducted on them with alcohol, xylene and then impregnation Burying with paraffin wax and making special L-Shaped molds using clean paraffin wax with a melting point of 60 °C, then cut with a rotary section 4-5 µm thickness, the sections were surfaced with 30% alcohol and then transferred to a special water bath to brush the sections at a temperature of 45 °C °, and then carried on glass slides, to prepare them for coloring, as they were stained with hematoxylin and eosin H & E according to (Bancroft & Stevens, 1982). It was examined under a light microscope

3. Statistical Analysis

The results were statistically analyzed using the Statistical Analysis Package for Social Science (spss) system to extract the significant differences between the experimental groups, emphasizing these differences by extracting the standard error (SE) (Dunken et al., 1982).

4. Results and Discussion

The results of this study showed that there were no significant differences at the level of significance (P≤0.05) in the group treated with aqueous extract of petroseliunm sativum which amounted to (5.17±0.62) when compared to the control group, while a significant decrease appeared at the level of significance (P≤0.0) in The total number of white blood cells (WBCs) in the cholesterol-treated group was (4.29±0.3) when compared to the control group, while the cholesterol-treated group for two weeks and then was treated with aqueous extract of petroseliunm sativum did not show any significant differences at the level of significance (P≤0.05), which It reached (5.52 ± 0.23) when compared to the control group, which amounted to (5.16 ± 0.15) cell /ml3 of blood , As for the results of examining the activity of the enzyme alanine aminotransferase (ALT), this study showed that there were no significant differences at a significant level (P≤0.05) in the group treated with aqueous extract of the petroseliunm sativum plant, which amounted to (47 ±1.14) when compared to the control group, while it showed the incidence of A significant decrease at a significant level (P≤0.0) in the concentration of ALT enzyme in the cholesterol-treated group, which

amounted to (91±2.73), when compared to the control group, while the cholesterol-treated group for two weeks and then treated with aqueous extract of petroseliunm sativum showed There was a significant increase at the level of significance (P≤0.0), which amounted to (50±2.55) when compared to the control, which amounted to (45±0.83) U/ Lbut it differed significantly from the group treated with cholesterol alone.

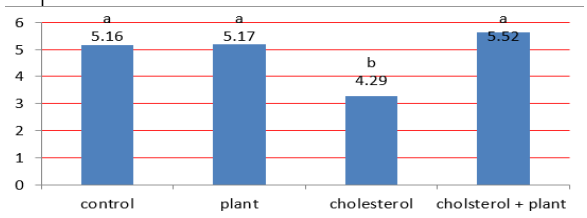


Diagram (1) Changes in the average number of white blood cells

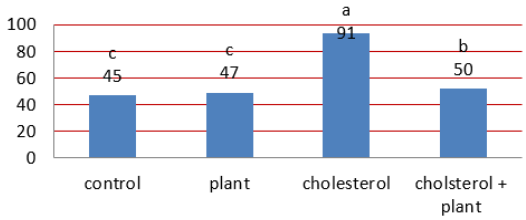


Diagram 2. Changes in the concentration of the enzyme alanine aminotransferase

The occurrence of a significant decrease in the total number of white blood cells in the cholesterol group may be due to the direct effect of oxidative stress and the effect of free radicals on the membranes of white blood cells, and thus acceleration and increase in the processes of apoptosis, which is one of the causes of exposure to chemical compounds and thus its effect On the immune response leading to a decrease in the number of cells, and this is consistent with (Lim et al., 2014) which indicated a decrease in the number of WBCs when treated with oxidants, while the group treated with aqueous extract of mineral did not show significant differences indicating the absence of any negative side effects when The level of this dose, as for the cholesterol group and the extract, it did not show any significant differences compared to the control, which indicates the high ability of the components of the water extract to repair the damage caused by cholesterol and reduce inflammation in the body and thus return the cells to their normal state and this is consistent with indicated to (Saeidi et al., 2012), A significant increase in the concentration of ALT enzyme in the cholesterol-treated group (G3) may be due to the destruction of most of the hepatocytes coverings, and consequently, the increase in the concentration of ALT enzyme in the blood serum as a result of oxidative stress caused by lipid peroxidation and the increase in the level of free radicals that resulted from cholesterol, and this is consistent with what was indicated by (Xu & Porter, 2015). As for the group treated with aqueous extract of metallic (G2), there were no significant differences indicative of its lack of effect at this dose level. Peroxide (G3) alone, and this indicates the possibility of the aqueous extract to capture the free radicals resulting from treatment with cholesterol and to compensate for damaged hepatocytes, and thus the concentration of the enzyme approaches the normal state without reaching the normal state, and this is consistent with what indicated (Jafar et al., 2012).

5. Histological Examination Results and Discussion

control group.

The results of microscopic examination showed the appearance of hepatocytes in the form of regular radial rows towards the central vein in the rat liver tissue of the control group. It is in Figure (1)

Cholesterol group

The results of the histological examination of the liver of a rat in the cholesterol-treated group showed cases of degeneration and vacuolated necrosis of some Vacuolated Necrosis and the areas of blood sinusoidal continuity with the central vein to drain the blood as in Figure (2), while there was a degeneration of the cytoplasm and nuclei and congestion inside the lumen of the central vein as in Figure (3). While the infiltration of lymphocytes appeared in the interstitial tissue between hepatocytes, as in Figure (4), the nuclei of some cells were divided as in Figure (5). The wall of the central vein appeared abnormally, as in Figure (6), and the decomposition of red blood cells appeared and their collection inside the lumen of the central vein, as in Figure (7)

The group treated with the *petroseliunm sativum*

The results of this study showed the appearance of the central vein wall with a normal appearance and close to the control group, but some cells appeared in a state of cell division as in Figure (8), and the majority of the hepatocytes appeared normal, and they were of a radial arrangement, and some cells appeared enlarged as in Figure (9).

-The group treated with cholesterol for two weeks, then it was treated with aqueous extract of the petroseliunm sativum for two weeks:

The results of the histological study showed the appearance of the wall of the central vein with a normal appearance, like that of the control group (Fig. 10), and the cells appeared in a radial arrangement around the central vein area, and the space appeared between the hepatocytes and the sinusoids, with a normal appearance as in the figure (11).

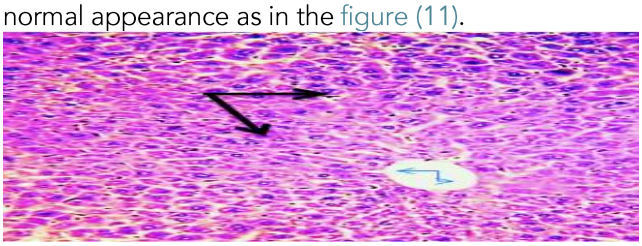
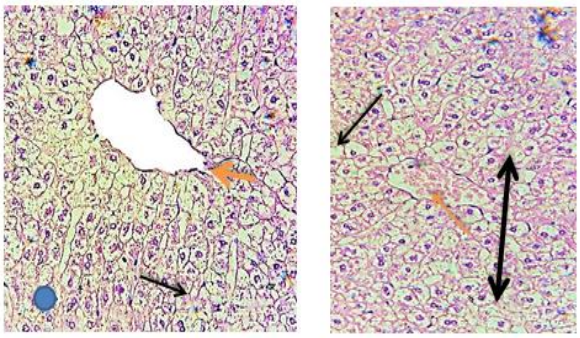


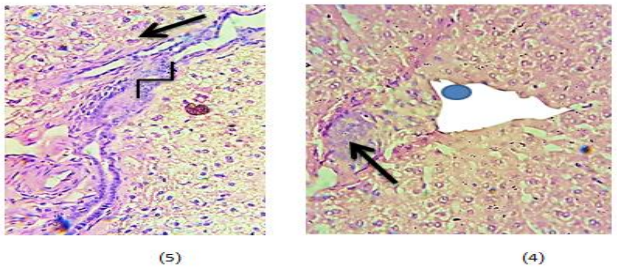
Figure (1): A section of the liver of a rat from the control group showing the normal appearance of hepatocytes ( ), central vein ( ), (H & E), (400X).



(2) (3)

Figure (2): Section of a cholesterol rat liver showing an abnormal appearance of the central venous wall ( ) , Vacuolated Necrosis ( ), (H & E), (400X).

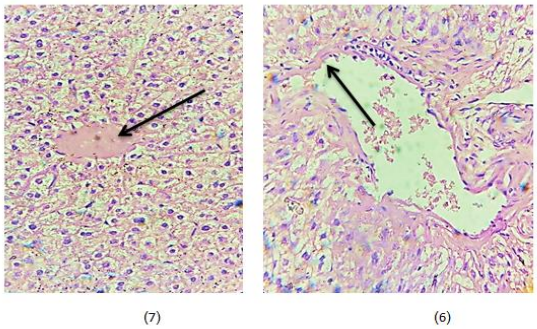
Figure (3): Section of a cholesterol rat liver showing an abnormal cell ( ), Congestion ( ), (H&E), (400X).



(5) (4)

Figure (4): Section of a cholesterol rat liver showing an Infiltration of Lymphocytes, (H&E), (400X).

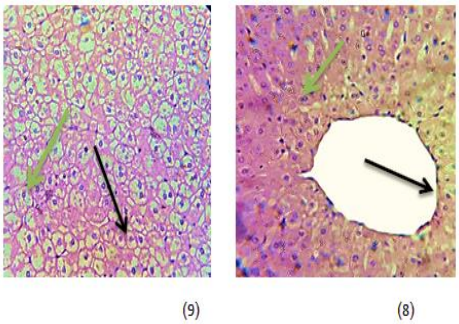
Figure (5): Section of a cholesterol rat liver showing an, cell nuclei division (H&E), (400X).



(7) (6)

Figure (6): Section of a cholesterol rat liver showing an abnormal appearance of the central venous wall & vaculation ( ) , (H&E), (400X).

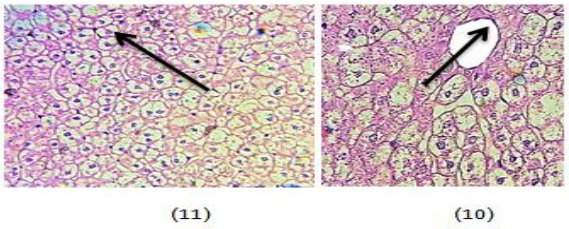
Figure (7): Section of a cholesterol rat liver showing an abnormal appearance of the central venous wall & cytolysis ( ) ,(H&E), (400X).



(9) (8)

Figure (8): A section of the liver of a rat from the Petroselinum sativum group showing the normal appearance of liver central vein ( ),(H & E), (400X).

Figure (9): A section of the liver of a rat from the Petroselinum sativum group showing the normal appearance of hepatocytes ( ), central vein ( ), (H & E), (400X).



(11) (10)

Figure (10): A section of the liver of a rat from the cholecterol& Petroselinum sativum group showing the normal appearance of hepatocyte ( ), (H & E), (400X).

Figure (11): A section of the liver of a rat from the cholecterol & Petroselinum sativum group showing the normal appearance of liver central vein ( ),(H & E), (400X).

Cholesterol group

The occurrence of these abnormal changes is due to the fact that oxidative stress works on the formation of free radicals that have the ability to interact with phospholipids in the cell membranes, resulting in lipid peroxides, which stimulates a series of membrane-lying reactions while affecting the vitality of the mitochondrial membrane, and the destruction of lysosomes leading to to cell necrosis and this is consistent with what was indicated by (Kocak et al., 2005), and the occurrence of degenerative changes leads to an inflammatory response, as the outputs of degenerate cells are Chemotactic factor that leads to attracting inflammatory cells to migrate to the degenerative area for the purpose of defending the tissue by devouring degenerative substances and removing the factor The cause of the damage and this is consistent with what he pointed out (Kumar et al., 2003), and congestion is the result of the accumulation of free radicals in the capillary blood vessels, and thus causes an increase in blood pressure in them and the occurrence of functional failure, and then the explosion of the endothelial lining of the capillary blood vessels leading to congestion Through the exit of red blood cells outside, and their deposition inside the central vein (Black et al., 2002) Free radicals also interact with proteins involved in the cellular and intracellular membrane and thus inhibit a number of membrane-specific biological activities. The breakdown of cells is caused by the induced oxidative stress that breaks down cellular membranes that interact with many of biomolecules, as indicated by him (Kocak et al., 2005).

Cholesterol group and *petroseliunm sativum* plant

The repair of tissue damage may be due to the effect of the components of the aqueous extract of the petroseliunm sativum on the epithelial cells and their stimulus to cell division, or because the mineral contains the compound Apgnin, which is characterized by its effectiveness in removing various free radicals from the body and then it reduces lipid disorders by reducing the concentration of cholesterol and glycerides Thus, the production of VLDL-C in the liver may be reduced, or the reason

may be due to the activity of phenolic compounds such as Merastin and flavonoids that increase the concentration and activity of catalase enzymes, superoxide dismutase (SOD) and glutathione peroxidase (Gpx), the liver antioxidant that reduces oxidative stress and prevents The process of lipid peroxidation and therefore an important role in regulating the immune system, which may be an indication of its ability to enhance the body's immunity to defense when an influential factor is present in the cells of the body. As indicated to him (Slighoua et al., 2021), this indicates the possibility of the aqueous extract of the petroseliunm sativum on cellular repair, because it possesses many effective secondary metabolic compounds such as phenols, whose effects have emerged in protecting various body tissues, including liver tissue, from damage by oxidants that lead to Damage to cellular components and membranes as indicated by (Gou et al., 2021).

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