Study of the biological efficacy of aqueous extract of *Teucrium Polium* on some histological parameters in male laboratory white rabbits of *Oryctolagus cuniculus*

Israa Khalaf Abdul Kareem  
Biology Department/College of Education for Pure Science/University of Kerbala  
Corresponding author email: asraa52141@gmail.com

Naseer Marza Hamza  
Biology Department/College of Education for Pure Science/University of Kerbala  
Email: naser.m@uokerbala.edu.iq

**Abstract**---The current study aims to know the protective effects of the aqueous extract of *Teucrium Polium* to reduce the harmful effects on liver tissue caused by the preservative sodium benzoate in *Oryctolagus cuniculus* male rabbits by studying some histological parameters, where the results of the current study showed that animal dosing with sodium benzoate at a concentration of (250) mg/kg of body weight for a period of 30 days caused tissue damage to the liver represented by congestion and expansion of the central vein and Necrosis of hepatocytes with irregular arrangement of hepatic cords in addition to the presence of congestion and expansion of the sinusoids with congestion of the central vein and infiltration of inflammatory cells around it, while treatment with aqueous extract of *Teucrium Polium* caused three concentrations (250-200-150) mg/kg body weight to reduce or suppress the toxic effects of benzoates, it was observed that the central vein was decongested with little expansion of the sinusoids, with the return of most of the hepatic cords to regularity, with the presence of degeneration in some hepatocytes in the treatment group, the first focus, while in the second and third focus group, the central vein congestion disappeared and decreased. The expansion of the sinusoids with the regularity of the hepatic chords, and the tissue became as close as possible to the normal tissue As for the protective groups that were dosed with aqueous extract of *Teucrium Polium* at three concentrations (250-200-150) mg/kg of body weight, in addition to sodium benzoate at a concentration of (250) mg/kg of body weight for a period of 30 days Where the central vein appeared slightly enlarged in addition to the
irregularity of some of the hepatic cords and the occurrence of a slight expansion in the sinusoids while the hepatocytes and their nuclei appeared naturally, while noting the presence of a slight degeneration in some cells, so it can be considered that the aqueous extract of Teucrium Polium plant has a preventive effectiveness in resisting the histological changes of the liver. The result of taking poisons intentionally or unintentionally, with the possibility of it being considered safe in terms of use.

**Keywords**---sodium benzoate, perennial plant, pathological changes.

**Introduction**

1- Medicinal plants:

The plant (*Teucrium Polium*) belongs to the Lamiaceae family and has many uses as a food and medicine. It has antioxidant properties. About 27 active compounds have been diagnosed in it that have increased effects on the concentrations of antioxidant enzymes. It also has antispasmodic effects, and digestive diseases. It is used in developing countries. It is widely used for the treatment of many different diseases, including liver problems, blood pressure, antipyretic, rheumatism, parasitic diseases, and it is an antimicrobial, and its importance lies in the fact that it contains many antioxidant elements, as many active compounds have been diagnosed in it that have inhibitory effects on the effect of free radicals in the body (1) (2).

The *Teucrium Polium* plant is a small herbaceous plant that is widely spread in the Mediterranean regions in rocky areas, hills, deserts and dry places (3) The scientific name of the plant is *Teucrium Polium*, which is derived from the Greek term Teucrum, which is associated with the ancient king of Troy, who was the first to use this plant for medicinal purposes as for the word Polium, it means grayish-white, and this is a reference to the color of the flowers (4).

*Teucrium Polium* plant contains many biologically active compounds, the most important of which are Alkaloids, including Stkadrin and Sisteron, in addition to essential oils and flavonoids, which are phenolic compounds and other active substances that have an important role in reducing the risk of disease, They act as an anticoagulant and reduce oxidative stress resulting from It generates free radicals and protects the body from cancer and heart diseases, The plant also contains many volatile oils, which have a major role in treating various infections, in addition to its role in increasing the body's immunity. The plant also contains carbohydrate compounds such as Fructose, Glucose, Sucrose, Ramenose, Ravenose and other sugars, which They are polyhydroxy compounds, which makes them of great importance in animal and plant organisms as they store and transfer energy in addition to being part of cellular components such as chitin in animals and cellulose in plants. (5) (6) (4).
2-Food additives

Food additives are defined as materials that are not consumed as food in themselves and are not used as an essential ingredient of food, whether they have nutritional value or not, but are intentionally added to food for a technological purpose or for preservation during transportation or storage. These additives or their by-products may become one of the components of this food. (7) (8).

Food additives are one of the important materials because they are linked to human health and safety, as many of them are linked to serious and chronic diseases, and the food industry has become linked to the addition of these materials, and food manufacturers use these materials to present their goods with a delicious taste, attractiveness and beautiful texture for the purpose of increasing their profits, and as a result of increased concentrations. These substances in food, food has become a threat to human health and safety (9).

At the present time, many preservatives have been added to food, because the development in the marketing of the various foods offered requires making food in one place and processing it in another place, and then exporting it later to many other countries. Food from spoilage, rotting and unwanted changes that may occur in it (10).

**Aim of the study**

The current study aims to demonstrate the protective effects of the cold aqueous extract of *Teucrium Polium* against the toxic effects of sodium benzoate on some biochemical parameters in male rabbits.

**Materials and Methods**

In this study, used 40 male rabbits, whose weight ranged from (1000-1500) grams, and their ages ranged between (7-11) months, approximately, they were raised in the animal house of the College of Pharmacy / University of Karbala for the period from October 2021 to December 2021, the animals were placed in special plastic cages covered with metal covers, their floors were brushed with fine sawdust, the cages were cleaned, the floors were constantly changed and sterilized with disinfectants, as well as care. Continuous cleaning of perfusion bottles, and the accommodation room, and all the experimental animals were subjected to appropriate laboratory conditions in terms of temperature of 25°C and the duration of lighting and ventilation, the animals were provided with water and a standard diet Ad libitum was freed for the duration of the research, and the animals were left for two weeks to adapt to the conditions before conducting the experiment and to ensure that they were free of diseases.

**Preparing the of Aqueous Extract of *Teucrium Polium***

The leaves of the *Teucrium Polium* plant were washed with running water, then dried and crushed using a grinder, then mixed (20) g of dry powder with (400) ml of distilled water using an electric mixer and left for (24) hours at room
temperature, then filtered the mixture using several layers of medical gauze To get rid of the plankton, then centrifugation at a speed of (3000) revolutions / min for a period of (10) minutes, then filter the extract using mesh filter papers (NO.0.1) type what man to obtain a clear solution, then dry the extract using an electric oven at 40°C Keep in a cool place until use (11)

**Experiment design**

The animals were divided into eight groups with five animals for each group. The first group was considered a negative control group and was dosed orally with tap water only, and the second group was a positive control group and was orally dosed with sodium benzoate at a dose of (250 mg/kg) body weight, and the third, fourth and fifth groups were considered extract groups and all dosed with aqueous extract for Teucrium Polium plant at a dose of (150,200,250mg/kg) of body weight, as for the sixth, seventh and eighth groups, they were considered protective groups and all dosed with aqueous extract for Teucrium Polium plant at a dose of (150,200,250mg/kg) of body weight for the last three groups in addition to sodium benzoate in the same previous dose, respectively, and after the end of the experiment period of (30) days, The animals were anesthetized and dissected, the liver was extracted and preserved in 10% formalin solution until the tissue slides were prepared from it according to the method (12)

**Result**

The liver generally consists of two main lobes: the right lobe and the left lobe, where the right lobe is larger than the left lobe Each lobe in turn is divided into smaller lobules called lobules where cross-sections of liver tissue in the control group showed that each lobule contained a central vein Surrounded by a large group of hepatocytes, which are arranged in the form of ribbons and contain clear nuclei located between them are gaps called (sinusoids) (Picture 1).

First: The effect of treatment with sodium benzoate on the histological structure of the liver when compared to the negative control group:

The histological sections of the liver in the group treated with sodium benzoate at a concentration of (250 mg/kg) body weight for 30 days were shown He suffers from an expansion and congestion in the central vein, necrosis and degeneration of hepatocytes, in addition to the irregularity of the hepatic cords And the presence of expansion and congestion in the sinusoids. Infiltration of inflammatory cells around the central vein is also noted, as in Figure (2) when compared to the negative control group, Picture (1).

Second: The effect of treatment with aqueous extract of *Teucrium Polium* plant on liver tissue in the extract groups when compared with the negative control group:

The results of the current study showed that the liver tissue in rabbits treated with aqueous extract of *Teucrium Polium* plant at a concentration of (250,200,150) mg/kg of body weight for a period of 30 days Where the concentration (150,200) mg / kg body weight showed a slight expansion of the central vein While hepatocytes appeared naturally free of any damage While some hepatic chords appeared regularly, while the sinusoids appeared normally, as in Figure (3) and Picture (4), While the concentration (250) mg / kg of body weight showed liver
tissue closer to its normal shape The central vein and hepatocytes appeared closer to normal, and some of the hepatic and sinusoidal cords were normal, as shown in Figure (5) Picture (1) when compared with liver tissue in the negative control group This indicates that *Teucrium Polium* plant does not have any side effects on the liver.

Third: The effect of treatment with aqueous extract of *Teucrium Polium* plant on the liver tissue in the protective groups when compared with the positive control group;

The results of the current study showed that the liver tissue in rabbits treated with aqueous extract of *Teucrium Polium* plant at a concentration of (250,200,150) mg/kg of body weight And then it was dosed with sodium benzoate at a concentration of (250) mg / kg of body weight for a period of 30 days, that the liver tissue in the preventive group with a concentration of (150) mg / kg of body weight The central vein appeared slightly enlarged, in addition to irregularities in some of the hepatic cords A slight expansion occurred in the sinusoids, while the hepatocytes and their nuclei appeared normally, as shown in Figure (6) while the liver tissue, upon microscopic examination, showed protective groups with a concentration of (250,200) mg / kg of body weight The central vein is normal and the hepatic chords are regular, in addition to the appearance of hepatocytes, nuclei and sinusoids closer to normal, as in Figure (7) and (8) When comparing with the tissue sections in the positive control group Picture No. (2) and this indicates that the aqueous extract of *Teucrium Polium* plant contributed to the protection of the liver tissue From the effect of sodium benzoate on the protective groups, and this role came through its possession of many active substances Which contributed to improving the functional performance of the liver in addition to its ability to remove free radicals and preventing its formation by reducing the level of inflammation that inflicts on cells due to the body's exposure to toxic substances, and this appears through its contribution to raising the concentrations of antioxidant enzymes in the body.
Picture (1), a cross-section of the tissue of the rabbit liver, *Oryctolagus cuniculus* for the control group. It is noted the presence of a normal central vein (→) and regularity of the hepatic cords (→) with the presence of sinusoids (→) and hepatocytes and their nuclei (H & E stain) (40X).

Picture (2) A cross-section of the rabbit liver tissue *Oryctolagus cuniculus* of the group dosed with sodium benzoate at a concentration of 250 mg/kg body weight. Hemorrhage was observed (→) infiltration of cells surrounding the central vein (→) Central vein dilatation and congestion (→) and hepatocyte necrosis (→) with irregular hepatic cords (→) (40X) (H & Estin)
| Picture (3) A cross-section of rabbit liver tissue *Oryctolagus cuniculus* of the group dosed with aqueous extract of *Teucrium Polium* plant at a concentration of 150 mg/kg body weight, the central vein is slightly dilated ( ) hepatocytes ( ) with regularity of hepatic cords ( ) in addition to the presence of sinusoids ( ) (40 x) (H & Estain) |
| Picture (4) A cross-section of rabbit liver tissue *Oryctolagus cuniculus* of the group dosed with aqueous extract of *Teucrium Polium* plant at a concentration of 200 mg/kg body weight, the central vein is observed in normal ( ) hepatocytes ( ) with regularity of some hepatic cords ( ) in addition to the presence of sinusoids ( ) (x40). (H & Estain) |
Picture (5) A cross-section of rabbit liver tissue *Oryctolagus cuniculus* of the group dosed with aqueous extract of *Teucrium Polium* plant at a concentration of 250 mg/kg body weight. The central vein is slightly dilated (→) hepatocytes (→) with regularity of the hepatic cords (→) in addition to the presence of sinusoids (→) (40x). (H & Estain)

Picture (6) A cross-section of rabbit liver tissue *Oryctolagus cuniculus* of the protective group treated with aqueous extract of *Teucrium Polium* plant at a concentration of 150 mg/kg body weight and sodium benzoate at a concentration of 250 mg/kg of body weight, in which the central vein is slightly dilated (→) necrosis of some hepatocytes (→) with Irregularity of some hepatic chord (→) in addition to the presence of sinusoids (→) (40x) (H & Estin)
Discussion

Firstly:
The current study, which showed the damage caused in the liver tissue, agreed with the results of the study (13) (14) Which showed necrosis in the liver tissue in addition to degenerative changes in hepatocytes in the group of animals treated with sodium benzoate. In the same direction, the researcher pointed out (15) That daily oral administration of sodium benzoate leads to obvious hepatotoxicity through structural damage to the liver And the promotion of oxidative stress that generates free radicals, which works to destroy the cytoskeleton of the liver, causing necrosis and degeneration of hepatocytes, loss of nuclei, and then cell death

While studying came(16) To confirm the toxicity of sodium benzoate on the liver through congestion and expansion of the central vein In addition to the expansion of the sinusoids and the necrosis of hepatocytes and their degeneration, Cellular infiltration in the portal ducts, weakness in the blood vessels within the liver tissue, and necrosis of the hepatic lobes. The current study also agreed with the study of (17) Who noted the presence of cellular infiltration, severe congestion of the central vein, and expansion of the sinusoids In addition, the microscopic
examination showed the presence of swollen hepatocytes due to the rupture of their cytoplasm and swelling of the mitochondria. While (18) indicated that the liver tissue of rats dosed with sodium benzoate in different concentrations and for different durations is 3 and 12 months. It was noticed after the end of the first period that there was significant damage to the blood vessels, but at the end of the second period, it was found that the liver lobules lost their normal shape, as well as the destruction of liver cells.

Secondly:
The current study showed that there were no side effects on the liver tissue when rabbits were dosed with aqueous extract of *Teucrium Polium* plant, and this is consistent with what was indicated by (19) who observed the safety of the liver tissue when mice were dosed with aqueous extract of the algae plant, and this was attributed to the fact that the algae contains effective compounds of great importance in maintaining the integrity of the structural and functional structure of the liver, as well as containing phenolic acids and flavonoids, thus acting as a protective substance for the liver.

The current study also agreed with what was indicated by (20), which noted that there were no damages to the liver tissue in groups dosed with aqueous extract of *Teucrium Polium* plant. The reason for this was shown to possess substances with protective activity against inflammation, oxidative stress and generated free radicals. In the same direction, the current study agreed with the study (21), which noted the presence of the central vein closer to the normal state and the emergence of The sinusoids and the presence of hepatocytes and their nuclei in a normal state, which indicates the integrity of the structural structure of the liver in the groups dosed with aqueous extract of *Teucrium Polium* plant.

Third:
This agreed with what the researcher (22) indicated that *Teucrium Polium* plant provides protection for liver cells against inflammatory effects and thus leads to preventing the release of free radicals. Thus, reducing the risk of oxidative stress as a result of the possession of the aqueous extract of *Teucrium Polium* plant on the phenolic compounds which play a protective role that protects the cells of the body, especially hepatocytes, from oxidative stress and liberates free radicals. This is in agreement with the results of our current study, which clarified the integrity of the liver tissue in the protective groups.

In the same direction, the researcher (23), who observed the integrity of the liver tissue in the protective groups dosed with the aqueous extract of *Teucrium Polium* plant, indicated in the same direction. When compared with the positive groups, this was attributed to the possession of *Teucrium Polium* plant on many effective compounds that contribute to protecting the liver tissue from toxic substances.

On the other hand, a study (24) revealed the protective role of *Teucrium Polium* plant by causing damage to the liver tissue by carbon tetrachloride, the aqueous extract of the plant showed a high protective effect. While the current study differed from the study (25), which observed an increase in the concentration of liver enzymes in the protective groups dosed with aqueous extract of the plant.
Teucrium Polium In addition to being dosed with a toxin, the increase in these enzymes is evidence of tissue and functional damage to the liver.

References


