Toxicity of Cadmium Chloride on White Rats Liver and the Protective Role of *Brassica Nigra* Seed Extract

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Abstract

The study designed for the toxic role of cadmium chloride on the liver of mice from both a physiological and histological aspects. The animals were treated with a dose of 5 mg/kg of body weight for 30 days. And the protective role of *Brassica nigra* seed extract against cadmium chloride toxicity. This pilot study was conducted on 20 adult white rats divided into 4 equivalent groups including the control group: Animals in this group received a dosed of distilled water for 30 days. The placebo group was treated with cadmium chloride at a dose of 5 mg/kg of body weight for 30 days and returned as an infected control group. While in the third group, animals treated with cadmium chloride were dosed with *Brassica nigra* seed extract at a dose of 200 mg/kg of body weight for 30 days. The fourth group was dosed with *Brassica nigra* seed extract at a dose of 200 mg/kg of body weight for 30 days. After 30 days, liver enzymes including aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were measured by spectrophotometric method. In addition to making tissue sections of the liver. The treatment of rats led to a significant increase ($P \leq 0.05$) in liver enzymes compared with the control group. It also led to histopathology in the liver tissue, while the *Brassica nigra* seed extract acted as a protective role against the toxicity of cadmium chloride.

Keyword*: cadmium chloride, Brassica nigra, aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP).*

Introduction

The increased emissions of both minerals into the environment and their lack of ability to rapidly biodegrade have increased the risk of human exposure to them. The main methods of exposure to cadmium and lead are ingestion and inhalation due to their presence in food and air, as well as in tobacco smoke[1]. The American Agency for Toxic Substances and Disease Registry (ATSDR) has ranked cadmium seventh and lead second on its priority list of hazardous substances[2]. Cadmium poisoning is a global health problem that leads to infection in many organs, and in some cases it may cause death. Cadmium production, consumption and release into the environment without proper treatment resulted in air, water and soil pollution. And that long-term exposure to cadmium through water, air and diet leads to the problem of different organs of cancer problem for these organs[3]. Cadmium is a heavy metal, which is very toxic and its use in various forms in the chemical industry (dye, paint) as well as the metallurgical industries (alloys) [4]. Numerous studies have demonstrated its toxic nature to the liver by administering cadmium chloride to mice that produce cell necrosis and lipid changes [5].

The chemical is highly toxic the liver generally metabolizes it into a non-toxic chemical, but sometimes the non-toxic or less toxic substance also activates to the highly toxic metabolite. Therefore, the chemical burden increases stress on hepatocytes and leads to hepatic disturbance due to degenerative cells, necrosis, tumors, etc. [6] . Cadmium has the ability to spread through various organs of the body quickly, and most of the cadmium ingested enters the liver and kidneys [7] . The liver is the organ most targeted by cadmium toxicity

and hepatotoxicity is the main cause of death in the organism [8] .

Medicinal plants and processed natural products have an important role in eliminating many of the harmful liver effects caused by exposure to environmental pollutants such as cadmium [9,10] . A medicinal plant can be defined as if it has the use of any part of the plant or its seeds as a treatment [11] . People all over the world use the mustard plant. And it has been mentioned as the greatest herb ever. It is used in the treatment and prevention of many ailments such as cancer, as well as has antidiabetic , antioxidant and antimicrobial properties [12,13]. The therapeutic efficacy of the mustard plant is due to the fact that it contains selenium as well as magnesium and B-complex vitamins are also among its main ingredients [14] . *Brassica nigra* seeds also have important medicinal uses in treating rheumatism, joint pain, liver and spleen diseases, oral tumors, and a laxative. Recent studies reveal that the mustard plant has antioxidant and antimicrobial properties [15,16] .

MATERIALANDMETHODS :

A- **Prepare the animals :**

Use 20 white rats. Their ages ranged between (2.5-3) months, and their weights ranged between (190-220 grams). The animal was bred in the College of Veterinary Medicine, University of Tikrit. All animals were exposed to uniform environmental conditions in terms of ventilation and temperature, where the temperature ranged between (20-25) C , and the lighting period was 12 hours of light and 12 hours of darkness (natural lighting). Animals were fed with standard feed.

B- Prepare mustard seed extract :

After obtaining the mustard seeds, they were left to dry, after which they were ground by an electric grinder, and the extraction was carried out according to the method Parthiban *et al*. [17] .

C- Experience design :

The animals were divided randomly into four groups (5 animals in each group). After the preparation period ended, the animals were dosed daily for 30 days through tube feeding, as follows :

1- Group I : This group was injected with distilled water for 30 days.

2- Group II : This group was treated with cadmium chloride at a dose of 5 mg / kg of body weight for 30 days [18] .

3- Group III : This group was dosed with mustard extract at a dose of 200 mg / kg of body weight for 30 days [17] .

4- Group IV : This group was dosed with cadmium chloride with mustard seed extract at a dose of 200 mg / kg of body weight for 30 days.

D- Collecting blood samples :

At the end of the experiment, the animals were silent for 12 hours, after which they were anesthetized with chloroform. Blood samples were taken directly by heart stab, and (3-5) ml of blood were collected using a medical syringe and placed in plastic tubes with dry caps. Using centrifugation, serum samples were obtained. Samples were collected to determine the enzymes of aspartate and alanine transporter (AST and ALT).) And alkaline phosphatase (ALP).

E- Prepare histological sections:

After dissecting the animals, liver tissue was obtained, and then cleaned with water were fixed in 10% formalin for 24 hours, dehydration in increasing concentrations (70%, 80%, 95%, 100% and 100%), purified with xylene and then mixed with paraffin. . Upon analysis, all paraffin-embedded tissues were divided at 5 µM , and stained with eosin and hematoxylin. Samples were examined under an optical microscope with a magnification of 400 X [19].

Results

It is noted from Table No. (1) that the treatment of rats with cadmium chloride led to a significant increase ($P \leq 0.05$) in the level of ALT, AST and ALK compared with the control group. Whereas, dosing of rats treated with CdCl2 with Brassica nigra seed extract resulted in a significant decrease in the level of ALT and AST ALK compared with the group treated with cadmium chloride, However, no statistically significant differences were observed upon dosing of Brassica nigra seed extract on healthy animals.

Table (1) concentration of liver enzymes in blood serum.

Parameters / Groups	AST (IU/ml)	ALT (IU/ml)	ALP (IU/ml)
Control group	42.2±4 b	62.3 ± 5 b	29.4±2 c
cadmium chloride	86.3±6 a	97.6 ± 5 a	57.2±4 a
CdCl2 and Brassica nigra seed extract	33.4±7 b	66.4 ± 2 b	46.2±8 b
Brassica nigra seed extract	30.6±4 b	58.2 ± 4 b	44.1±3 b

-Values represent arithmetic mean ± standard error.

-Vertically different letters mean that there is a significant difference at a significant

level ($P \leq 0.05$) .

- The number of animals is 5 in each group.

Histological:

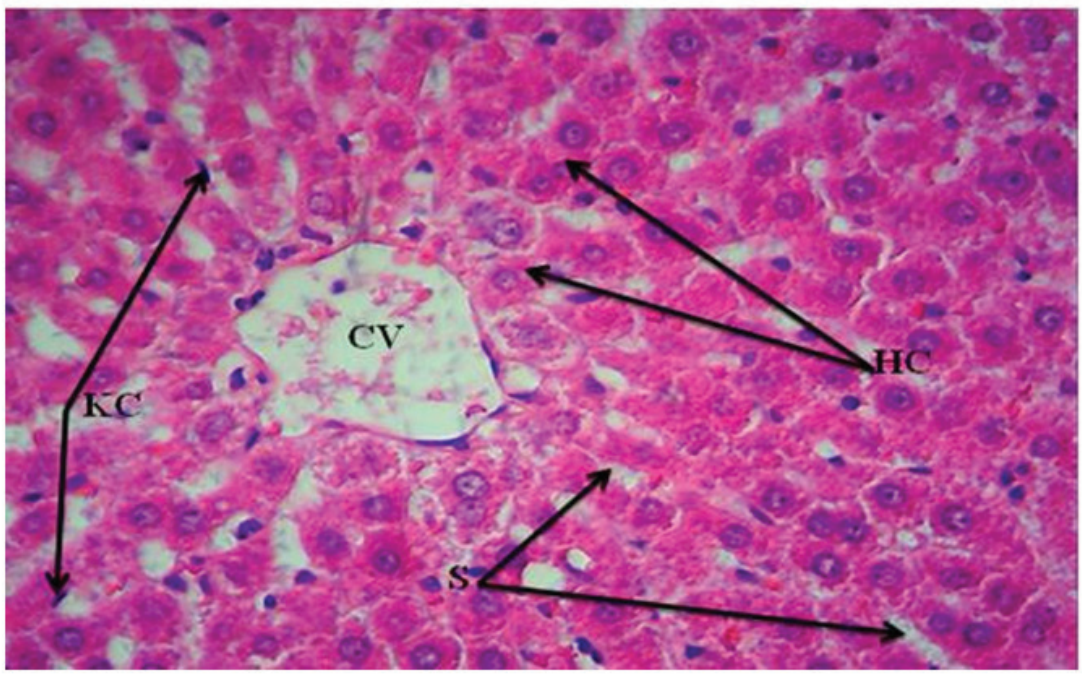

Image (1) of a rat liver section from the control group showing the central vein (CV), hepatocytes (HC) arranged in the blood vessels as ropes, as well as blood sinusoids cells (S) and Koffer cells (KC). H & E 400X.

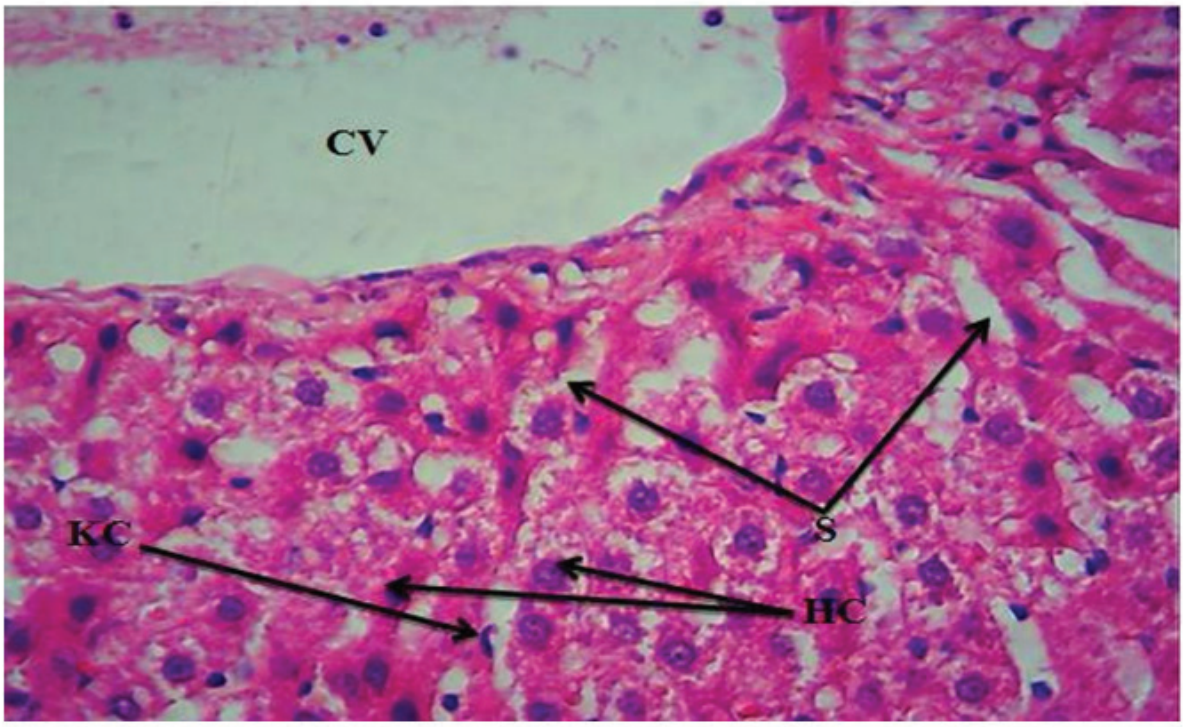

Image (2) section of rat liver of the group treated with brassica nigra seed extract shows the central vein (CV) and hepatocytes (HC) arranged vascularized in the form of cellular cords as well as blood sinusoids (S) and Koffer cells (KC) in their normal form.

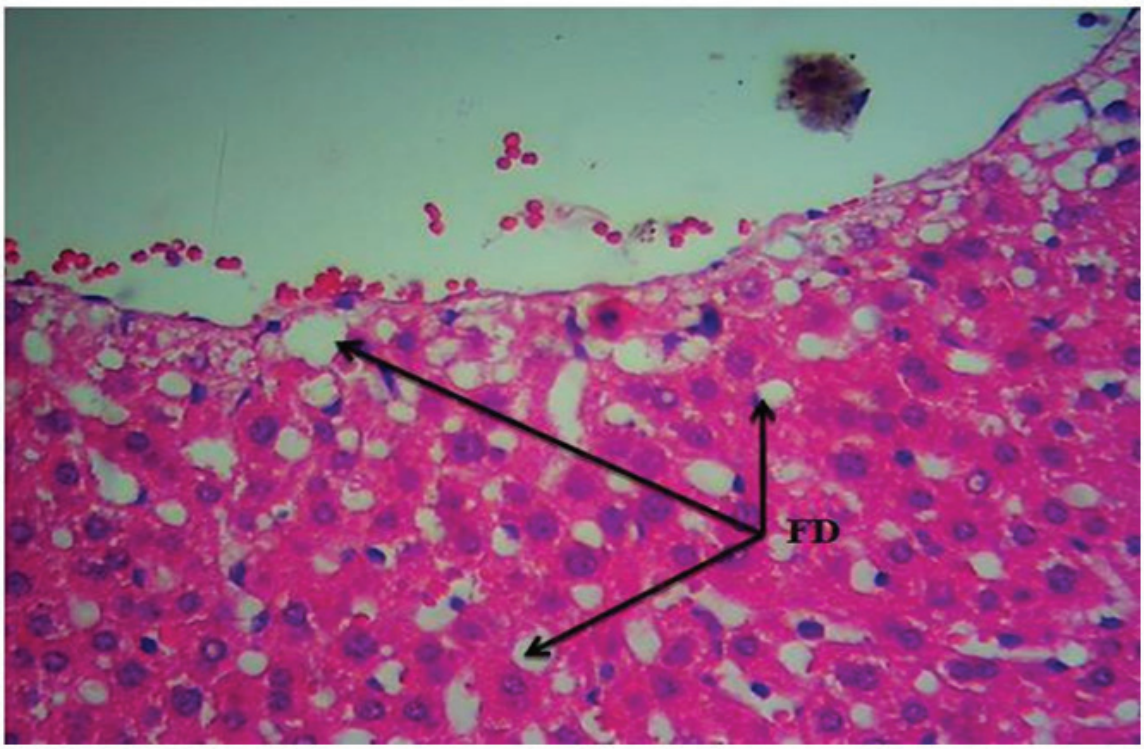

Image (3) of a group of rat liver section treated with cadmium chloride shows fatty degeneration in hepatocytes (FD). H & E 400X .

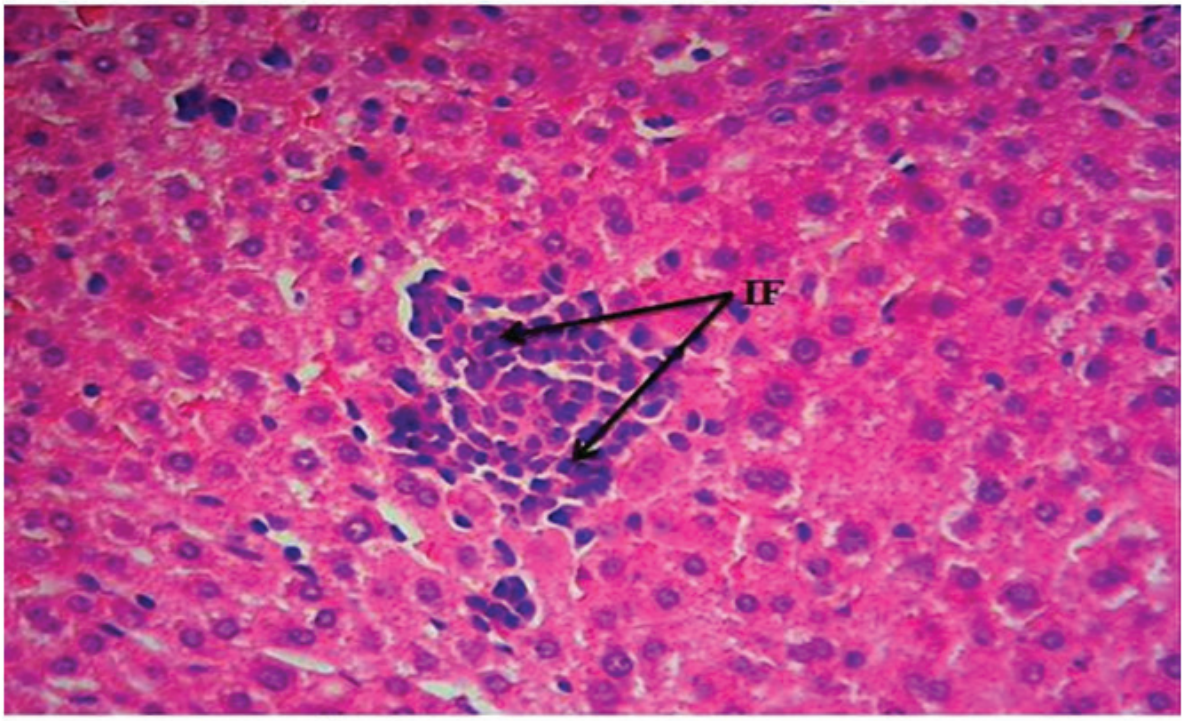

Image (4) of a group of rat liver section treated with cadmium chloride showing a focal infiltration of inflammatory cells (IF) H&E 400X .

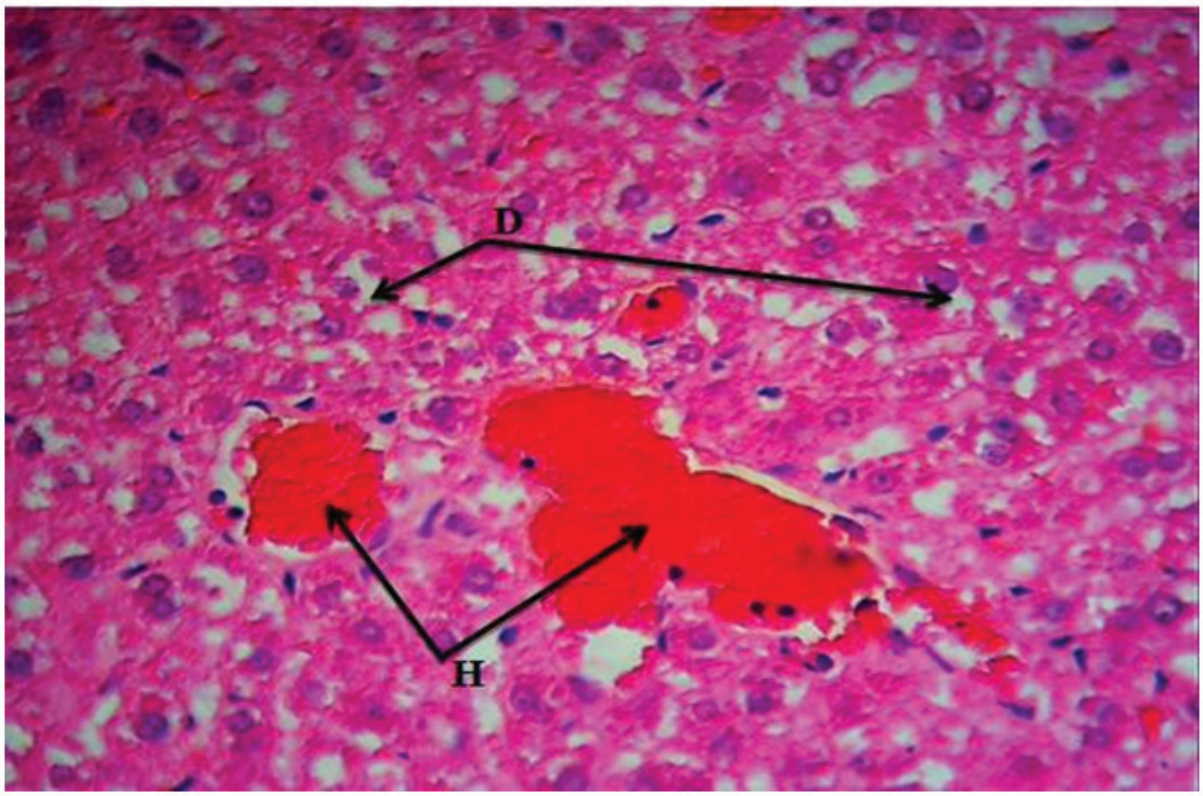

Image (5) of a group of rat liver section treated with cadmium chloride showing the degeneration of some hepatocytes (D) with hemorrhage (H) within the liver tissue H&E 400X.

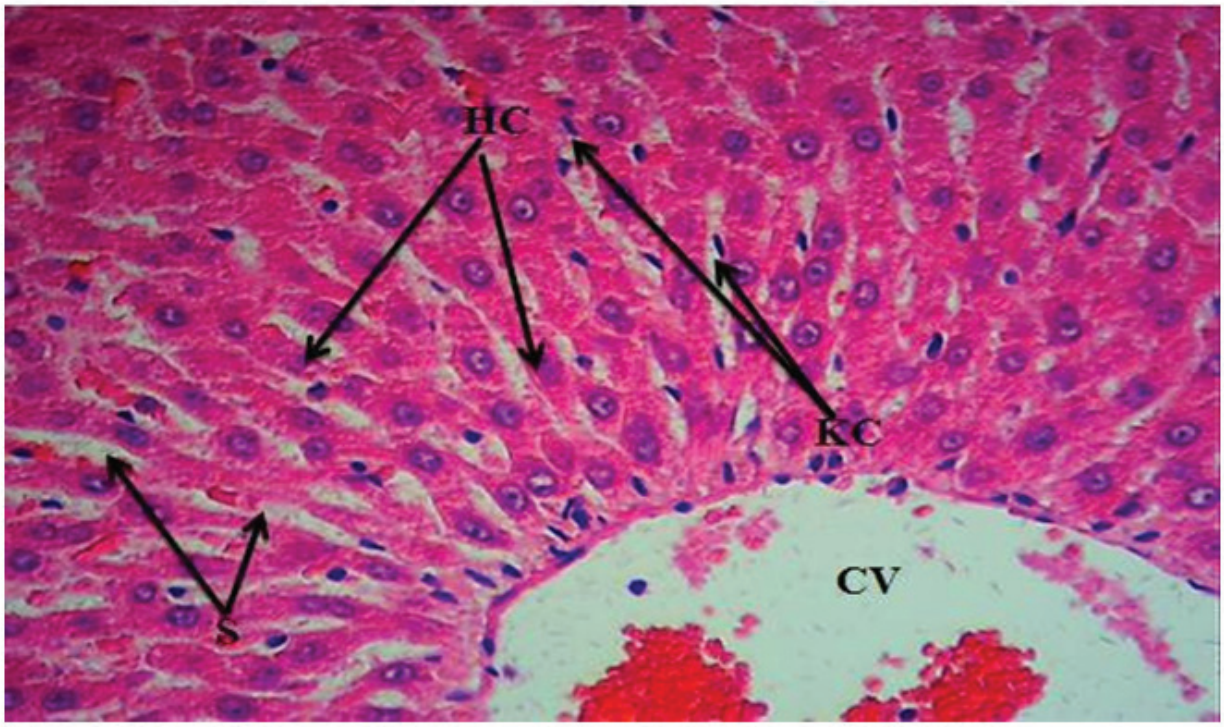

Image (6) section of the rat liver of the group treated with cadmium chloride and mustard seed extract showing the central vein (CV) and hepatocytes (HC) arranged vesicles in the form of cellular cords as well as blood sinusoids (S) and Koffer cells (KC) naturally. H & E

400X,

Discussion

Through Table No. (1) it is noticed that the treatment of rats with cadmium chloride led to a significant increase ($P \leq 0.05$) in the level of ALT, AST and ALP compared to the control group. These results agreed with the results of Mohamed *et al* [20] , And Sajjad *et al.* [21] . who found that levels of liver enzymes increased significantly in the group of animals treated with cadmium chloride due to the occurrence of hepatocyte toxicity.

As they observed that cadmium chloride caused a significant increase in liver enzymes . Cadmium causes toxicity to hepatocytes and its enzymes AST, ALP and ALT that are released into the bloodstream, and thus the level of these enzymes in the blood rises above the normal range [22].

As the evaluation of the activity of the enzymes AST and ALT is important for evaluating the function of cells of some tissues, especially the liver, it is known that both are present in hepatocytes, renal, muscle, and others. Therefore, their high activity outside the cell indicates the presence of functional impairment or the presence of damage to the cells of these tissues [23] , So the treatment with cadmium chloride to the use of radicals and active oxygen species (ROS) , which worked on loading neurons, [24] , And the increase in the level of these two enzymes explained the effect of cadmium in changing the permeability of the membranes of the liver cells, which leads to the leakage of these enzymes into the blood, or due to the occurrence of Hepatocellular necrosis that occurs as a result of toxic substances or the occurrence of liver cirrhosis and also leads to leakage of these Enzymes from broken cells and their arrival into the blood [25,26] .

However, when rats treated with cadmium chloride with mustard extract resulted in a significant decrease ($P \leq 0.05$) in the level of liver enzymes (ALT, AST and ALP) compared to group of rats treated with cadmium chloride . These results are in agreement with those of Rajamurugan *et al.* [27] . Who used *Brassica nigra* against liver and kidney damage . Rajamurugan *et al.* [28] . found that *Brassica nigra* extract has an antioxidant

effect in vivo. Also, *Brassica nigra* extract has been shown to have antioxidant activity in vitro . Several studies by previous researchers have confirmed that cruciferous vegetables can work as a good source of natural antioxidants because they contain high levels of carotenoids and phenolic compounds. Most of the antioxidant effects have been associated with the phenolic compounds that reduce levels of oxidative stress [29] . Kuyutork *et al*. [30] found that the hepatotoxicity of cadmium was affected in two ways, first by the onset of the inflammatory condition, and second by the toxic and direct effect of cadmium on liver cells. The present study showed that administration of *Brassica nigra* seed extract after cadmium chloride restored enzyme levels in animal serum as an indicator of the productive effect of mustard seed extract against cadmium-induced liver damage due to the presence of high levels of carotenoids and phenolic compounds.

Tissue sections of rat liver treated with cadmium chloride show clear fatty degeneration in hepatocyte (FD) . and Degeneration of some hepatocytes (D) with hemorrhage (H) . These results are consistent with several studies, including the study of Zhai *et al*. [31] and Albasha and Azab [24] . Who found that cadmium toxicity causes vacuoles in liver cells, increased nuclear chromatin density with compression of the nuclei in cells, in addition to degeneration of the fat mass of cells with large vacuoles occurring in the cytoplasm of nuclei of hepatocytes, In addition to other studies, it was found that cadmium causes the generation of active oxygen species (ROS) that lead to damage to cellular membranes due to oxidative damage to their lipid contents, and this leads to cell toxicity in the liver [32,33] .

While histopathological sections of the liver in rats treated with cadmium chloride and dosed with aqueous extract of *Brassica nigra* seeds showed that it has a protective role against toxic damage to cadmium chloride as it contains effective compounds against oxidative stress [34,29].

Conclusions

Through the research, we noticed that cadmium chloride has a toxic effect on hepatocytes histologically and physiologically, while *Brassica nigra* seed extract proved a good protective role against cadmium chloride toxicity.

Ethical Considerations: All Research participants haven't been subjected to any kind of harm in any way.

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Conflict of Interest: The author declare no conflict of interest regarding this research.

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