Histological and Biochemical Effects of Cadmium chloride on Kidney and Heart Tissues and Curative Roles of Brassica nigra Seeds Extract in Male Rats

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ABSTRACT

Cadmium (Cd) is known as an inorganic highly toxicant and widely distributed in the environment and causes various disease conditions, and it is widely used in numerous industrial processes and it is a component of many commercial products. The major pharmacological producers are currently running excessive research on plants to introduced new drugs in the medical treatments. Brassica nigra has antioxidant, anti-inflammatory, antiepileptic, antidiabetic and many other pharmacological properties.

Keywords: Cadmium chloride, Brassica nigra, Kidney, Heart.

Introduction

Cadmium is a heavy metal, colored bluish-white and has soft touch; It is naturally present in the environment, and it was given the chemical symbol (Cd). Its amount is about 25,000 tons in the planet, [1]. It is a widely spread environmental pollutant, due to its widespread usage in industries across the world. Among all of the above, cadmium is considered to be the cause of many cancerous tumors, embryonic malformations and mutations in general. [2].

Cd can be the causative agent to a critical toxicity in number of organs especially the kidney, liver and Heart which may cause a dysfunction For each of urinary, digestive and circulatory system and other human health risks, prolonged cadmium ingestion leads to chronic cadmium poisoning [3]. Cd induce an oxidative stress at several tissues by progressing peroxidation of lipids in plasma membrane of cells which cause alterations in their antioxidant systems [4].

Risks of human exposure to Cd are increased because of the increased emissions of it in the environment, and their hard biodegradability have increased these risks, the main pathways of exposure are either ingestion or inhalation due to its presence in water, food, air, and even in tobacco leaves [5]. Cd is an ecological pollutant if present in soil and predominatingly related with Zn, Cu, or Pb, and its absorption may be via roots of plants that subsequent may be intake by human or animal [6].

There are many researches discussed the preventive characteristics of a number of natural anti-oxidants versus mineral toxicity, meanwhile herbs are generally considered as a safe and affective against various human diseases, and their medical usage has increased gradually in many countries, Natural antioxidants enhance the innate antioxidants mechanisms to reactive oxygen species (ROS) and neutralize the reactive

species to restore the optimal cellular balance [7].

Phytochemical examination showed that Brasica nigra contains each of carbohydrates, glycosides, flavonoids , albumin, sinabin, inositate synegrin, myrosin , alkaloids. Total phenol values is about 6.67 mg/g gallic acid, and abort (30-35%) of fatty acids, and about (40%) of proteins, derivatives of vinyl Propane which includes: glucosinolates mainly synegrine (Allyl glucosinolates, 1-5%), and sinapine (choline ester cinnapic acid, 1%) [8].

Furthermore, B. nigrs seeds contain Vitamin A, members of Vitamin B complex (B1-Thiamin, B2-Riboflavin, B3-Niacen and B6-Pyridoxine), Vitamin C, Vitamin D, also contain large levels of minerals such as – Calcium (Ca) – Iron (Fe) – Zinc (Zn) - Copper (Cu)- Manganese (Ma)– Selenium (Se), in addition to antioxidants, nutritional fibers and oils [9].

Aim of the study

is to evaluate the protective role of aqueous extract of mustard (Brasica nigra) seeds toward the harmful effects of CdCl2 on several physiological parameters and histological changes of kidney and heart of white male rats.

Materials and Methods:

Animals and breeding: In this study, 20 healthy adult male rats were used, they were weighed about 200-220 g and their age 11-12 weeks. Animals of experiment gained from the animal house of the college of Veterinary medicine at Tikrit University and the breeding process was carried out in a small room with dimensions of 3 x 3 m, air-conditioned at a temperature of (25 ± 2) Celsius,

and the light period was 12 hours of light and 12 hours of darkness.. All animals were placed in plastic cages with metal mesh covers, dimensions 30 cm long, 20 cm wide and 20 cm high.

Experience design: The animals which used in the experiment were divided into 4 groups, each one included 5 males in good health, as follows:-

1 - The first group (G1) : (control 1) 5 male rats, which were fed a normal diet for the duration of the experiment and dosed with distilled water.

2- The second group (G2): 5 male rats, were dosed 100 mg/kg of body weight of mustard seed extract for 30 days [9]

3- The third group (G3): (control 2) 5 male rats were dosed CdCl2 5 mg/kg of body weight for 30 days [10] ..

4- Fourth group (G4): 5 male rats which were dosed with 5 mg/kg body weight of CdCl2 for 30 days and treated by 100 mg/kg body weight of mustard seed extract for another 30 days.

Collection and extraction of experimental seeds : The mustard plant (B. nigra) seeds obtained from territorial markets, they were cleaned by a brush to get a clean seeds without any impurities, The seeds left to dry in the lab. Away from sun light, then they crushed by an electrical grinder, the mixture was placed in airtight and opaque cans and kept at room temperature, the seeds were extracted by the warm water method [11], and about 50 grams of the powder (crushed seeds) were taken and putted in a container with 500 milliliters of warm water and via kind stirring the mixture was mixed and then the container was placed in the vibrator shaker for 24 hours, by using of several layers of medical gauze the mixture had been filtered and the large parts removed, after filtering the mixture was putted in several glass dishes which transferred to the oven, the mixture was completely dried at 40°C, and stored in packages which were opaque sealed glass bottles and placed in the refrigerator (-18) until usage [9].

Hematological samples: After the end of the 30-day experiment, the animals were starved for 24 h after which blood samples were drawn through jugular vein transection. The blood putted in test tubes which placed in incubator at a temp. of 37 °C to 30 min., after that blood transported to a centrifuge at 3000 rpm for 15 min., a micropipette was used to separate the serum from the other components. In the case of deep freezing at a temp. of -18 ° C until biochemical tests are performed to determine the concentrations of urea, creatinine, cholesterol, triglycerides, HDL-c, LDL-c, VLDL-c.

Histological samples: Histological sampled of kidney and heart were handled according to the known protocol of routine stain to eosin and hematoxylin. The specimens were diagnosed via a light microscope at 400x magnification power [12] .

Results:

Kidney: Table (1) reveals that treating animals with CdCl2 causes a significant increase ($P \leq 0.05$) in urea concentrations, with a significant decrease in creatinine concentration compared with the control group, while the cadmium chloride-treated rats dosed with mustard seed extract led to a clear decrease in the concentration of urea and creatinine compared with the group treated with CdCl2 and it was close to its concentration in control.

changes represented by the presence of a clear infiltration of

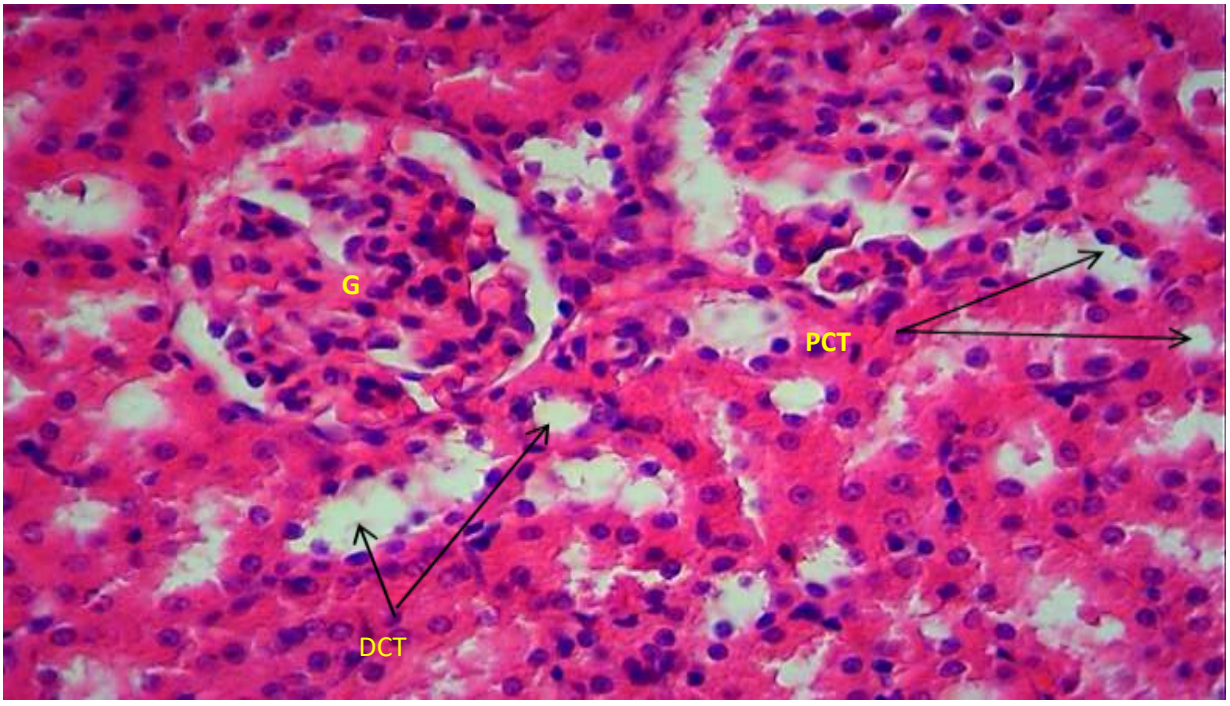
criteria / group	Urea (mg/dl)	Creatinine (mg/dl)
Control G1	**45±1 B**	**1.2±0.15 A**
mustard seed extract G2	**32±2 C**	**0.81±0.2 B**
$CdCl_2$ G3	**69±4 A**	**0.75±0.2 C**
$CdCl_2$ + mustard seed extract G4	**52±5 B**	**0.85±0.2 B**

- The values are mean ± standard error.
- Different letters vertically mean that there is a significant difference at the level of significance ($P \leq 0.05$).
- The number of animals is 5 in each group.

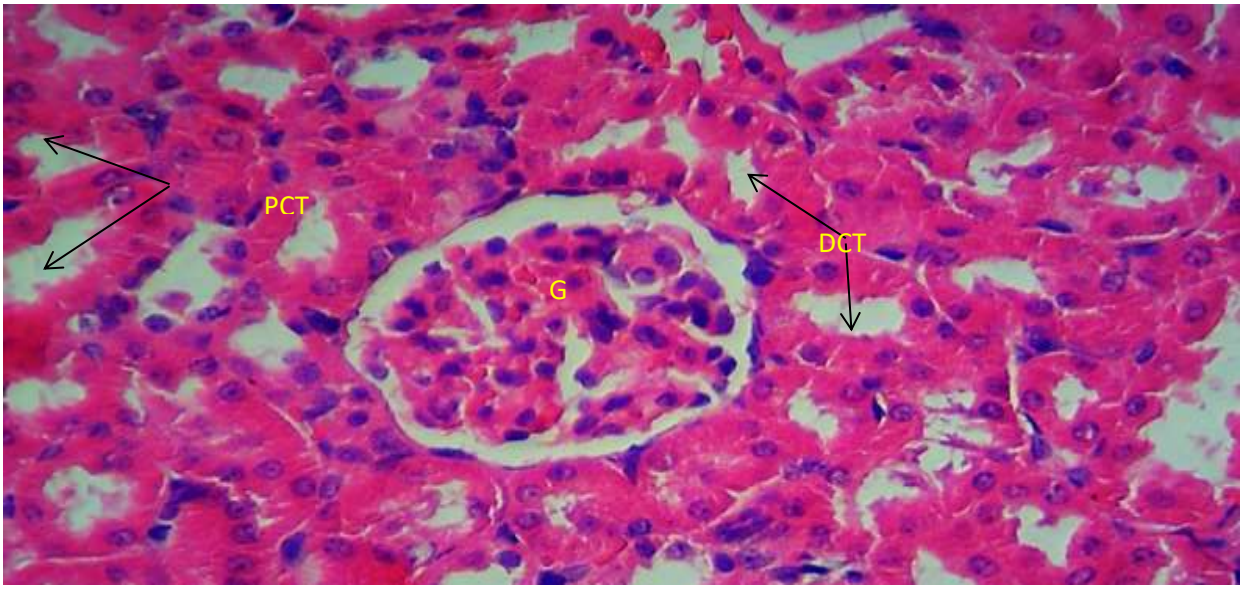
Histological results:

Through histological examination of the kidney tissue, the normal structure of the kidney tissue was observed, as it was possible to observe the proximal and distal convoluted tubules, as well as the renal glomeruli in the control group (G1) (Fig. 1) and the group treated with mustard seed extract (G2) (Fig. 2). While the tissues of animals treated with CdCl2 (G3) showed a number of histological inflammatory cells with the destruction of some renal glomeruli and shrinkage of some of them with the possibility of observing the presence of bleeding within the renal glomeruli in addition to general degeneration in all renal tissue, (Fig. 3, 4, 5).

While the histological examination of the kidney tissues in the animals of the 4th group which treated by CdCl2 and mustard seed extract (G4) showed that most of the kidney tissues returned to their normal state with presence of little hemorrhage , (Fig. 6).



(Fig.1) A section of the kidney of (G1) showing the proximal and distal convoluted tubules (PCT) and distal (DCT) as well as the renal glomeruli (G) clearly and naturally. H & E 400X,



(Fig.2) A section of the kidney of (G2) showing the proximal convoluted tubules (PCT) and distal tubules (DCT) as well as renal glomeruli (G) normally. H & E 400X,

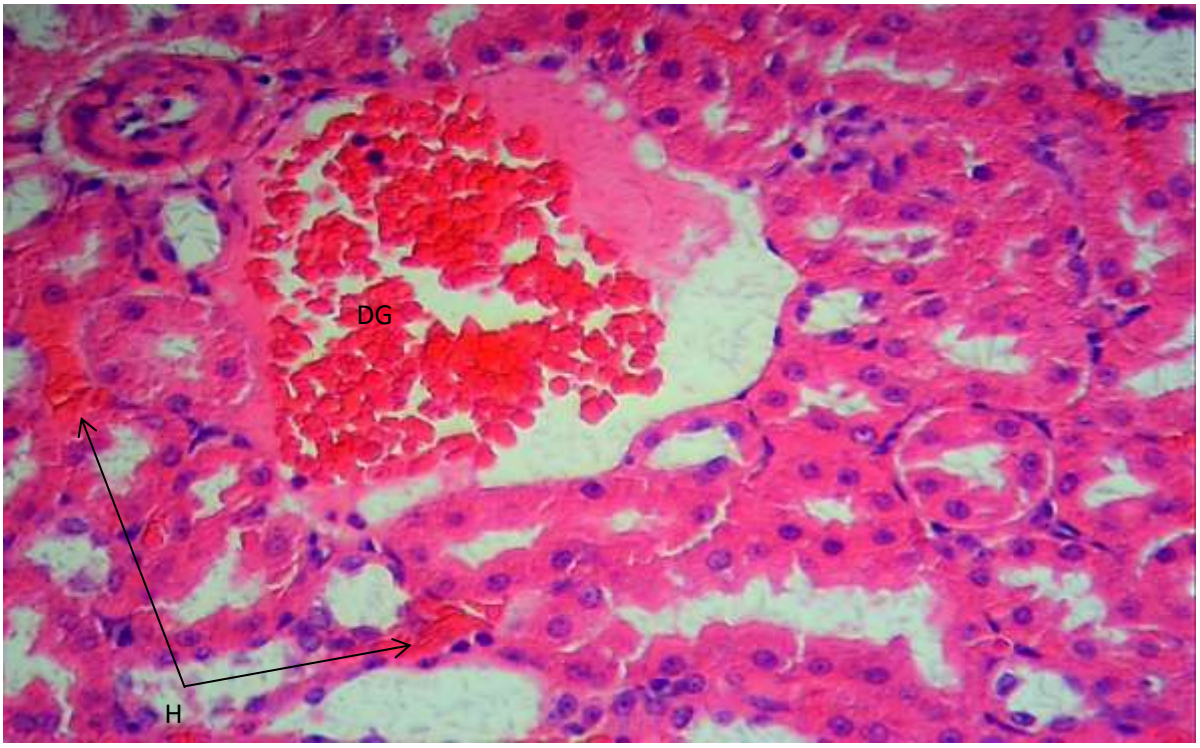
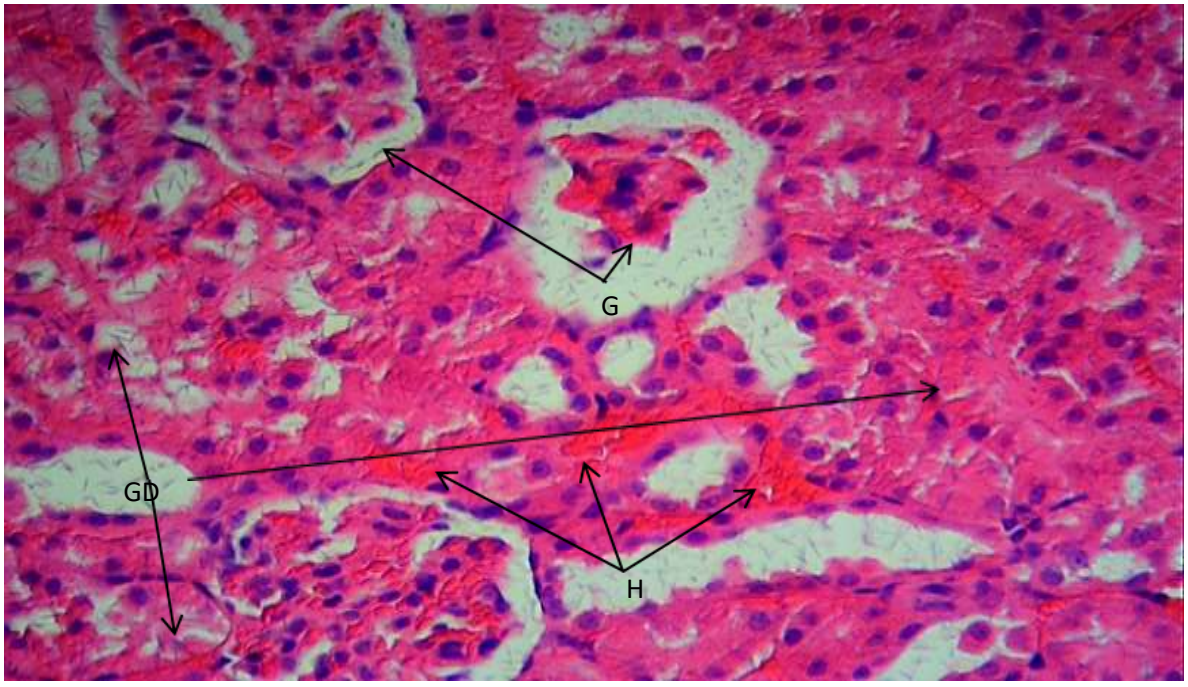
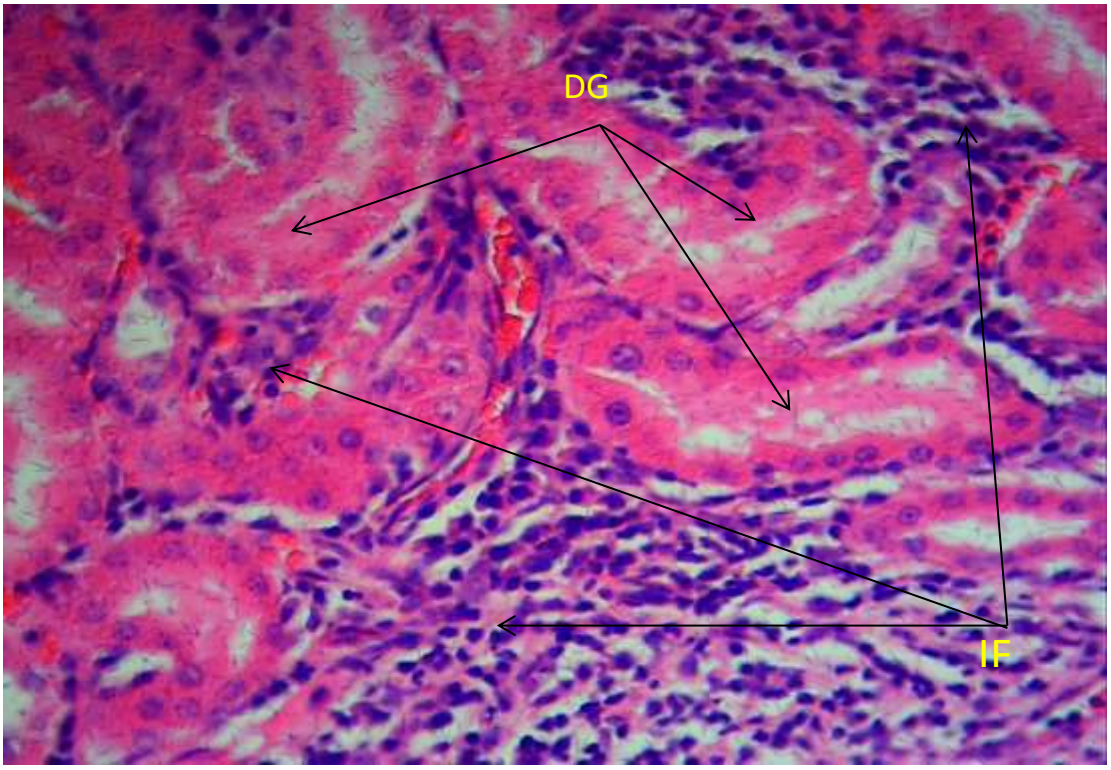


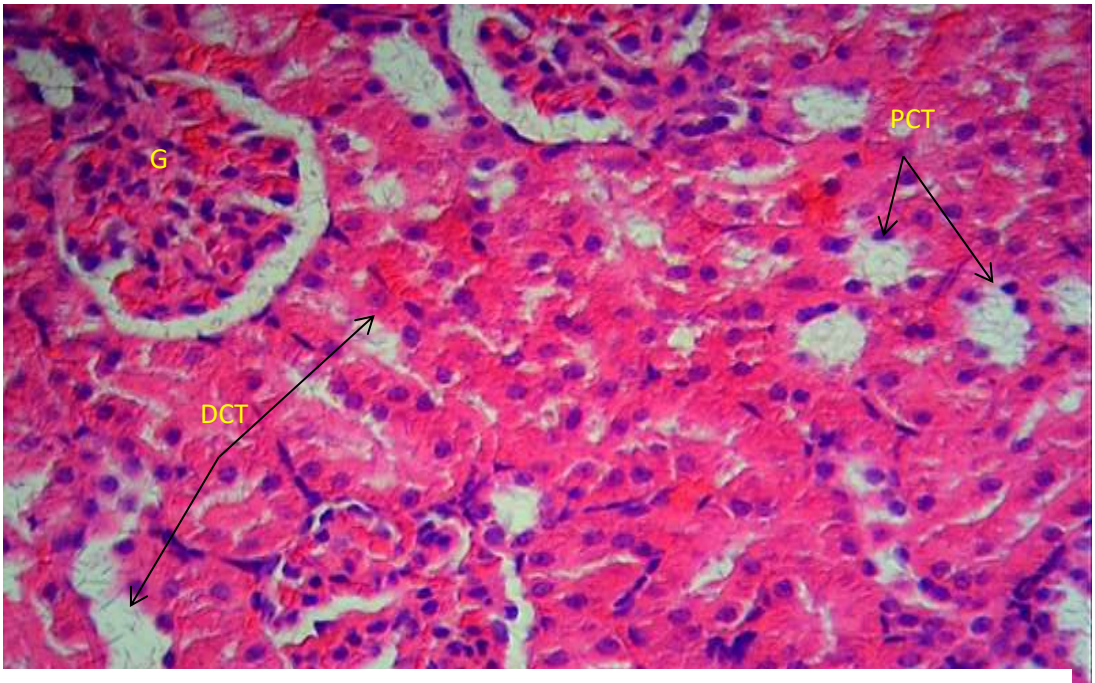
Fig.3) A section of the kidney of (G3) showing the destruction of the renal glomerulus (DG) with a hemorrhage in its place (H). H & E 400X.



(Fig.4) A section of the kidney of (G3) showing shrinkage and lose of architecture of the renal glomerulus (G) with hemorrhage within the kidney tissue that suffer from general degeneration (GD) and hemorrhage (H). H & E 400X.



(Fig.5) A section of the kidney of (G3) showing a clear infiltration of inflammatory cells (IF) and degeneration of urinary tubule epithelial cells (DG). H & E 400X,



(Fig.6) A section of the kidney of (G4) showing the proximal convoluted tubules (PCT) and distal tubules (DCT) as well as renal glomeruli (G) normally. H & E 400X,

Heart:

criteria / group	Cholesterol (mg/ dl)	Triglyceride (mg/dl)	HDL-c mg/ (dl)	LDL-c (mg/ dl)	VLDL-c mg/ (dl)
Control G1	**55± 4 a**	**32 ± 3 B**	**37 ± 2 a**	**14 ± 2 B**	**6.4 ± 1.3 b**
mustard seed extract G2	**38 ± 6 c**	**47 ± 4 A**	**18 ± 2 c**	**22 ± 4 A**	**9.4 ± 1.2 a**
$CdCl_2$ G3	**45 ± 3 b**	**33 ± 2 B**	**26 ± 3 b**	**21 ± 3 A**	**6.2 ± 1.2 b**
$CdCl_2$ + mustard seed extract G4	**43 ± 2 b**	**34 ± 3 B**	**29 ± 1 b**	**14 ± 3 B**	**7 ± 1 b**

-The values are mean ± standard error.

- Different letters vertically mean that there is a significant difference at the level of significance ($P \leq 0.05$).

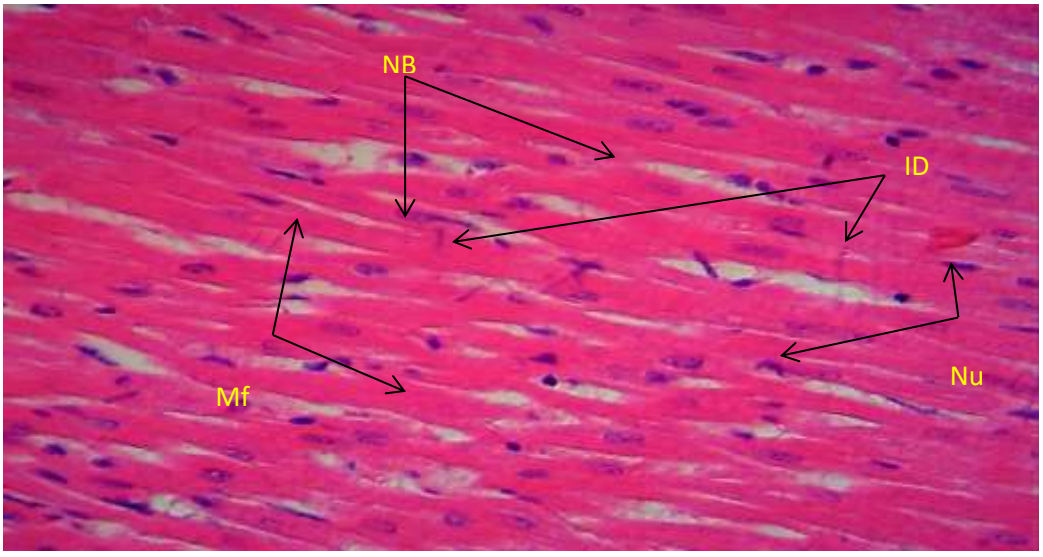
The number of animals is 5 in each group.

Histological results:

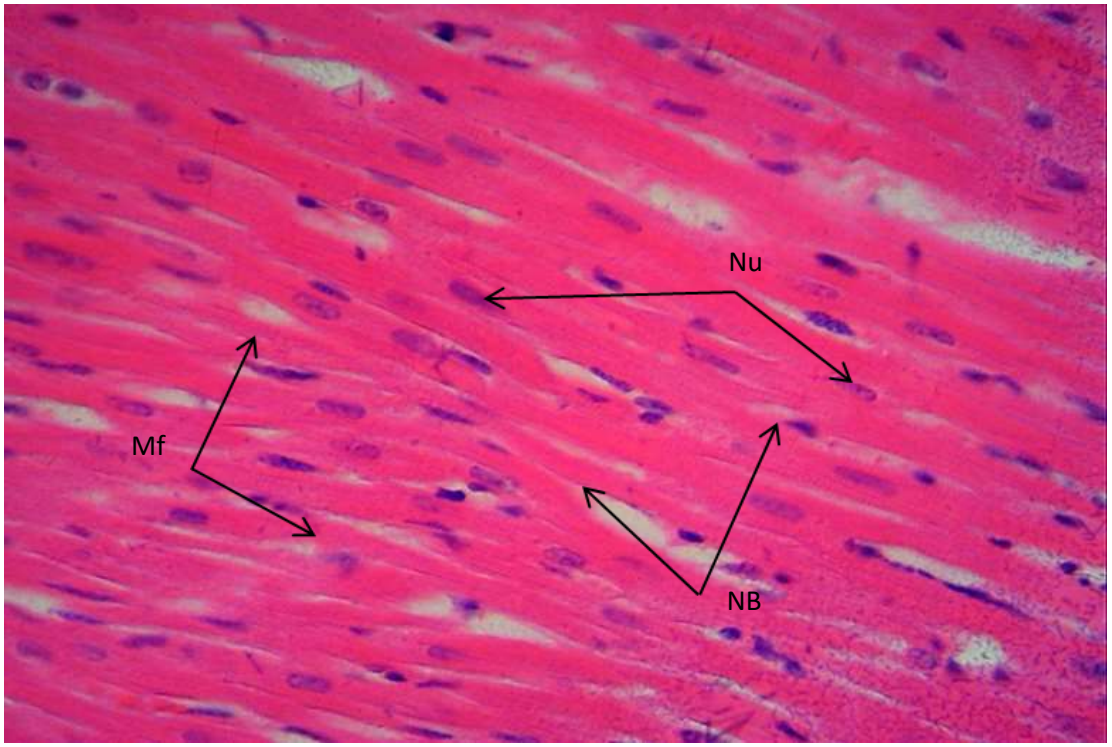
Through the histological examination of tissue slices taken from the hearts of animals, normal heart tissue was observed in the control group and the group treated with mustard seed extract consisting of cardiac muscle fibers arranged as (Fig.7&8).

In the group treated with CdCl2 at a concentration of 5 mg/kg of body was observed within the cardiac cells, infiltration of inflammatory cells, and hemorrhage within the heart tissue (Fig. 9, 10).

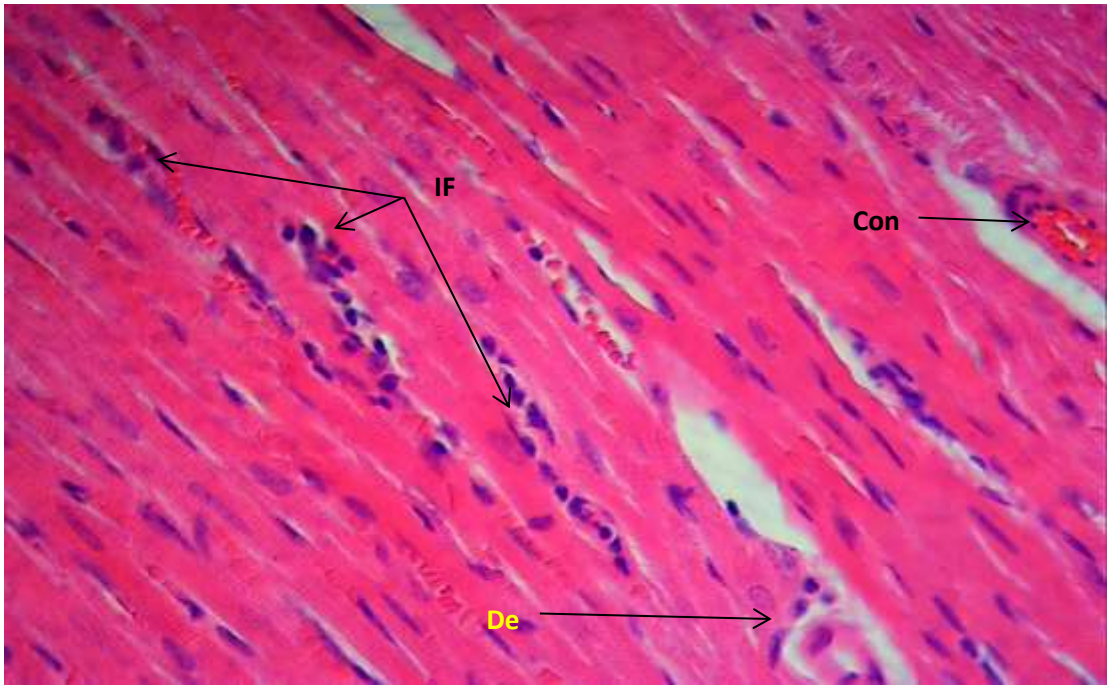
Histological examination of the tissue sections of the group of animals treated with CdCl2 and mustard seed extract showed that most of the heart tissue returned to its normal state. (Fig.11).



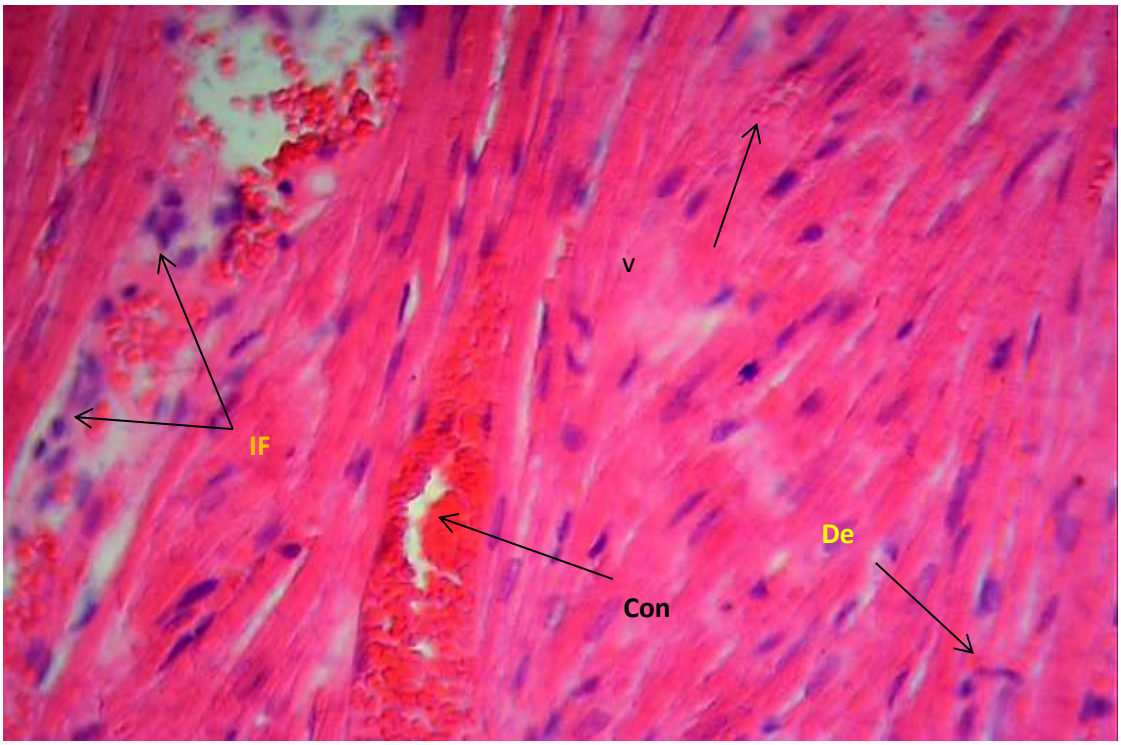
(Fig.7) A section of the heart of the control group (G1) showing cardiac muscle fibers (Mf) , their nuclei (Nu), normal branching of cardiac muscle fibers (NB), and intercalated discs (ID) H & E 400X,



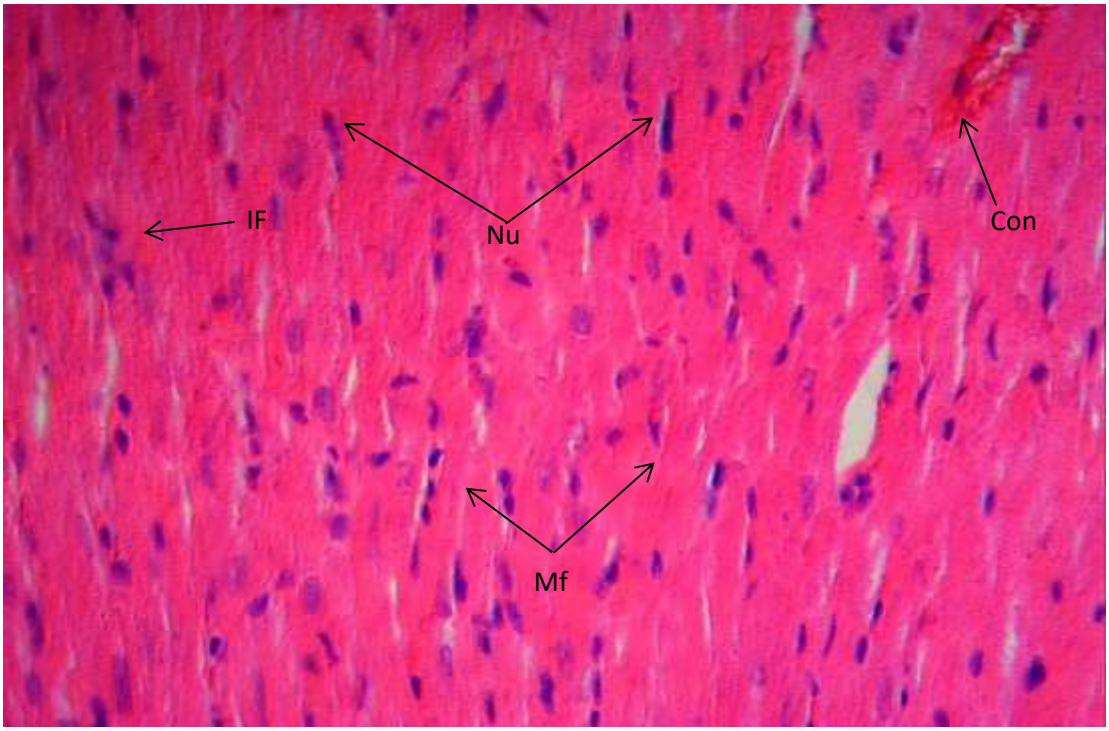
(Fig.8) A section of the heart of (G2) showing cardiac muscle fibers (Mf) and their nuclei (Nu), branching of cardiac muscle fibers (NB), appear normally H & E 400X.



(Fig.9) A section of the heart of (G3), showing a infiltration of inflammatory cells (IF), tissue degeneration (De), and congestion of blood vessels (Con) H & E 400X,



(Fig.10) A section of the heart of (G3), showing disarrangement of muscular fibers, infiltration of inflammatory cells (IF), tissue degeneration (De), congestion of blood vessels (Con), and vacuolated sarcoplasm (V) H & E 400X,



(Fig.11) A section of the heart of (G4) showing cardiac muscle (Mf) and its nuclei (Nu) appear normal, with presence of some congested blood vessels (Con) and little inflammatory cells infiltration (IF) . H&E 400X.

Dissection:-

The increase in the percentage of urea and triglycerides which resulted in production of free radicals and that lead to the occurrence of oxidative stress, which is recording as a one of the main factors causing histological changes in the kidney and heart tissues [13].

The results of the current study showed that the animals treated with CdCl2 at a dose of 5 mg / kg of body weight had lesions in both of kidney tissues represented by cellular degeneration with the destruction of some renal glomeruli and some shrinkage with the presence of hemorrhage [14], and the heart tissues represented by blistering with hemorrhage within the heart tissues. Cadmium can cause severe tissue changes due to lipid oxidation and generation of free radicals[20].

CdCl2 (25) mg/L after 45 days in drinking water also reduced glutathione in liver tissue. This result was confirmed by the study that showed similar results in adult rabbits given 200 ppm of CdCl2 in the drinking water [15]. Others reported similar results in the liver and kidneys of rats [16]. The decreased levels of glutathione may be due to a lack of internalization of the mechanism by which Cd is uptaken, transported and uptaken by cells [14].

It has been suggested that acute nephrotoxic and cardiotoxic injury, involving a direct toxic effect of the metal, ischemia due to endothelial cell injury, and latter inflammatory injury, in which the activation of Coverer cells plays a major role through a series of inflammatory mediators [17].

Some histological changes were observed such as loss of tissue architecture, cellular degeneration and necrosis, lipocytosis, acute glycogen depletion, lipofuscin pigments, and collagen fiber formation. By exposing mice to CdCl2 for 22 days [18].

The aqueous extract of mustard seeds has an effective role in protecting the kidneys from the toxicity of oxidative stress by reducing the level of urea in the blood serum, and the active compounds present in the plant work to reduce free radicals [19].

The protective effect might be due to presence of various antioxidant present in the extract, which was reported from our recent investigation [21].

Conclusion

The previous results of this study showed clear harmful effects of cadmium chloride on the functions and tissues of both kidneys and heart, as it led to an significant negative changes in the level of urea and creatinine and triglycerides, LDL-C, VLDL-C, with a significant increase in cholesterol and HDL-C concentrations, in addition to severe bleeding and infiltration of inflammatory cells with severe congestion. And also the results showed the impressive protective role of the Brassica nigra seed extract in reducing and removing most of the negative effects.

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