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HISTOPATHOLOGICAL STUDY OF ORAL MAGNESIUM (MG) ADMINISTRATION AGAINST CHRONIC CADMIUM (CD) TOXICITY IN WISTAR RATS ON (LIVER, KIDNEY, SPLEEN AND BRAIN)

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. Author SD designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors MK and MB managed the analyses and histology of the study. Author AT managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Toxicity by cadmium induced damages and histopathological changes in liver, kidneys, spleen and brain of Wistar rats. This research aimed at investigating the potential protective effects of magnesium on the histoarchitectural integrity of tissues and organs.

Forty male Wistar rats were randomly divided into 4 groups during 3 months: Control group received water, Cd group water and oral administration at dose 15 mg/Kg of $CdCl_2$, (Cd+Mg) group water and oral administration at doses (15 mg / kg of $CdCl_2$+ 10 mg/kg of $MgCl_2$) and Mg group water and oral administration (10 mg/kg of $MgCl_2$).

A histopahtological study was carried to show the beneficial effect of magnesium treatment against chronic cadmium toxicity.

Administration of cadmium for 3 months led to apparent histological abnormalities, damages and alterations to liver, kidney, spleen and brain. A supplementation of magnesium showed significant histological improvements and markedly reduced tissue damage when compared with Cadmium Group.

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Results of this study demonstrate that the oral administration of magnesium in rats exposure to cadmium chronic toxicity ameliorated the histopathological changes observed in the liver, kidney, spleen and brain with no observable alteration in the histoarchitecture in the organs of the magnesium-supplemented rats.

Keywords: Cadmium; magnesium; chronic; histopathological; rats.

1. INTRODUCTION

Cadmium (Cd) is a toxic heavy metal. In humans, acute Cd exposure via inhalation results in pulmonary edema and respiratory tract irritation, while chronic exposure often leads to renal dysfunction, anemia, osteoporosis, and bone fractures [1]. Cd is also a potent carcinogen in a number of tissues and is classified by IARC as a human carcinogen [2]. Chronic exposure to Cd affect kidney and liver [3,4].

Magnesium (Mg) is an essential cofactor to activate many enzyme systems in humans. It is involved in carbohydrate, lipid, protein, and DNA metabolism, interacting either with the substrate or the enzyme directly [5]. Increasing evidence suggests that Mg modifies Cd absorption in the gastrointestinal system and thus reduces peripheral blood Cd [6,7].

It has been suggested that Mg could decreases free radicals and LPO generation by increasing levels of reduced GSH and the activity of superoxide dismutase, [8]. Moreover, Mg inhibits the activity of reduced NADPH oxidase and xanthine oxidase, the enzymes that produce superoxide radicals [9].

The objective of the present study was to examine the possible histopathological changes and damages of chronic Cd liver, kidneys toxicity in Wistar rats testes and brain and the protective effect of magnesium against poisoning rats with cadmium.

2. MATERIALS AND METHODS

2.1 Animal

Forty Male Wistar rats (procured from the Algiers Institute of Pasteur Research, Algeria) weighing approximately 155-200 g were used in this study during an experimental period of three months (Chronic exposure). The animals were housed in polypropylene cages under natural photoperiod. Food and tap water were given during the study period. All treatments were started after almost two weeks of stabilization from arrival of the rats in the laboratory. The animals were randomly divided into 4 groups, each consisting of 10 animals. Control group only water, Cadmium chloride group administered at a dose of 15 mg/kg [10] was dissolved in distilled water and administered orally; (Cd+Mg) group (15 mg/kg+10 mg/Kg) orally administration in water, magnesium (Mg) group at dose 10 mg/kg [11] administered orally.

2.2 Histopathological Study

The sections of the liver, kidneys, spleen and brain were taken for histopathological preparation and examination. The technique used is that described by Baker et al. [12] which include the following steps: Fixation: The fixation of the samples was made in the alcoholic Bouin. This fixer has the advantage of softening purchased. The liquid is prepared at go:

- A picric acid solution (75%) 45 ml.
- Acetic acid (5%) 7 ml.
- Of formaldehyde 26 ml.
- Distilled water 22 ml.

Dehydration: Once fixed, the samples are presented in baths successive (48 hours) of ethanol (70%, 80%, 90% and 100%), they are thus dehydrated by removal of water from the tissues. This operation also allows to stop the action of Bouin. Paraffin inclusion: The samples are then reserved in cassettes (plastic boxes with an opening on the top), which are immersed in liquid paraffin at 56°C for inclusion. The cassettes are labeled to allow their identification before to be placed in contact with a refrigerated plate, the paraffin then solidifies in a few seconds, we unmold the cassette to obtain a solid block close for cutting. Sections of 3 to 5 m are made on a microtome (Microtome Reicher-Jung) from the cooled blocks. The cuts thus made are spread on microscope slides, then unfolded and fixed with gelatinous water heated to 40°C. They are then stored in an oven at 37°C 24 hour Staining: After drying, the slides are stained with EO (Hematoxylin-Eosin), standard stain used in histology. This staining was performed according to the protocol following:

- Deparaffinize and hydrate the slides with tap water, then rinse with distilled water.
- Immerse in a Harris hematoxylin bath (15 min) which colors the basophilic structures (nuclei). and viewed under light microscope at × 400 magnifications.
- Differentiate the cuts in acid alcohol (100 ml 70% ethanol + 50 ml HCl), then the rinse with tap water.

- Blue in an ammoniacal water bath (100 ml of distilled water + 2 ml of ammonia).
- Immerse in an Eosin bath (15 seconds to 2 min) which stains the acidophilic structures (cytoplasm).
- Rapid dehydration in alcohol and mounting of the blades in Eukitt. All these baths are separated by washes with tap water.

After drying, the slides can be observed under a microscope. The photos of the cuts have been taken through a microscope coupled to a digital camera [12].

3. RESULTS

3.1 Liver

The liver of rats in the group showed no observable microscopic changes as seen in control group normal heptocytes (Fig. 1).

There were degenerative changes in some hepatocytes especially at the portal areas of the livers of rats in group Cd (Fig. 2). The livers of rats exposed to (Cd+Mg) and magnesium group showed no observable microscopic lesions (Fig. 3).

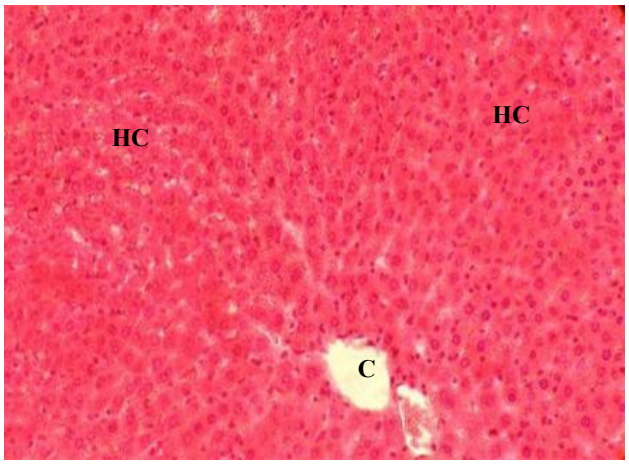

Fig. 1. Histology of section of the liver of rat control group
No observable microscopic lesions in the hepatic cells (HC) and the central vein (C) (H&E staining)

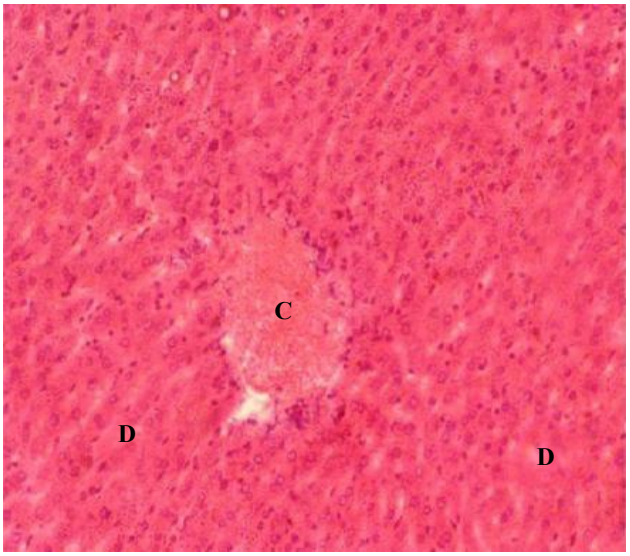

Fig. 2. Histology of section of the liver of rat Cd group, hepatic degenerated cells (D) and congested central vein (C) (H&E staining)

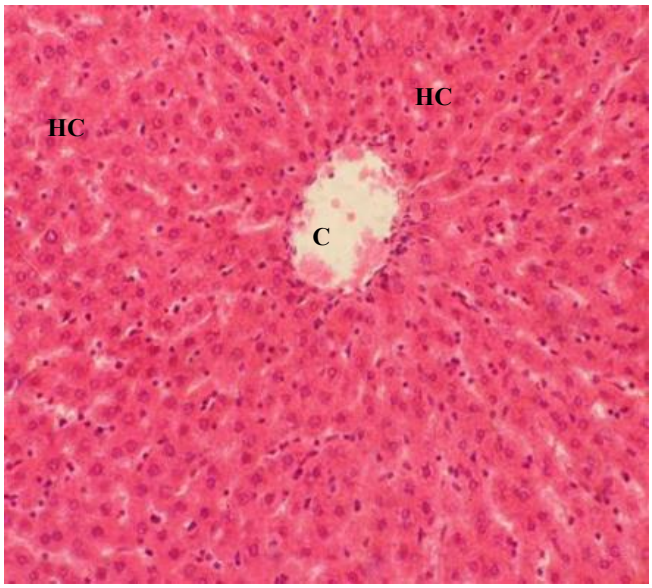

Fig. 3. Histology of section of the liver of rat (Cd +Mg) group
The relatively preserved architectural morphology of hepatic cells (HC) and the central vein of liver. (H&E staining)

3.2 Kidney

There were glomerular degeneration, mononuclear cells infiltration into the interstices and tubular necrosis in the kidneys of rats in Cd group (Fig. 4). The kidneys of rats exposed to Cd and treated with Mg showed no observable microscopic lesions (Fig. 5). The kidneys of rats in control group showed no observable microscopic changes normal glommerular (Fig. 6).

3.3 Spleen

There were depopulated splenic cells (lymphoblast and lymphocytes) in both the red and the white pulps of the rats in Cd group (Fig. 7). There was also haemosiderosis in the spleen of rats in (Cd+Mg) group (Fig. 8). The spleen of rats in the control group showed no observable microscopic lesion (Fig. 9).

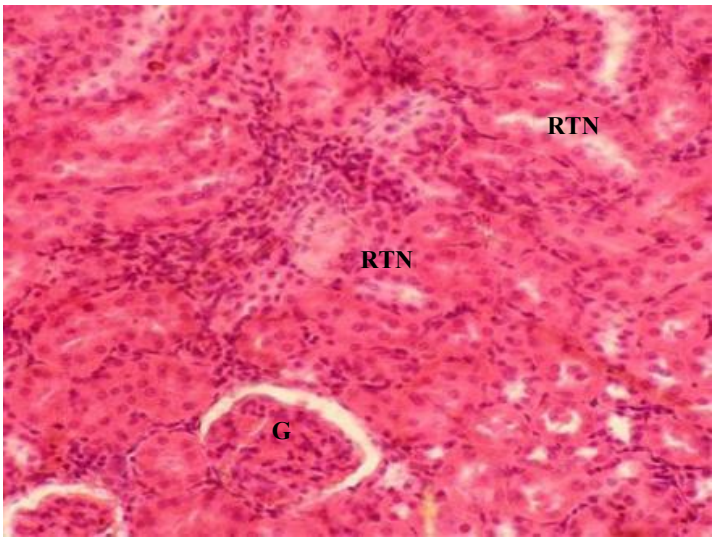

Fig. 4. Histology of section of the kidney of rats Cd group. The glomerural degeneration (G) and renal tubular necrosis (RTN) with mononuclear cellular infiltration into the interstices

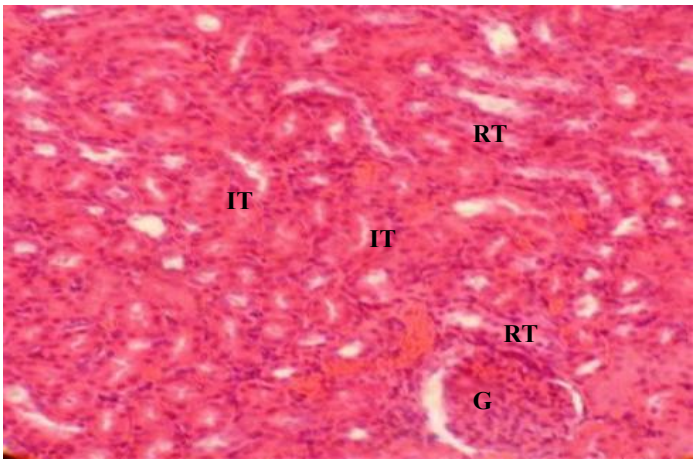

Fig. 5. Histology of section of the kidney of rats (Cd + Mg) group
No observable microscopic lesions in the glomerulus (G), renal tubules (RT) and the intersticises of tubules (IT). (H&E staining)

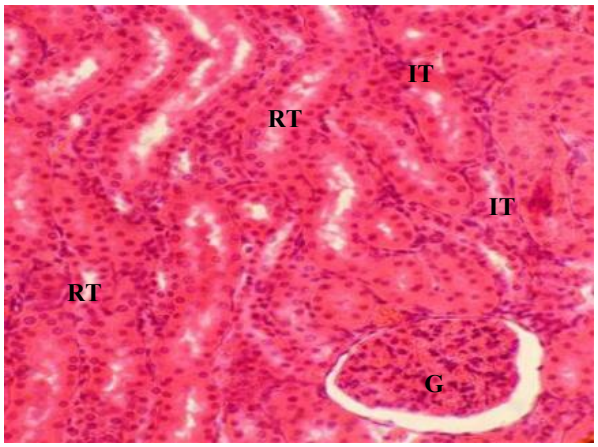

Fig. 6. Histology of section of the kidney of rats control group
No observable microscopic lesions in the glomerulus (G), renal tubules (RT) and the intersticises of tubules (IT). (H&E staining)

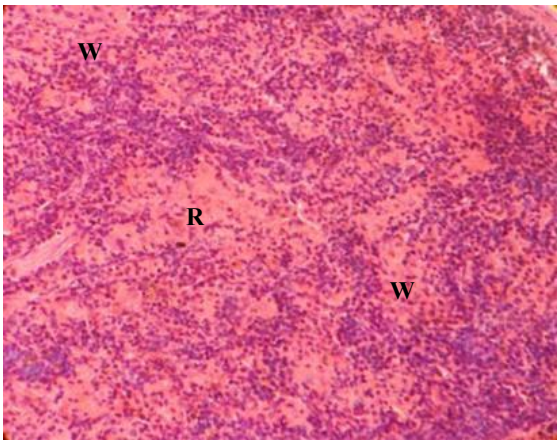

Fig. 7. Histology of section of the spleen of rats cadmium group
Note the depopulated splenic cells in both the white (W) and the red (R) pulps. (H&E staining)

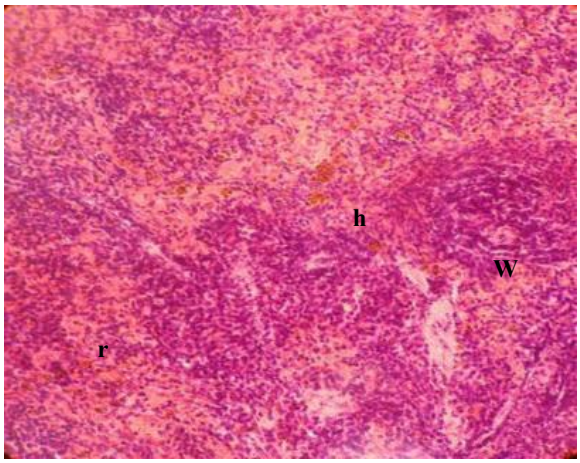

Fig. 8. Histology of section of the spleen of rats (Cd +Mg) group
Hemosiderosis (h) with no other observable microscopic lesions in both the red (r) and the white (w) pulps. (H&E staining)

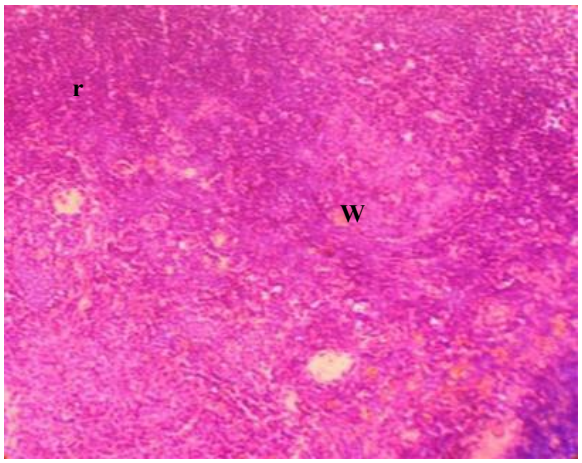

Fig. 9. Histology of section of the spleen of rats control group
No observable microscopic lesions in both the red (r) and the white (w) pulps. (H&E staining)

3.4 Brain

There was neuronal degeneration in the brains of rats in Cd group (Fig. 10) with vacuolations in the brain of rats treated with Mg (Fig. 11). The brains of rats in (Cd+Mg) group showed reduced vacuolations (Fig. 12). The brains of rats in control group showed no observable microscopic lesion.

4. DISCUSSION

Cadmium causes the great disorders in the body functions [13]. It is a known environmental and industrial pollutant with an enormous neuroendocrine disrupting potential. This study showed the efficacy of magnesium in preventing/ameliorating the toxic effects of cadmium on the histoarchitecture of the liver, kidney, spleen and brain in adult Wistar rats. At the moment, this is the first report showing these effects.

Histopathology is considered the most reliable parameter for the detection of toxic effects on male reproduction [14,15] and as such histological investigation was carried out to ascertain the extent of cellular and tissue disruption induced by cadmium on the different organs.

The present study evaluated the histomorphological damages of cadmium chronic toxicity on the tissues and the protective effect of magnesium as antioxidant to reduce the histopathological changes in (liver, kidney, spleen and brain).

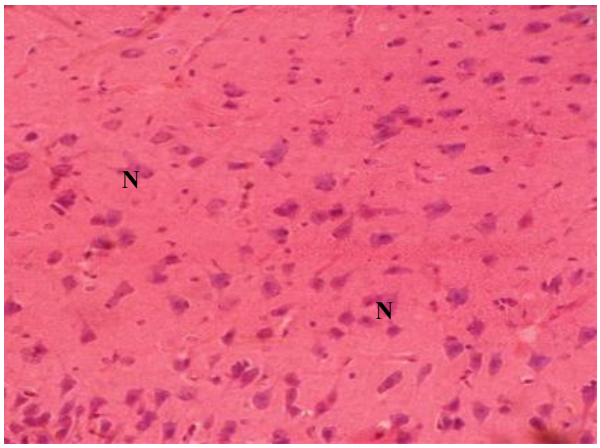

Fig. 10. Histology of section of the brain of rats Cd group
We note neuronal degeneration (N). (H&E staining)

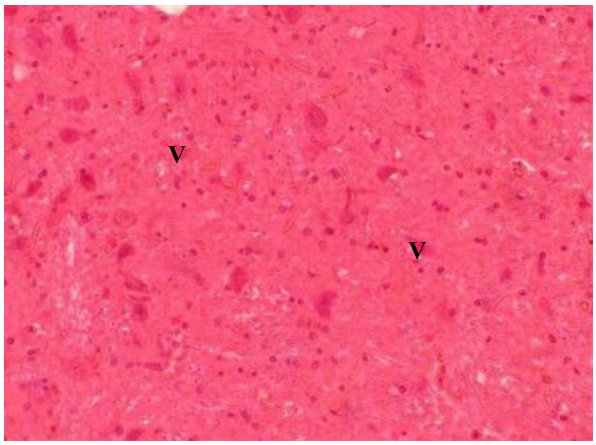

Fig. 11. Histology of section of the brain of rats (Cd+Mg) group
We note the reduce vacualations. (H&E staining)

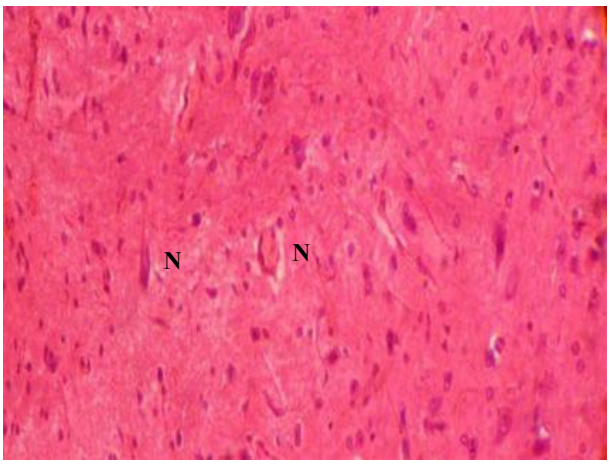

Fig. 12. Histology of section of the brain of rats control group
No observable microscopic lesions in the neurons (N). (H&E staining)

In the liver of Wistar rats histomorphologically, the portal areas, of the livers had degeneration, focal degeneration to diffusely degenerated hepatocytes in some rats chronically exposed to cadmium which might be due to oxidative stress [16].

The infiltration of Kupffer cells were followed by large deposition of reticular fibers, mononuclear cell infiltration and congestion of the liver tissues [17] reported that these could have contributed to the impairment of regular function of the liver due to xenobiotics modification in detoxification processes.

The supplementation with magnesium abolishing effect of magnesium in the hepatotoxicity induced by the cadmium, thus demonstrating the antioxidant effect of magnesium since there was a positive relationship between the oxidative damage due to cadmium and the ameliorative effect therein by the magnesium [18]. The hepatoprotective effect of magnesium had been previously observed in an oxidative stress study [19].

There were no observable microscopic lesions and damages in liver control group.

Histopathologic lesions seen in the kidney of the rats chronically exposed to cadmium group were glomerular and renal tubular degeneration with infiltration of mononuclear cells to the interstices of the kidney which might be consequent upon increased free radicals production in the rats which overwhelmed the endogenous antioxidants produced by the body [20].

In a recent study, mild congestion and haemorrhage, mild focal coagulative necrosis and sloughing of tubular epithelial cells in oxidative stress condition in rats followed chronic cdamium exposure [21]. Oxidative stress was also said to cause shrinkage of the glomerular network, necrosis of proximal tubules and ruptured distal collecting tubules [22].

The renal tubules are particularly sensitive to toxic influences because they have high oxygen consumption, vulnerable enzyme systems and complicated transport mechanisms that may be used for transport of toxins and may be damaged by such toxins during excretion and/or elimination [23].

The presence of necrosis might be related to the depletion of ATP [24], possibly through the direct toxic effect on the cell function, which might involve reactive free radicals, oxidative stress or both [25].

Such nephrotoxic effects induced by cadmium were absent in the rats of control group and might have been ameliorated in the group exposed to cadmium and treated with magnesium, (the antioxidant effect of magnesium).

Histopathological examination of spleen in the present study showed depopulation of splenic cells which involved the lymphoblasts and lymphocytes in both red and white pulps of rats in the cadmium group. These changes might be associated with the oxidative stress caused by the exposure to cadmium [26].

The rats had no observable microscopic changes in their spleens in the control group, normal tissue.

The absence of microscopic lesions in the spleens of rats in the in rats exposed to cadmium and treated with magnesium groups and the lymphocytes proliferation in both red and white pulps of the spleens from rats with administration of magnesium groups tend to suggest that there was an ameliorative effect, probably due to magnesium supplementation which prevented the immuno-suppressive effect that was observed in the cadmium group by its antioxidant function [27].

The histopathologic changes induced chronically in rats brains exposed to cadmium showed that was neuronal cells degeneration which was believed to be caused by oxidative stress. Such neuronal degeneration and mild degeneration in purkinge cells had been similarly observed in oxidative stress condition in chickens [28].

The brain of rats in magneium group showed vacuolations probably due to the pro-oxidant function of magnesium [29]. There was no recognizable lesion in the brains of rats in the control group.

Supplementation with magnesium showed ameliorative effects in the brain of the rats since there were relatively preserved neuronal cells in the brains of the rats in the group. Thus, the degeneration of neuronal cells in both the magnesium-treated group and the combination of the cadmium with magnesium-treated group indicated that its toxic effects in the brains were not abolished, but ameliorated and that probably, the severity of the neurotoxicity were not reversible or abolished by the antioxidant effect of magnesium within the experimental period and condition. Similar oxidative stress condition was shown to be attenuated by the antioxidant effect of magnesium [30].

5. CONCLUSION

The current research demonstrated that magnesium has protective, as well as restorative capacity on the

histoarchitecture of the liver, kidney, spleen and brain in Wistar rats exposed to the cadmium chronic toxicity.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All experiments were performed in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80–23), revised 1996.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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