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Original Article

Histopathological Study of Liver and Kidney Tissues in C57 Mice via Chronic Exposure to Cadmium and Zinc

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Abstract

Heavy metals have a wide application in the industrial world, affecting the health and longevity of living organisms. The current study assessed the possible effects of Cadmium (Cd) and Zinc (Zn) on the liver and kidney. Therefore, 150 male and female white mice C57BL were treated in three different groups with 0.685 mg/L $CdCl_2$. $2.5H_2O$ (group 1), and 0.567 mg/L $ZnSO_4.7H_2O$ (group 2) in drinking water, while the control group only received water for 90 days to investigate how these elements accumulated in the liver/kidney and evaluate the possible histological changes in the liver and kidney. During 90 days, the histopathological consequences of Cd and Zn on the liver and kidneys were recorded. The results pointed out that exposure to heavy metals, such as Cd and Zn, led to organ accumulation of these elements. The histological evaluations demonstrated significant detrimental effects on the liver and kidney. Under the influence of Cd, light microscopic examination revealed significant histological alterations in both organs. In the animals exposed to Cd and Zn, histopathological alterations were observed in the liver, including extensive degeneration, necrosis, depletion, and necrosis of hepatocytes with significant nuclear hypertrophy. When animals are exposed to Cd and Zn, histological alterations in the kidneys include severe vascular degeneration and renal tubule necrosis. In conclusion, heavy metal intoxication has been shown to cause histopathological changes in the liver and kidneys of experimental animal models.

Keywords: Heavy metal, Accumulation, Liver, Kidney, Histopathology

1. Introduction

The development of new materials and technology has resulted in pollution of the air, land, and water as human civilization has progressed. At levels of milligrams or micrograms per kg of environmentally hazardous compounds, natural or man-made compounds, as biological unities, harm plants, animals, and humans (1). Metal melting, mine drainage, other anthropogenic activities and industrial processes, as well as natural ones, such as volcanic eruptions, forest fires, sea-salt sprays, rock erosion, and borne soil particles, distribute inorganic contaminants throughout the environment (2). Heavy metals are among the most dangerous inorganic contaminants due to their toxicity to both terrestrial and aquatic life.

Animals require metals, such as Cadmium (Cd), Cobalt (Co), Manganese (Mn), and Zink (Zn), which are involved in cellular metabolism, antioxidant, anti-inflammatory defenses, gene expression, and protein synthesis (3, 4). Heavy metal concentrations in the environment are too excessive and become dangerous to animals and plants. The Cd and Lead (Pb) are primarily stored in the kidneys and liver, although they can be found in high concentrations in the digestive system and spleen (5). Heavy metals are difficult to degrade and continue their presence in the environment

for long periods of time, and once they enter the human body through air, water, or polluted food, they can have long-term and permanent harmful impacts (6).

The Cd, along with other toxicants, causes hepatotoxicity to the tissues of the liver, which is a target organ, and disrupts several plasmatic enzymes, such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, and lactate dehydrogenase (7, 8). Moreover, Cd-related hepatotoxicity mediates the increase of reactive oxygen species (ROS), hydrogen peroxides, hydroxyl groups, and superoxide, inducing oxidative damage to membrane lipids. The Cd is a carcinogenic contaminant present in the environment with a half-life of more than 20 years. When it enters the human body, it gently accumulates in tissues and bounds to metallothionein thiols; thereafter, it disrupts antioxidant enzyme activity, breaks the natural structure of nucleic acid DNA, and eventually, causes teratogenicity and carcinogenicity (9, 10).

Copper (Cu), Mn, and Zn are essential microelements for physiological processes and perform a major role in the natural development and maintenance of homeostasis in organisms. On the other hand, exposure to these components with high levels may bring about negative health consequences (11, 12). These different metals exert harmful effects on molecular processes, such as the disruption of intracellular homeostasis, and limit proteinase activity when entering organisms, resulting in an excess of DNA damage, reactive oxygen species (ROS), and carcinogenicity (13, 14).

The Cd induces the synthesis of metallothionein (MT) in the organs and creates MT-Cd. It is initially absorbed into the liver tissues when reaching the digestive tract and is transmitted to the kidneys via the bloodstream; thereafter, it is filtered by the glomerular membrane and reabsorbed by tubular cells, particularly in the proximal convoluted tubules (15). The MT-Cd causes severe nephrotoxicity after pinocytosis and aggregation in proximal tubular cells. Numerous studies demonstrated comparable evident changes in renal tissue when Cd was detected (16, 17). In light of the aforementioned issues, the present study aimed to identify histopathological changes in C57BL mice due to heavy metal exposure after accumulation in liver and kidney tissues.

2. Materials and Methods

A total of 150 C57BL mice, male and female, aged 8 weeks with an average weight of 20-25 g were used in this experiment. The animals were monitored for 90 days from (01/07/2019) in the Laboratories of Environment and Water Directorate, Ministry of Sciences and Technology. The mice were put into three groups (n=50 in each group). The first group received 0.685 mg/L of Cd salts ($CdCl_2$. $2.5H_2O$) in the water supply, the second group received 0.567 mg/L of Zn salts ($ZnSO_4$. $7H_2O$) in drinking water, and the third group (the control group) received only pure drinking water. All these experimental animals were kept in a temperature-controlled environment at 25°C with a 12-hour light/dark cycle. They had free access to food and water; moreover, they were treated humanely according to the Principles of the Experimental Animal Ethics Committee at the Animal House at Alrazi Center for medical and diagnostic kits. The National Research Council's Animal Nutrition Committee recommended that they should be fed a regular diet (1995). To avoid precipitation, each heavy metal compound was dissolved as a stock solution and then diluted with water to the required concentration.

2.1. Histopathological Examination

Standard histology laboratory techniques were used to process the tissues after fixing hematoxylin and eosins stains by formalin and were kept at 4°C until they were embedded in paraffin wax and cut into 3-4 µm pieces.

3. Results

The pathological effects of numerous heavy metals on tissues were evaluated using hematoxylin and eosin staining. Inflammatory cell infiltration and histological alterations were observed in liver and kidney tissues of the treated groups.

3.1. Effect of Zn on liver and kidneys tissue

Liver: In comparison with the control group (Figures 1 and 2), the sections of the liver tissues showed a marked disarrangement of hepatic cords with marked compressed sinusoids. The magnified sections exhibited severe cellar swelling of hepatocytes, marked nuclear hypertrophy, and necrosis of hepatocytes with tissue depletion (Figures 3 and 4).

Kidney: In comparison with the sections obtained from the control group (Figures 5 and 6), the sections of the renal cortex and medulla displayed mild vascular degeneration and necrosis of renal tubules with tubular cast formation (Figures 7 and 8).

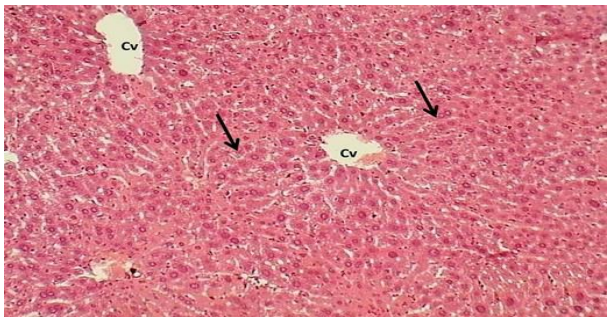

Figure 1. Sections of liver tissue (control) showing central vein (Cv) and hepatic cords (Arrows) (H&E stain at 400x magnification)

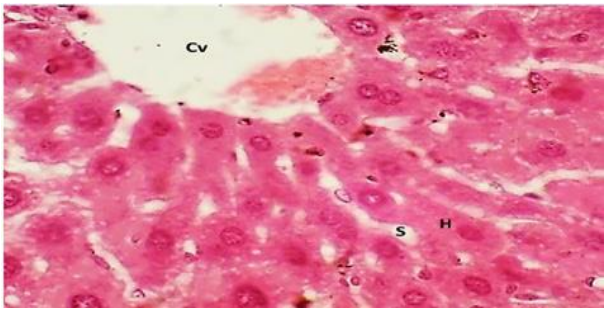

Figure 2. Magnified sections of the liver lobule (control) illustrating central vein (Cv), hepatocytes (H), and sinusoids (S) (H&E stain at 400x magnification)

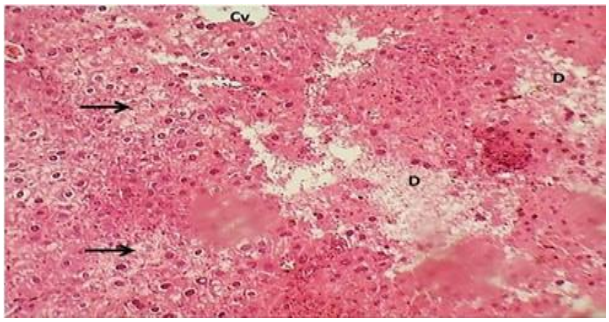

Figure 3. Section of the liver (Zn group) showing generalized degeneration, necrosis (arrows), depletion (H&E stain at 400x magnification)

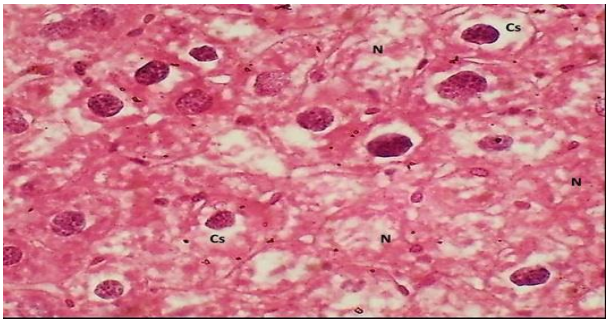

Figure 4. Magnified sections of the liver (Zn group) showing cellular swelling (Cs) and necrosis of hepatocytes with marked nuclear hypertrophy (N) (H&E stain at 400x magnification)

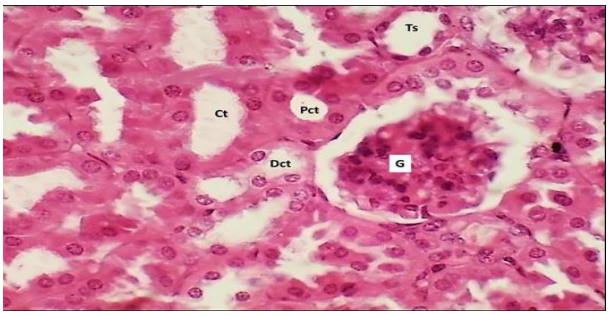

Figure 5. Section of the renal cortex (Control) showing normal glomerulus tuft (G), thin segment (Ts), collecting tubules (Ct), proximal (Pct), and distal convoluted tubules (Dct) (H&E stain at 400x magnification)

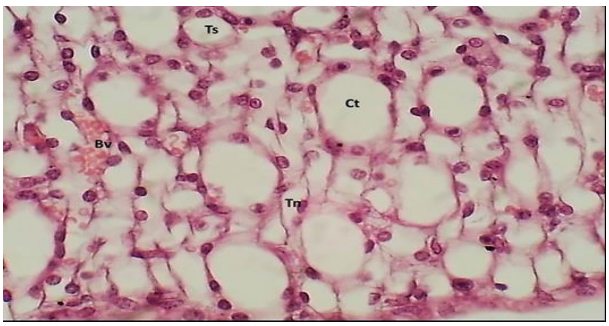

Figure 6. Section of the renal medulla (Control) showing normal thick segment (Ts), thin segment (Tn), collecting tubules (Ct), proximal (Pct), and blood vessel (Bv) (H&E stain at 400x magnification)

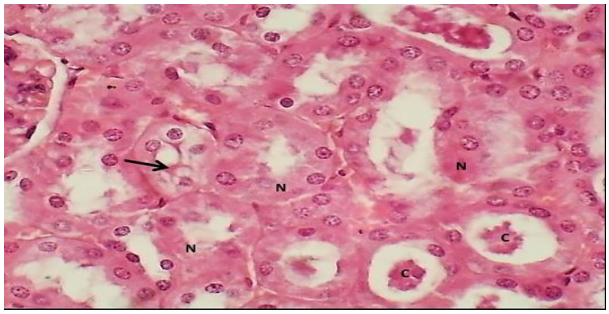

Figure 7. Section of the renal cortex (Zn group) showing mild vascular degeneration (arrows) and necrosis of renal tubules (N) with tubular cast formation (c) (H&E stain at 400x magnification)

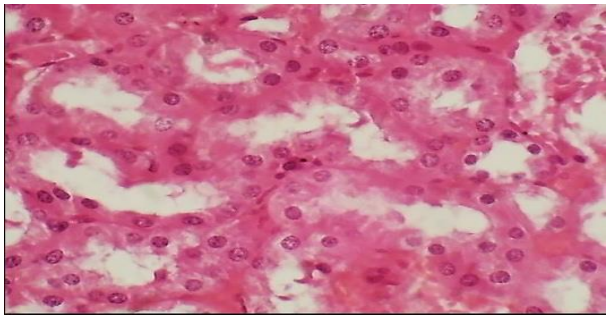

Figure 8. Section of the renal medulla (Zn group) showing severe vascular degeneration and necrosis of renal tubules (H&E stain at 400x magnification)

3.2. Effect of Cd on liver and kidneys tissue

Liver: In comparing with a control group (Figures 1 and 2), the sections of the liver show moderate cellular swelling and necrosis of hepatocytes with congestion and dilation of the central vein (Figures 9 and 10).

Kidney: In comparison with the sections of control (Figures 5 and 6), the sections of renal cortex and medulla demonstrate severe vascular degeneration and necrosis of renal tubules with glomerular deterioration (Figures 11 and 12).

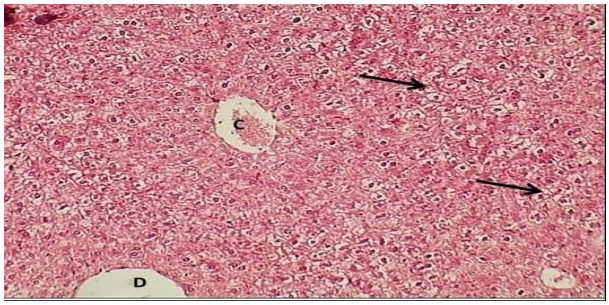

Figure 9. Section of the liver (group-d) showing generalized moderate cellular swelling and necrosis (arrows), and congestion (C) with dilation of central vein (D) (H&E stain at 100x magnification)

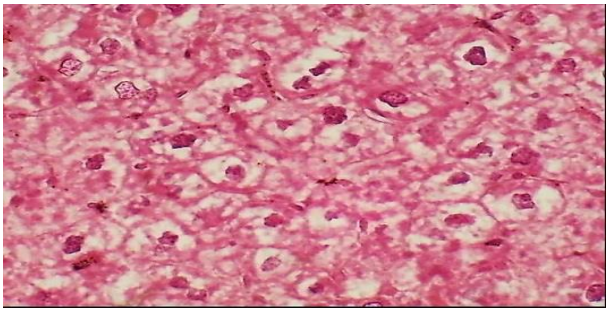

Figure 10. Magnified sections of the liver (group-d) showing moderate cellular swelling and necrosis of hepatocytes with marked nuclear pyknosis (H&E stain at 400x magnification)

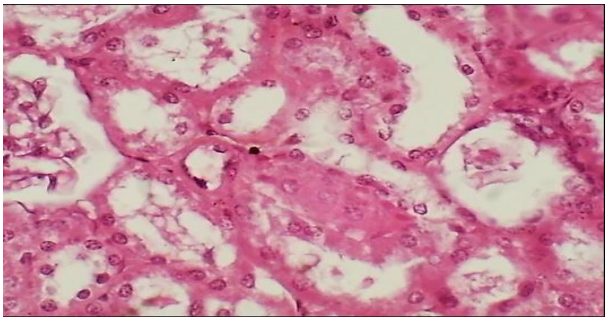

Figure 11. Section of the renal cortex (Group-d) showing severe glomerular degeneration with degeneration and necrosis of renal tubules (H&E stain at 400x magnification)

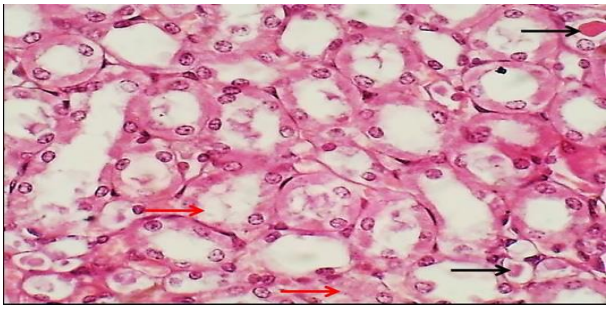

Figure 12. Section of the renal medulla (Group-d) showing vascular degeneration (Black arrows) and cast formation of renal tubules (Red arrows) (H&E stain at 400x magnification)

4. Discussion

Even though numerous studies have focused on the toxicity of one or two specific heavy metals, the majority of people are exposed to multiple heavy metals at the same time, whether through contaminated air, food, or water. Heavy metal exposure can lead to a variety of problems, such as accumulation, as well as tissue or organ damage. As a result of heavy metal exposure, the tissues of the liver and kidneys are

broken down and changed. The concentrations of several heavy metals, such as Zn and Cd, showed a decreasing trend when compared to the control. This decline was detected in the kidney and liver in the current investigation.

The results indicated that a high Cd concentration in vivo could affect the concentration of biological elements, such as Zn (18, 19), and the toxicity of Cd is inhibited by an appropriate dose of Zn. Researchers (20) similarly have pointed out that when Cd and Zn were joined, the content of the Cd was raised, while the Zn concentration reduced, indicating antagonistic actions within the two elements. The findings suggested that Cd and Zn could accumulate in the tissues of the liver. After histological examination, heavy metal effects on the liver and kidney as depicted in the figures of tissue sections.

The kidneys and liver are the principal organs involved in drug and xenobiotic metabolism. Chemical toxicity usually manifests in the kidney and liver before other organs (21). The Cd is an extremely detrimental contaminant to the environment since it consumes glutathione and protein-bound sulfhydryl groups, leading to an increase in ROS, such as hydroxyl radicals, superoxide ions, and hydrogen peroxide formation. Heavy metals induce significant alterations in the liver, including swelling and extensive fatty degeneration in hepatocytes, as well as a large vacuole in the cytoplasm.

Due to hepatic cell injury following Cd treatment, the cytoplasm of hepatocytes revealed vacuoles and the nuclei were fairly poor in staining with pycontic. It was discovered that the damage to liver cells increases as the dose is increased. Apoptosis was also observed when Cd was administered at a dose of 10 mg/kg body weight (22). This result was in line with those obtained by Brzoska, Moniuszko-Jakoniuk (23) who reported that following Cd administration, the liver weight of Wistar rats decreased by 8% ($P< 0.05$). The histopathological changes in the liver tissue of Cd-treated animals were described as a breakdown of liver plates, the disintegration of liver indicated by nuclei pycnosis, breakdown of the cell membrane, and cytoplasmic vacuolization (24). These findings are in agreement with those of El-Refaiy and Eissa (25), who found severe liver fibrosis, adipose changes, deterioration symptoms, as well as inflammatory cell infiltrations, in Cd-affected mice.

The formation of extremely reactive radicals and subsequent lipid peroxidation may be responsible for histopathological alterations observed in Cd-treated livers. Excessive bleeding in the Cd-treated liver may be attributed to congested blood arteries and blood sinusoids, allowing blood to escape. Furthermore, some researchers have indicated that sinusoid walls contain a large number of Kupffer cells. Proliferation and expansion in the number of Kupffer cells may indicate that Cd-treated animals have developed a defense mechanism (25, 26).

Significant Cd pathological changes in the kidney structure were observed in mice after three months of Cd administration. When compared to control groups, they showed evidence of tubular necrosis and fibrosis, swollen renal glomeruli, and decreased glomerular space with some capsular fibrosis following eight weeks of Cd and Zn exposure. For the Cd-contaminated mice, these alterations were related to a decrease in the density of the microvillus membrane surface per cell volume. The proximal tubular brush border of the mouse kidney, Cd inhibits the vacuolar hydrogen ion-ATPase and endocytosis, endocytosis of filtering proteins may be impeded, and vesicle-mediated recycling of specific membranes may be disturbed (27).

Histoarchitectural abnormalities detected in the foregoing results were found to be consistent with the findings of the study by El-Refaiy and Eissa (25) who pointed to lesions in the cortex and medulla of the kidney of Cd-treated white mice. After the Feulgen reaction, the nuclei in the tubular cells and collecting ducts of treated mice were stained brilliant pink, suggesting the likelihood of Cd-induced DNA damage.

Similar findings were described by Garba (28) who observed histopathological alterations in the rat kidney that included proteinaceous release inside channels, severe multifocal congestion, cystic dilatation in the medulla, and hemorrhage associated with interstitial mononuclear cellular infiltration.

These degenerative alterations in kidneys could be caused by changes in metabolic activities or interactions between metal ions and renal tissue. Heavy metals nephrotoxicity primarily affects the renal glomerulus and tubules. The renal capillaries were affected by Cd in favor of Bowman's space, resulting in glomerulus atrophy (29). In addition, histopathological studies demonstrated that Cd toxicity affects proximal tubular necrosis, causing the kidneys to go through apoptosis and tubular disintegration. The Cd causes renal function loss when it passes through the kidney, primarily in the cortical region.

These effects could be caused by the accumulation of MT-Cd or free Cd as a result of elevated lipid peroxidation in renal tissues of animal models (30). Other studies of Cd-treated mice demonstrated the breakdown of certain cells in the proximal and distal tubules, as well as severe degeneration and cytoplasmic vacuolation, in the collecting tubules (31). Chronic heavy metal treatment in mice leads to accumulation at various levels in different tissues or organs, for instance, Zn accumulates in the kidneys and liver, while Cd accumulates in the liver, kidneys, serum, heart, lungs, and spleen (32). In addition, it exhibited histological damage due to a redox imbalance that induces oxidative stress in the tissues of the kidneys and liver (33).

As evidenced by the obtained results, heavy metal intoxication has been shown to cause histopathological changes in the liver and kidneys of experimental animal models. The disintegration of hepatocytes, rupture of the hepatic cell membrane, vocalization of cytoplasm, pycnosis of nuclei, and acclamation of nuclei, are some of the histological alterations caused by Cd and Zn in the liver. Tubular necrosis, fibrosis, shrinkage of glomeruli, pycnotic nuclei of tubular cells, and hemorrhaging were also observed in Cd and Zn-intoxicated kidneys.

Authors' Contribution

Study concept and design: Z. G. A.
Acquisition of data: A. H. A.
Analysis and interpretation of data: H. K. O.
Drafting of the manuscript: H. S. N.
Critical revision of the manuscript for important intellectual content: S. A. A.
Statistical analysis: A. H. A.
Administrative, technical, and material support: A. H. A.

Ethics

Animals were treated humanely according to the principles of the Experimental Animal Ethics Committee at the Animal House, at Alrazi Center for medical and diagnostic kits.

Conflict of Interest

The authors declare that they have no conflict of interest.

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References

1. Sánchez-Bayo F. Sources and toxicity of pollutants. Ecological Impacts of Toxic Chemicals Online: Bentham Science Publishers. 2011:3-12.
2. Nriagu JO. A global assessment of natural sources of atmospheric trace metals. Nature. 1989;338(6210):47-9.

3. Prashanth L, Kattapagari KK, Chitturi RT, Baddam VRR, Prasad LK. A review on role of essential trace elements in health and disease. J Dr NTR Univ Health Sci. 2015;4(2):75.
4. Abaspour Aporvari MH, Mamoei M, Tabatabaei Vakili S, Zareei M, Dadashpour Davachi N. The Effect of Oral Administration of Zinc Oxide Nanoparticles on Quantitative and Qualitative Properties of Arabic Ram Sperm and Some Antioxidant Parameters of Seminal Plasma in the Non-Breeding Season. Arch Razi Inst. 2018;73(2):121-9.
5. Jezierska B, Witeska M. The metal uptake and accumulation in fish living in polluted waters. Soil and water pollution monitoring, protection and remediation: Springer; 2006. p. 107-14.
6. Mahboob S, Kausar S, Jabeen F, Sultana S, Sultana T, Al-Ghanim K, et al. Effect of heavy metals on liver, kidney, gills and muscles of Cyprinus carpio and Wallago attu inhabited in the Indus. Braz Arch Biol Technol. 2016;59.
7. Koyu A, Gokcimen A, Ozguner F, Bayram DS, Kocak A. Evaluation of the effects of cadmium on rat liver. Mol Cell Biochem. 2006;284(1):81-5.
8. Yannai S, Sachs K. Absorption and accumulation of cadmium, lead and mercury from foods by rats. Food Chem Toxicol.1993;31(5):351-5.
9. Abdel-Wahab A, Abdel-Razik A-RH, Aziz RLA. Rescue effects of aqueous seed extracts of Foeniculum vulgare and Carum carvi against cadmium-induced hepatic, renal and gonadal damage in female albino rats. Asian Pacific J Tropic med. 2017;10(12):1123-33.
10. Odewumi CO, Badisa VL, Le UT, Latinwo LM, Ikediobi CO, Badisa RB, et al. Protective effects of N-acetylcysteine against cadmium-induced damage in cultured rat normal liver cells. Int J Mol Med. 2011;27(2):243-8.
11. Crossgrove J, Zheng W. Manganese toxicity upon overexposure. NMR Biomed. 2004;17(8):544-53.
12. Dos Santos Carvalho C, Fernandes MN. Effect of copper on liver key enzymes of anaerobic glucose metabolism from freshwater tropical fish Prochilodus lineatus. Comp Biochem Physiol A Mol Integr Physiol 2008;151(3):437-42.
13. O'Brien TJ, Jiang G, Chun G, Mandel HG, Westphal CS, Kahen K, et al. Incision of trivalent chromium [Cr (III)]-induced DNA damage by Bacillus caldotenax UvrABC endonuclease. Mutat Res Genet Toxicol Environ Mutagen. 2006;610(1-2):85-92.
14. Siu ER, Mruk DD, Porto CS, Cheng CY. Cadmium-induced testicular injury. Toxicol Appl Pharmacol. 2009;238(3):240-9.
15. Nordberg M. General aspects of cadmium: transport, uptake and metabolism by the kidney. Environ Health Perspect. 1984;54:13-20.
16. Imed M, Fatima H, Abdelhamid K. Protective effects of selenium (Se) and zinc (Zn) on cadmium (Cd) toxicity in the liver and kidney of the rat: histology and Cd accumulation. Food Chem Toxicol. 2008;46(11):3522-7.
17. Mahran A, Osman H, El-Mawla A, Attia A. Protective effect of zinc (Zn) on the histology and histochemistry of liver and kidney of albino rat treated with cadmium. J Cytol Histol. 2011;2(4):2-9.
18. Erdem O, Yazihan N, Kocak MK, Sayal A, Akcil E. Influence of chronic cadmium exposure on the tissue distribution of copper and zinc and oxidative stress parameters in rats. Toxicol Ind Health. 2016;32(8):1505-14.
19. Satarug S, Nishijo M, Ujjin P, Moore MR. Chronic exposure to low-level cadmium induced zinc-copper dysregulation. J Trace Elem Med Biol. 2018;46:32-8.
20. Saibu Y, Jamwal A, Feng R, Peak D, Niyogi S. Distribution and speciation of zinc in the gills of rainbow trout (Oncorhynchus mykiss) during acute waterborne zinc exposure: Interactions with cadmium or copper. Comp Biochem Physiol Part-C: Toxicol Pharmacol. 2018;206:23-31.
21. Abdel-Daim MM, Abuzead SM, Halawa SM. Protective role of Spirulina platensis against acute deltamethrin-induced toxicity in rats. PloS One. 2013;8(9): 72991.
22. Gathwan KH, Al Ameri QMA, Zaidan HK, Al Saadi AH, Ewadh MJ. Heavy metals induce apoptosis in liver of mice. Inter J Appl Biol Pharmacol Technol. 2012;3(2):146-50.
23. Brzoska M, Moniuszko-Jakoniuk J, Piłat-Marcinkiewicz B, Sawicki B. Liver and kidney function and histology in rats exposed to cadmium and ethanol. Alcohol Alcohol. 2003;38(1):2-10.
24. Mohapatra AK, Datta S, Kumari P. Genotoxic and histopathological effects of cadmium in male swiss albino mice, Mus musculus. BioScan. 2013;8(2):391-401.
25. El-Refaiy AI, Eissa FI. Histopathology and cytotoxicity as biomarkers in treated rats with cadmium and some therapeutic agents. Saudi J Biol Sci. 2013;20(3):265-80.

26. Omar A. Histopathological and physiological effects of liver and kidney in rats exposed to cadmium and ethanol. Glob Adv Res J Environ. 2013;2(3):93-106.
27. Herak-Kramberger CM, Brown D, Sabolić I. Cadmium inhibits vacuolar H+-ATPase and endocytosis in rat kidney cortex. Kidney Int. 1998;53(6):1713-26.
28. Garba SH. Toxicological Effects of Inhaled Mosquito Coil Smoke on the Rat Spleen: A Haematological and Histological Study" SH Garba," MM Shehu and "AB Adelaiye. J Med Sci. 2007;7(1):94-9.
29. Jemai H, Lachkar HA, Messaoudi I, Kerkeni A. Effects of zinc pre-treatment on blood glutathione, serum zinc and kidney histological organisation in male rats exposed to cadmium. J Trace Elem Med Biol.2010;24(4):277-82.
30. Renugadevi J, Prabu SM. Cadmium-induced hepatotoxicity in rats and the protective effect of naringenin. Exp Toxicol Pathol. 2010;62(2):171-81.
31. Hefny HA, Ahmed SH. Histopatological effect of CdSo4 on foetal liver, kidneys and heart of albino rats. Egyptian British biol. 2004;6:111-8.
32. Wang Y, Tang Y, Li Z, Hua Q, Wang L, Song X, et al. Joint toxicity of a multi-heavy metal mixture and chemoprevention in sprague dawley rats. Int J Environ Res Public Health 2020;17(4):1451.
33. Desta G, Selemun H, Mekbeb A, Abraha T, Mihret G. Histopathological effects of cadmium on liver, kidneys and testis. Asian J SciTechnol. 2018;09(17):8355-60.