Journal of Agricultural Chemistry and Environment, 2016, 5, 159-169
http://www.scirp.org/journal/jacen
ISSN Online: 2325-744X
ISSN Print: 2325-7458

Combined Effects of Cd and Hg on Liver and Kidney Histology and Function in Wistar Rats

Karima Dardouri[1], Samir Haouem[1], Ines Gharbi[2], Badreddine Sriha[3], Zohra Haouas[4], Abdelhamid El Hani[1], Mohamed Hammami[2]

[1]Department of Physiology, Faculty of Medicines of Monastir, University of Monastir, Monastir, Tunisia
[2]Laboratory of Biochemistry, Research Laboratory LR12 ES 05 Lab-NAFS "Nutrition-Functional Food & Vascular Health", Faculty of Medicines, University of Monastir, Monastir, Tunisia
[3]Laboratory of Pathology, University Hospital Farhat Hached, Sousse, Tunisia
[4]Laboratory of Histology, Cytology and Genetics, Faculty of Medicines of Monastir, University of Monastir, Monastir, Tunisia
Email: karima.dardouri@yahoo.fr

How to cite this paper: Dardouri, K., Haouem, S., Gharbi, I., Sriha, B., Haouas, Z., El Hani, A. and Hammami, M. (2016) Combined Effects of Cd and Hg on Liver and Kidney Histology and Function in Wistar Rats. *Journal of Agricultural Chemistry and Environment*, **5**, 159-169.
http://dx.doi.org/10.4236/jacen.2016.54017

Received: May 4, 2016
Accepted: October 16, 2016
Published: October 19, 2016

Abstract

The present study was performed in order to discern the effects of combined exposure to cadmium and mercury on liver function and histopathological alterations in male adult Wistar rats. In the present investigation, cadmium (100 mg/l) and mercury (25 mg/l) were administered orally for 10 weeks separately or in combination. The rational for studying cadmium and mercury is that both of these metals are encountered frequently in the same contaminated areas. In liver, the activities of serum alanine aminotransferase (ALT) and aspartate amino tranferase (AST) increased significantly in the cadmium (Cd) and mercury (Hg) alone or in combination (Cd + Hg) compared to the control suggesting that both cadmium and mercury cause hepatotoxicity spatially when co-administrated. We noted an increase in serum lactate dehydrogenase (LDH) activity in Cd and combined Cd + Hg treated groups while it decreased in Hg treated group. There was no statistically significant change in the level of total bilirubilin. Serum urea concentration showed a significant increase in the Cd and Hg groups compared to the control group. However an increase in serum creatinine concentration was noted only in the combined treated rats showing that renal insufficiency is more serious in the co-exposed group. Light microscopic examination indicated severe histological changes in the two organs under Cd and mercury influence. Results of the present investigation clearly showed that mercury has profound effects of hepatic handling of cadmium (synergistic effect) as shown by histological and biochemical results. Moreover, we observed a antagonist effect between these two toxic metals on kidney markers such as urea.

Keywords

Cadmium Chloride, Mercuric Chloride, Hepatotoxicity, Kidney Damage, Rat

1. Introduction

Human activities play a major role in polluting the environment by toxic and carcinogenic metal compounds. There are evidences that these metals by accumulating contaminate water sources and food chain with their compounds. Hence, industrial pollution of the environment with metal compounds is becoming a serious problem. Unlike most organic pollutants, heavy metals are not degraded rather accumulate in the environment and food chain [1]. The interaction between cadmium (Cd) and mercury (Hg) can be a good example. In fact, cadmium and mercury have proved to be extremely toxic to mankind while their usage in various industries has increased rapidly in this century [2].

Cadmium contamination of environment is a subject of serious international concern since the metal is known to enter the food chain and undergo bioaccumulation, endangering human health [3]. After absorption, cadmium is circulated in blood, bound mainly to blood cells and albumin. It is primarily distributed to the liver and then redistributes progressively to the kidney as cadmium-metallothionein (Cd-MT). After distribution, approximately 50% of the total-body burden is found in the liver and kidney [4].

Mercury, identified thousands of years ago is one of the oldesttoxicants known [5]. Although in recent years, environmental and occupational exposures to mercury have been greatly reduced, this metal still remains a threat to human health from multiple sources: air, water and food [6]. Once absorbed, mercury distributes widely to all tissues. The principal target organs of the inorganic mercury are kidney and liver [7]. Previous studies have revealed that $HgCl_2$ caused histopathological and ultrastructural lesions in the liver evidenced by periportal fatty degeneration and cell necrosis [8].

In real life, the human population is exposed to complex mixtures of contaminants. So, the experimental work with combination of contaminants is more relevant on the human exposure than the work with a single substance. However, for cadmium and mercury there is, to our knowledge, no information regarding the effect of simultaneous intoxication with these two metals on liver and kidney function and structure. Consequently, this study was performed to elucidate the effects induced by cadmium and mercury on the liver and the kidney function and histology.

2. Materials and Methods

2.1. Animals and Treatment

The study comprised 19 male Wistar rats weighing 126 ± 11 g and randomly separated into four groups. The first group (n = 4) was the control group, consumed distilled water as drinking water. The second group (n = 5) received drinking water with cadmium chloride (100 mg/l). The third group (n = 5) rats exposed to mercury chloride (25 mg/l) in drinking water. The forth group (n = 5) was co-exposed to cadmium chloride (100 mg/l) *and* mercury chloride (25 mg/l) in drinking water.

After 10 weeks of treatment, the animals were weighted then sacrificed by decapitation under euther anesthesia. Blood was collected and centrifuged, and the serum was

conserved at −80˚. The livers were quickly excised, rinsed in ice-cold physiological saline to clean them of blood, weighted, finely minced in the same solution and homogenized (approximately 10% w/v) in a Potter Elvehjem homogenizer with a Teflon pestle. Liver homogenate were stored at −80˚.

2.2. Serum Biochemical Parameters

Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) activities, bilitubilin, creatinine, and urea concentrations were determined by automate (Synchrom CX9 PRO Beckman coulter).

2.3. Procedures for Histopathology

Immediately after sacrifice, a representative sample of liver and renal tissue from each individual was quickly removed and instantly fixed in 10% phosphate buffered formalin. Fixed liver samples were embedded in paraffin blocks and sections of 5 mm were prepared and stained with Haematoxylin (Al-Haematein)-Eosin (H&E).

2.4. Statistics

Data are exposed as means ± SE. statistical analysis was performed to compare treated groups with control group and the combined metal treatment group with the metal treatment groups using a one-way analysis of variance (ANOVA). Differences at $P \leq 0.05$ were considered statistically significant.

3. Results

3.1. Serum Hepatic Marker Enzymes Status and Bilirubin

Our long-term, combined exposure to metals caused changes in rat liver function (**Figure 1**). A combined effect was observed with ALT, which peaked in the Cd + Hg (differing significantly from the control and Hg groups), and with AST, whose levels in the Cd and Cd + Hg groups significantly differed from control group (**Figure 1(a)**) ($P < 0.05$).

LDH increased with co-exposure to metals, peaking in the Cd + Hg group (**Figure 1(b)**). Where as non-significant changes was observed in serum total bilirubin levelas compared to control group (**Figure 1(c)**).

3.2. Assessement of Kidney Function Markers

Figure 2 demonstrates the recovery pattern by alone and combination therapy of Cd and Hg through some biochemical markers of kidney function. The release of urea was increased in the circulation after acute exposure of cadmium or mercury intoxication ($P < 0.05$). Where as no change was observed in combined metal treated group compared to the control group (**Figure 2(a)**).

Serum creatinine concentration in Cd treated rats and combined Cd + Hg rats werehighercompared to the control group whereas itwas found to be decreased in Hg treated group (**Figure 2(b)**) ($P < 0.05$).

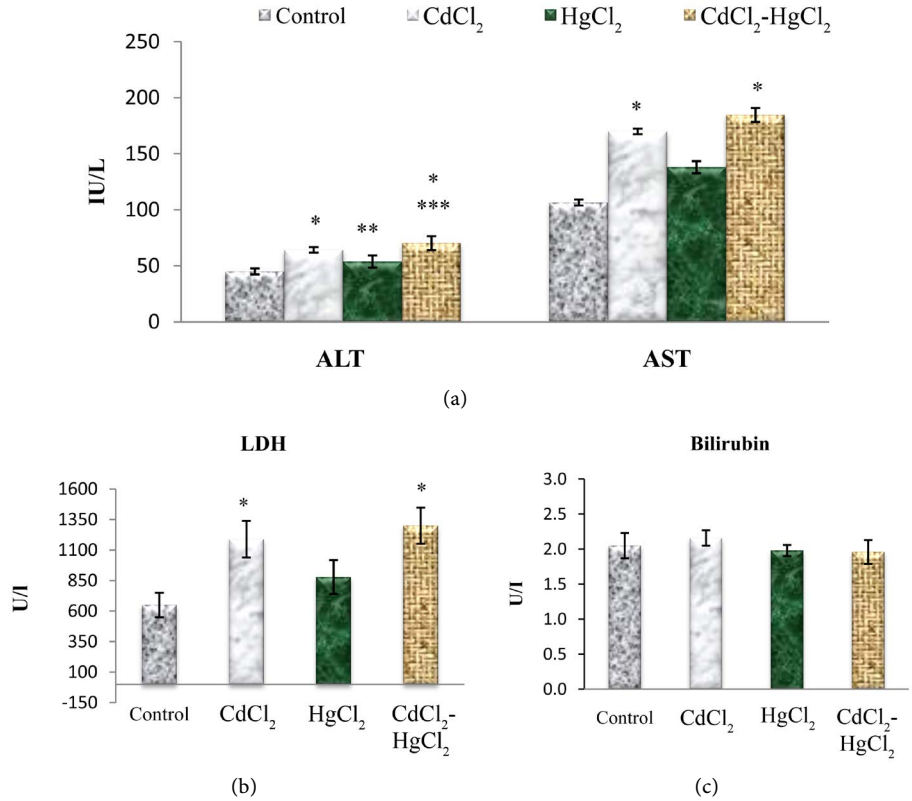

Figure 1. Effect of cadmium and mercury alone or in combination on the levels of plasma ALT and AST (a), LDH (b), and Bilirubin (c) in control and experimental rats. Each point represents the mean value ± SE. **P* < 0.05: Student's t-test, significant levels shown for difference between control and treated groups. ***P* < 0.05: Student's t-test, significant levels shown for difference between ratstreated with Cd and Hg alone or Cd + Hg. ****P* < 0.05: Student's t-test, significant levels shown for difference between ratstreated with Hg and Cd + Hg.

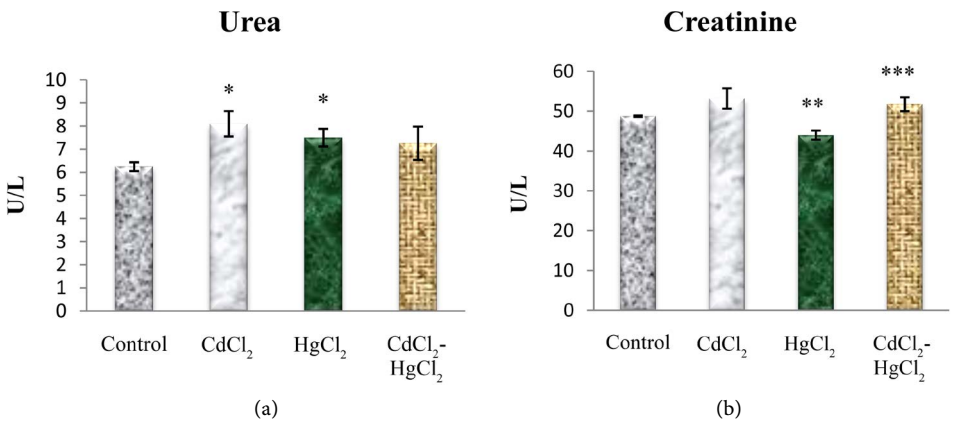

Figure 2. Effect of cadmium and mercury alone or in combination on the levels of plasma creatinine and urea in control and experimental rats. mean ± SE for each group. Experiment. Values are mean ± SE for 5 rats in each group. **P* < 0.05: Student's t-test, significant levels shown for difference between control and treated groups. ***P* < 0.05: Student's t-test, significant levels shown for difference between ratstreated with Cd and Hg alone or Cd + Hg. ****P* < 0.05: Student's t-test, significant levels shown for difference between rats treated with Hg and Cd + Hg.

Depending on the parameter studied, the alterations in the indicators of kidney function were either significantly related to the intake of cadmium or mercury, or were a result of interaction effect between the two metals.

3.3. Histopathological Effects on the Liver and Kidney

Administration of Cd or Hg alone caused moderated destraction of the histology of liver and kidney of the male Wistar rats compared with the rats co-exposed to Cd + Hg which shows more toxicity effects.

3.3.1. Histological Effects on the Liver

At light microscopical level the liver of the control animals is found to be composed of a continuous compact field of hepatacytes interspersed with blood sinusoids and intermittent islands of connective tissues enclosing the bile ducts, venous and arterial vessels. The hepatocytes are radially organised in hepatic lobules, which in most cases are very tortuous and branched, with a large central vein. Close to the blood sinusoids, other structures are located like endothelial cells, bile canaliculi and reticular fibers (**Figure 3(a)**).

Treatment with Cd or Hg caused severe liver damage including sinusoidal dilatation of central vein, degenerated hepatocytes, focal necrosis, congestion of sinusoidal spaces, vacuolization, inflammatory cell infiltration, proliferation of kupffer cells and bile ductless (**Figure 3(b)** and **Figure 3(c)**) when compared with control liver (**Figure 3(a)**). After Cd + Hgco-exposure, the liver tissue was completely damaged and qualitative degenerative and necrotic changes were observed in almost all structures of the liver tissues (**Figure 3(d)**).

3.3.2. Histological Effects on the Kidney

Kidney of control group showed normal features of renal tubules and Bowman's capsules (**Figure 4(a)**) while in Cd exposed animals kidney (cortex) showed glomerular atrophy, dilatation of the Bowman capsule and degeneration of tubular cells with picnotic nuclei (**Figure 4(b)**). The $HgCl_2$ group illustrated necrosis of hepatocytes, vacuolization of the cytoplasm of the hepatocytes, moderate infiltration of lymphoides, vessel congestions and dilated sinusoidal spacesand increased pycnotic nuclei (**Figure 4(c)**). Rats treated withcadmium and mercury in combinaison rats showed local areas of quite severe tubular injury associated with glomerulus with a thickened capsule and a small area of edema (**Figure 4(d)**).

4. Discussion

Due to human activities, such as mining and smelting, metal pollution is becoming a major risk to many ecosystems. Among the pollution-producing metals, cadmium and mercury are regarded as non-essential elements, with no known physiological functions. They are extremely toxic to plants and animals, have a long half-life and are extremely persistent in the environment [9].

Through this study, we investigated some effects of simultaneous coexposure to

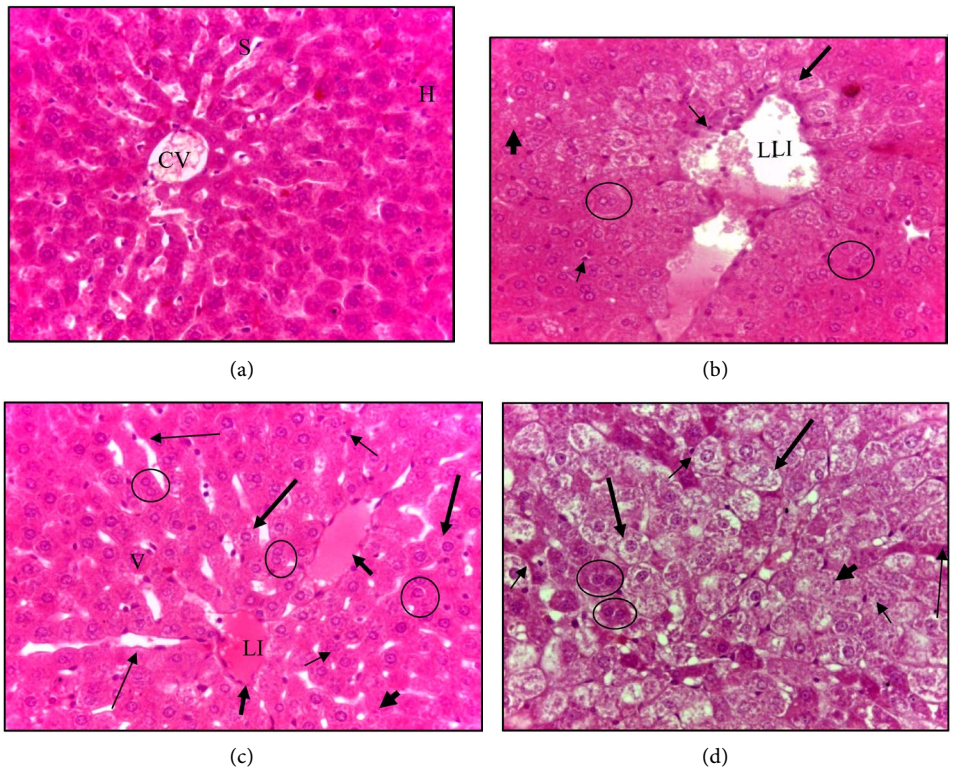

Figure 3. Liver section of rats: (a) control rats showing normal liver architecture with Central vein (CV), hepatocytes (H), Sinusoid (S) and kupffer cells (K) (H&E ×400); (b) cadmium-treated rats showing mild inflammation, sinusoidal dilatation and micro and macro steatosis. Arrows show the macro-and micro-vesicular fatty changes. (H&E, ×400); (c) mercury-treated rats showing hypertrophied hepatocytes (big bold arrows), vacuolization of the cytoplasm of the hepatocytes (V), moderate infiltration of lymphoides (LI), vessel congestions, increased Kupffer cells (short arrows), dilated sinusoidal spaces (short bold arrows) and increased pycnotic nuclei (round) (H&E, ×400); (d) cadmium and mercury-treated rats showing inflammation, necrosis, sinusoidal dilation, and degenerative changes with frequent apoptotic cells at the same time evidence of beginning regeneration is seen with the appearance of binucleated cells (round),. Arrow shows Kupffer cell hyperplasia highly cellular (H&E, ×400).

inorganic cadmium and mercury in the function of the liver and kidney and their histological structure in the rat. Both substances are hepato- and nephrotoxic, but they affect these organs in different ways [10].

Liver injury followed by Cadmium [3] and mercuryexposure [11] is well established dy the elevated levels of serum hepatic marker enzymes indicating the cellular leakage and loss of functional integrity of hepatic membrane architecture. High levels of aspartate transaminase (AST) and alanine transaminase (ALT) are the crucial parameters to detect liver damage [12].

In the present study, the results demonstrated that cadmium chloride alone increased biochemical parameters such as ALT and AST. Hwang *et al.* [13] have alsoobserved similar type of results in rat serum when treated with cadmium. Other studies also observed that the liver damage in Cdtreated mice was mainly due to the elevation of AST and ALT levels in serum [14].

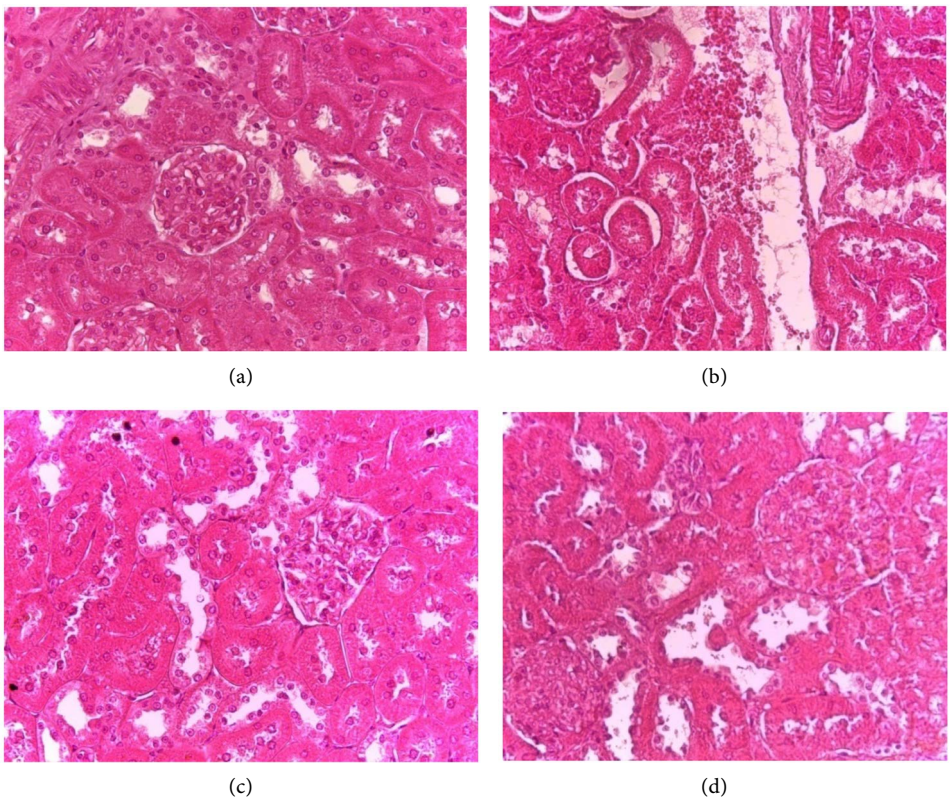

Figure 4. (a)-(d) Histological changes of kidney in each group. (a) Renal cortex of a control rat showing well developed glomerulus with normal tubular cells (H&E, 40×); (b) Renal cortex of cadmium-treated ratsshowing acute tubular necrosis and glomerular widening (H&E, 40×); (c) Renal cortex of mercury-treated rats showing tubular necrosis and glomerular swelling (H&E, 200×); (d) Renal cortex of cadmium and mercury-treated rats showing local areas of quite severe tubular injury associated with glomerulus with a thickened capsule and a small area of edema (H&E, 200×).

Further, mercury intoxication induces a significant elevation in serum AST and ALT activities [1] [15]. The increase in these enzymes may be due to cellular necrosis of hepatocytes, which causes increases in the permeability of cell [16]. In rats co-exposed to Cd and Hg, the serum level of AST and ALT were higher compared to individual metals treated rats.

LDH is an index of the cell damage including hepatotoxicity and the endothelial disruption in blood vessel. In the present study, in increased level of LDH was substantially detected in all experimental animals compared with the control group. The changes in serum LDH activity observed in the combined Cd and Hg exposed rats were higher than compared to individual metals treated rats.

This result is suggestive of the beginning of the cytolysis, which is a possible indication of membrane damage including the endothelial membranes in blood vessels. This disruption of endothelial membrane, directly or indirectly, as reported earlier by Järup *et al.* [17], induces the generation of reactive oxygen species in endothelial cells.

Cadmium is one of the most dangerous occupational and environmental toxins. It

accumulates in the human organism mainly in liver and kidneys, where it causes functional changes and then interstitial fibrosis [18] [19]. Cadmium chlorid ingestion at 100 ml/l for a period of 10 weeks produced a cumulative effect in rats. It has been repeatedly shown that the kidney is one of the tissues most sensitive to the toxic effects of cadmium. In fact, it has been reported that, after its absorption, Cd is taken up by the hepatocytes, and then from the liver it circulates in blood bound to metallothionein. The Cd-metallothionein complex (Cd-Mt), because of its small molecular size, is easily filtered through the glomerular membrane and taken up by renal tubular cells [20]. On its way through the kidney, this complex causes injury, mainly in the cortical region, reaching the proximal tubule and causing a gradual loss of the organ's function [21]. Moreover, these changes may be due to the accumulation of free radicals as the consequence of increased lipid peroxidation by free Cd ions in the renal tissues of Cd-treated rats [22].

In cadmium administered rats, the cadmium gets accumulated in the kidney, hence there is a defect in glomerular filtration. According to Oluwafemi *et al.* [23] rise in urea and creatinine values is an indication of renal-tubular damage due to cadmium induced nephrotoxicity. This study showed that the level of creatinine and urea gets increased in serum in Cdtreated ratswhen compared to the control rats.

Mercuric chloride treatment has been shown to cause a significant increase in serum creatinine and serum urea indicating an impaired renal function. The increased blood urea and creatinine are in agreement with the results obtained by Sheikh *et al.* [24] and Alam *et al.* [25] in male rats treated with mercury.

In the present study, the liver of mercury-treated rats showed congestion of hepato-portal blood vessels, congestion of central vein, edema in the portal tract and fatty changes indicating the toxic effect of mercuric chloride. It has been shown that in chronically diseased liver, some cells are activated by factors released by the liver hepatocytes and Kupffer cells, proliferate, and acquire the features of myofibroblasts, with or without the lipid droplets [26].

The histopathological lesions described avobe conform to the generally accepted classification of tubulo-interstitial nephritis. The pattern which emerges in the cadmium-exposed rat is that of a progressive nephrotic syndrome affecting first the tubules and surrounding blood vessels and connective tissue with secondarily a glomerular lesion involving the epithelial cells and the capillary loop [27].

5. Conclusions

Our experiment constitutes a new approach to the study of biological effects associated with environmental exposure to heavy metals. Our goal was to establish the effect of prolonged combined exposure to low environmental doses of Cd and Hg in the liver and the kidney histology and function indicators.

Our study has confirmed that Cd and Hg interact in environmental conditions. This interaction is mostly synergistic in liver as shown by histological and biochemical results. Moreover, we observed a antagonist effect between these two toxic metals on

kidney markers such as urea.

As our study uses an animal experimental model, its results are limited in terms of extrapolation to humans. Even so, our results are very important, as they help to better understand how low environmental doses of Cd and Hg interact in their biological effects on tissues.

Prolonged combined exposure to low-dose metals is present in many populations across the world and may contribute to the development of various diseases. Therefore, it is very important to continue and expand this kind of research, particularly of low-dose metal interaction.

References

[1] Jagadeesan, G. and Sankarsami, P.S. (2007) Hepatoprotective Effects of Taurine against Mercury Induced Toxicity in Rats. *Journal of Environmental Biology*, **28**, 753-756.

[2] Chul-Whan, C.M.D. (1987) A Study on Effect of Garlic to the Heavy Metal Poisoning of Rat. *Journal of Korean Medical Science*, **2**, 213-223. http://dx.doi.org/10.3346/jkms.1987.2.4.213

[3] Renugadevi, J. and Milton, P.S. (2010) Cadmium-Induced Hepatotoxicity in Rats and the Protective Effect of Naringenin. *Experimental and Toxicological Pathology*, **62**, 171-181. http://dx.doi.org/10.1016/j.etp.2009.03.010

[4] Akyolcu, M.C., Ozcelik, D., Dursun, S., Toplan, S. and Kahraman, R. (2003) Accumulation of Cadmium in Tissue and Its Effect on Live Performance. *Journal de Physique IV France*, **107**, 33-36. http://dx.doi.org/10.1051/jp4:20030236

[5] Mandava, V.R. and Chhunchha, B. (2010) Protective Role of Melatonin against the Mercury Induced Oxidative Stress in the Rat Thyroid. *Food and Chemical Toxicology*, **48**, 7-10. http://dx.doi.org/10.1016/j.fct.2009.06.038

[6] Brkljacic, J.J., Milutinovic, D.V., Dundjerski, J. and Matic, G. (2004) Mercury Inhibits Rat Liver and Kidney Glucocorticoid Receptor Hormone Binding Activity. *Cell Biology and Toxicology*, **20**, 171-182. http://dx.doi.org/10.1023/B:CBTO.0000029467.21231.12

[7] Sanchez, D.J., Belles, M., Albina, L.M., Sirvent, J.J. and Domingo, J.L. (2001) Nephrotoxicity of Simultaneous Exposure to Mercury and Uranium in Comparison to Individual Effects on These Metals in Rats. *Biological Trace Element Research*, **84**, 139-154. http://dx.doi.org/10.1385/BTER:84:1-3:139

[8] Waan, M.A.M. (2009) Effects of Mercury Exposure on Blood Chemistry and Liver Histopathology of Male Rats. *Journal of Pharmacology and Toxicology*, **4**, 126-131. http://dx.doi.org/10.3923/jpt.2009.126.131

[9] Cristina, O.V., Rubén, R.A., Francisca, F.D.C., Ramon, O.C. and Luis, E.H. (2005) Cellular Damage Induced by Cadmium and Mercury in Medicago Sativa. *Journal of Experimental Botany*, **56**, 2239-2251. http://dx.doi.org/10.1093/jxb/eri223

[10] Brzóska, M.M., Moniuszko, J.J., Marcinkiewicz, B.P. and Sawicki, B. (2003) Liver and Kidney Function and Histology in Rats Exposed to Cadmium and Ethanol. *Alcohol and Alcoholism*, **38**, 2-10. http://dx.doi.org/10.1093/alcalc/agg006

[11] Bharat, B.P., Atish, R., Soumik, A. and Shelley, B. (2010) Induction of Oxidative Stress by Non-Lethal Dose of Mercury in Rat Liver: Possible Relationships between Apoptosis and Necrosis. *Journal of Environmental Biology*, **31**, 413-416.

[12] Ford, E.J.H. and Boyd, J.W. (1962) Cellular Damage and Changes in Biliary Excretion in a Liver Lesion of Cattle. *Journal of Pathology*, **83**, 39-48.

http://dx.doi.org/10.1002/path.1700830106

[13] Hwang, D.F., Hour, J.L. and Cheng, H.M. (2000) Effect of Taurine on Toxicity of Oxidized Fish Oil in Rats. *Food and Chemical Toxicology*, **38**, 585-591. http://dx.doi.org/10.1016/S0278-6915(00)00052-1

[14] Hu, C.C., Yem, C.J., Jang, M.L., Liu, C.B., Chen, W.K. and Chung, C. (1991) Cadmium Induced Serum Biochemicals Changes in Subchromically Exposed Rats. *Chung Shan Medical Journal*, **2**, 97-102.

[15] In Sug, O., Datar, S., Koch, C.J., Shapiro, I.M. and Shenker, B.J. (1997) Mercuric Compounds Inhibit Human Monocyte Function by Inducing Apoptosis: Evidence for Formation of Reactive Oxygen Species, Development of Mitochondrial Membrane Permeability Transition and Loss of Reductive Reserve. *Toxicology*, **124**, 211-224. http://dx.doi.org/10.1016/S0300-483X(97)00153-4

[16] Youcef, N., Ahlem, B. and Sakina, Z. (2013) Amelioration of Mercuric Chloride Toxicity on Rat Liver with Argan Oil and Sodium Selenite Supplements. *International Journal of Pharma and Bio Sciences*, **4**, 839-849.

[17] Järup, L., Berglund, M., Elinder, C.G., Nordberg, G. and Vahter, M. (1998) Health Effects of Cadmium Exposure—A Review of the Literature and a Risk Estimate. *Scandinavian Journal of Work*, *Environment & Health*, **24**, 1-51.

[18] Kowalczyk, E., Jankowski, A., Niedworok, J., Smigielski, J. and Tyslerowicz, P. (2002) Effect of Long-Term Cadmium Intoxication on Selected Biochemical Parameters in Experimental Animals. *Polish Journal of Environment Studies*, **11**, 599-601.

[19] Yasuda, M., Miwa, A. and Kitagawa, M. (1995) Morphotometric Studies of Renal Lesions in Itai-Itai Disease: Chronic Cadmium Nephropathy. *Nephon*, **69**, 14-19. http://dx.doi.org/10.1159/000188354

[20] Jihen, E.H., Imed, M., Fatima, H. and Abdelamid, K. (2008) Protective Effects of Selenium (Se) and Zink (Zn) on Cadmium (Cd) Toxicity in the Liver and Kidney of the Rat: Histology and Cd Accumulalion. *Food and Chemical Toxicology*, **46**, 3522-3527. http://dx.doi.org/10.1016/j.fct.2008.08.037

[21] Haouem, S., Chargui, I., Najar, M.F., Sriha, B. and El-Hani, A. (2013) Liver Function and Structure in Rats Treated Simultaneously with Cadmium and Mercury. *Open Journal of Pathology*, **3**, 26-31. http://dx.doi.org/10.4236/ojpathology.2013.31005

[22] Renugadevi, J. and Prabu, S.M. (2009) Naringenin Protects against Cadmium Induced Renal Dysfunction in Rats. *Toxicology*, **256**, 128-134. http://dx.doi.org/10.1016/j.tox.2008.11.012

[23] Oluwafemi, A.O., Basiru, O.A., Babatunji, E.O., Adebola, B.O. and Olaide, I.O. (2014) Protective Effect of *Irvingia gabonensis* Stem Bark Extract on Cadmium-Induced Nephrotoxicity in Rats. *Interdisciplinary Toxicology*, **7**, 208-214.

[24] Sheikh, T.J., Patel, B.J., Joshi, D.V., Patel, R.B. and Jegoda, M.D. (2013) Repeated Dose Oral Toxicity of Inorganic Mercury in Wistar Rats: Biochemical and Morphological Alterations. *Veterinary World*, **6**, 563-567. http://dx.doi.org/10.5455/vetworld.2013.563-567

[25] Alam, M.S., Kaur, G., Jabbar, Z., Javed, K. and Athar, M. (2007) Eruca Sativa Seeds Possess Antioxidant Activity and Exert a Protective Effect on Mercuric Chloride Induced Renal Toxicity. *Food and Chemical Toxicology*, **45**, 910-920. http://dx.doi.org/10.1016/j.fct.2006.11.013

[26] Ibegbu, A.O., Ayuba, M., Animoku, A.A., Daniel, B., Sadeeq, A.A., Peter, A., Hamman, W.O., Umana, U.E. and Musa, S.A. (2014) Effect of Ascorbic Acid on Mercury-Induced Changes on the Liver in Adult Wistar Rats. *IOSR Journal of Dental and Medical Sciences*,

13, 10-16. http://dx.doi.org/10.9790/0853-131021016

[27] Aughey, E., Fell, G.S., Scott, R. and Black M. (1984) Histopathology of Early Effects of Oral Cadmium in the Rat Kidneys. *Environmental Health Perspectives*, **54**, 153-161. http://dx.doi.org/10.1289/ehp.8454153