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Research Article

Histological and Biochemical Effects of Mercury chloride on the Kidney of Adult wistar Rats

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Abstract: The study was conducted to assess the possible toxic effect of mercuric chloride (HgCl) on the histopathological and biochemical changes in the Wistar rats. Thirty-two adult wistar rats (weighing between 125 – 240g) of both sexes were separated randomly into 4 groups of eight animals each group. Group B, C and D, received 4g/kg, 6g/kg and 8g/kg body weight of mercury chloride solution respectively for 27 days while Group A was the control which received distilled water as contained in the experimental doses during the period. On the 28th day of administration, the rats in all the Groups were sacrificed including the control group by cervical dislocation. . The trunk of each rat was dissected and blood was collected from the heart for biochemical evaluation. The kidneys were removed, rinsed and weighed before fixing in 10% formol saline for histological studies using H&E staining techniques. Biochemical evaluation shows A significantly ($P < 0.05$) higher level of serum alanine amino transferase (ALT), Aspartate aminotransferase (AST) and alkaline phosphtase (ALP), creatinine and urea were recorded in treatment groups.. Gross morphological changes include congestion, severe haemorrhage, necrosis, degenerative changes in kidneys, depletion of lymphocyte in spleen,. It was notable that kidney was adversely affected in mercury-treated group.. Morphometric study shows insignificant difference in the organ weights between kidney weight of the control and mercury-treated rats **Conclusion:** Mercuric chloride (HgCl) exposure in wistar rat adversely affected the kidney morphology in adult wistar rats which result in a compromise of renal functions in treated wistar rats**.**

Keywords: *biochemical, haematological, mercuric chloride, morphological, toxicity, wistar rat*.

INTRODUCTION

Mercury (Hg) is a highly toxic metal that results in a variety of adverse neurological, renal, respiratory, immune, dermatological, reproductive and developmental disorders. Its wide industry related effects on human and animal biosystems have been well documented (W.H.O.1991) and general exposure to this biologically-active chemical agent has been shown to be exacerbated through contaminated water and food (Magos, L., & Clarkson, T.W .2006).

Nowadays, large populations worldwide are exposed to relatively low levels of Hg, especially via the use of pesticides in agriculture and of fluorescent light bulbs as well. In this context, Hg exists in a wide variety of physical and chemical states, each of which has specific characteristics for target organs. For example, exposure to Hg vapor as well as to organic Hg compounds specifically affects the central nervous system while kidneys, liver and gastrointestinal tract are mainly targeted by inorganic Hg compounds, such as mercuric chloride (Schurz, F. et al., 2000; Ghosh, A., & Sil, P.C. 2008). In this respect, multiple mechanisms have been proposed to explain the biological toxicity of HgCl2 by investigating the biochemical fate of various Hg forms (Aleo, M. F. 2005). Indeed, the Hg2+ form has shown a great affinity for endogenous biomolecules-associated thiol (-SH) groups (Clarkson, T.W. 1997) and it is invariably found attached to SH-containing proteins, small-molecular weight peptides (such as glutathione) and amino acids (such as cysteine), leading to a profound deterioration of vital metabolic processes (Şener, G. *et al.*, 2003; Wiggers GA. *et al.*, 2002). Consequently, the oxidative stress was strongly suggested as one of the crucial mechanisms in Hg-induced pathological aspects (Lund, B. O. *et al.*, 1993). However, biochemical parameters are still more indicative of early physiological changes

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following sub chronic and chronic Hg exposure (Wadaan, M. A. 2009).

Mercury is present in the earth crust and we are all exposed to some form of mercury through the air we inhale, the water we drink and the food we eat. Adding to that, mercury has been used in a wide range of products ranging through seed treatment, consumer applications, dental fillings and preservatives in vaccines. Thus, we are all exposed to mercury in some form and at some concentration. However, the Western world has transferred hazardous industries to countries characterized by less developed economies and industrial regulations. In these countries processes including potential mercury release will continue to be a challenge to both man and the environment. In addition to dental amalgam mercury has been of considerable use in laboratory instruments, which during the last decades have been replaced by other technologies.

Mercury and its compounds are currently used in a number of countries, especially in industrial countries such as Iran. Mercury is applied in several different aspects including Batteries (Bernardes, A. M. *et al.,* 2003)

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Acute inhalation exposure to mercury, at high concentrations, may induce respiratory distress including dyspnea. Chronic exposure may induce symptoms from the central nervous system (CNS) including tremors, delusions, memory loss and neurocognitive disorders.

Many of the signs and symptoms associated with slight poisonings will eventually disappear after the exposure ends. However, severe exposure may result in a lasting effect on brain function. Additionally, long-term exposure may also cause effects in kidney (Hursh, J. B. *et al.,* 1 976).

MATERIALS AND METHODS

Experimental Animals

Thirty-two adult presumably, healthy wistar rats (weighing between 125 – 240g) of either sex and water was given throughout the experimental and acclimatization used for this study. The rats were fed daily with normal rat chow purchased from Bovajay mill. The rat were given distilled water *ad libitum*

All the rats were carefully and routinely screened, inspected and confirmed to be healthy during the period of acclimatization. The animals were housed in serene and conducive cross- ventilated room in the Animal Holding of Anatomy Department, Ladoke Akintola University of Technology, Ogbomoso, Nigeria and treated in accordance with the 'Guide for the Care and Use of Laboratory animals' prepared and compiled by the National Academy of Science and published by the National Institute of Health (1985).

Reagents

The reagents used are:

- Mercury Chloride obtained from the Department of Biochemistry, Ladoke Akintola University of Technology, Ogbomoso.
- Distilled water from the Department of Food Science and Engineering, Ladoke Akintola University of Technology, Ogbomoso.
- Formol saline obtained from the department of Anatomy, Ladoke Akintola University of Technology, Ogbomoso.

Experimental design

The rats were separated randomly into 4 groups of eight animals each group. Group B, C and D, received 4g/kg, 6g/kg and 8g/kg body weight of mercury chloride solution respectively while Group A was the control which received distilled water as contained in the experimental doses during the period. On the 28th day of administration, the rats in all the Groups were sacrificed including the control group by cervical dislocation.

Collection of organs

The animals were sacrificed after the last dose of mercury chloride administration to them using the cervical dislocation method. The cervical regions of the wistar rats were dislocated to elicit a fracture at the cervical region thereby rendering the animals temporally unconscious. This method was carried out quickly so as to prevent the process of autolysis. The kidneys were carefully dissected out and fixed in 10% formol saline for routine tissue processing by microscope. Serial sections of 5um thick were obtained using a rotary microtome and the sections were subjected to histological staining following the method of Carleton (Carleton, B. *et al.,* 2005).

Statistical analysis

All data were expressed as mean ± SD of number of experiment (n= 4). The statistical analysis of the result obtained in this study was evaluated and tested for significance using t- test. If p – value of the t – test in less than 0.05 ($p<0.05$), then result is significant. If p – value of the t – test is greater than 0.05 ($p>0.05$), then that means that the result is not significant.

RESULTS

The body weights of the rats before, during and after administration are shown in **Table1.** There was no significant difference observed in rats treated with mercuric chloride compared to the control rats.

The organ weights of the rats are shown in **Table 2.** This shows no significant difference between the control and treated rats.

The serum analyses of biochemical parameters showed significant differences between treated and controls as shown in the groups are shown in **Table 3.** The effects of mercuric chloride on renal biochemical parameters (Alkaline phosphate, alkaline transferase Creatinine and urea) in the rat are shown in chat diagram **Plate 3.** Administration of mercuric chloride to the rats resulted in a statistically significant increase of ALP, creatinine and urea levels in mercury-treated group.

Table-1.Body Weights Before, During and After Mercuric Chloride administration

WEEKS	GROUP A	GROUP B	GROUP C
WEEK 0	159.7 ± 2.136	159.4 ± 7.745	171.9 ± 4.575
WEEK 1	151.6 ± 6.442	162.5 ± 6.682	165.9 ± 5.153
WEEK 2	151.1 ± 7.884	151.6 ± 10.680	165.0 ± 6.730
WEEK 3	166.7 ± 4.167	166.1 ± 8.929	172.8 ± 5.313
WEEK 4	173.3 ± 9.718	172.1 ± 13.090	180.6 ± 10.020

Table 2. Organs Weights after Mercuric Chloride Administration

GROUPS	LEFT KIDNEY	RIGHT KIDNEY
A	0.5367 ± 0.01476	0.4883 ± 0.028
B	0.5700 ± 0.0399	0.5171 ± 0.036
C	0.5625 ± 0.023	0.5063 ± 0.021
D	0.5388 ± 0.041	0.5138 ± 0.039

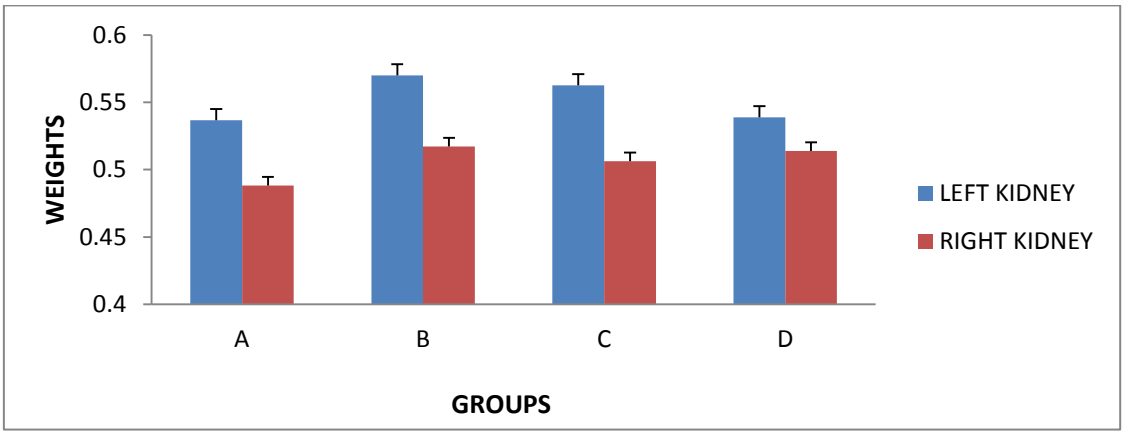

Fig.-1. This shows variation in the weight of the organs. Data are reported as mean ± SEM. There was no significant difference observed

BIOCHEMICAL ANALYSIS.

Table-3: .Effect Of Mercuric Chloride Administration on the Levels Ofalt, Ast, Alp, Crt and Urea.

	A	B	C	D
ALT	42.10 ± 1.77	54.56 ± 5.53	87.06 ± 7.78**	75.93 ± 9.09*
AST	178.00 ± 2.03	159.9 ± 13.34	187.5 ± 7.00	222.20 ± 11.84**
ALP	4.258 ± 0.73	7.358 ± 0.84*	6.133 ± 0.67	10.85 ± 1.35**
CRT	86.13 ± 6.37	103.0 ± 1.42*	120.5 ± 11.15*	131.8 ± 4.55**
UREA	6.705 ± 0.24	7.665 ± 0.16*	8.663 ± 0.50*	11.28 ± 0.88**

***Significantly different from the control**

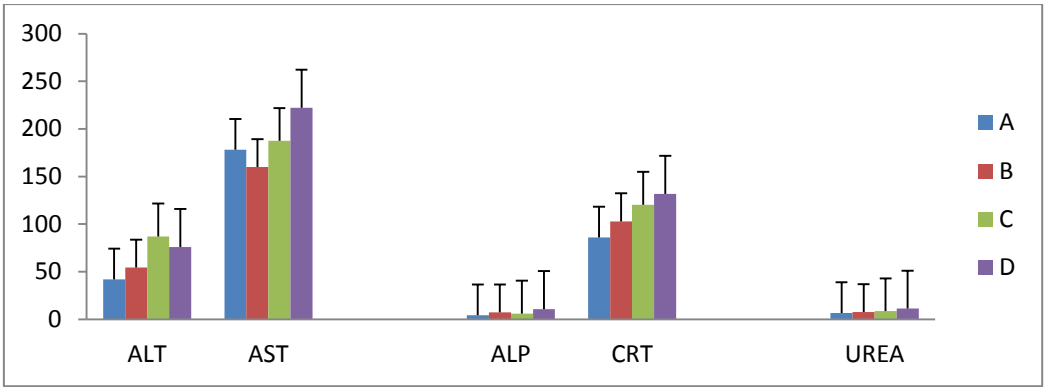

Fig.-2. This shows variation in serum analyzed concentration of the rats treated with mercuric chloride compared to the control rats. Data are reported as mean ± SEM and significant difference ($p \leq 0.05$) was observed.

HISTOLOGICAL OBSERVATIONS

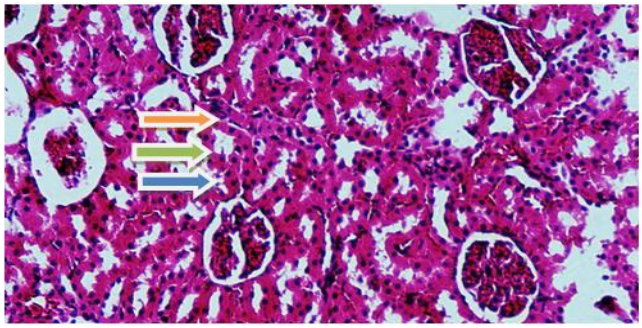

Plate1a (H&E X100) Photomicrograph of a normal histology of kidney of the control rat (group A) showing normal glomerulus and Bow's man capsule (green arrow) intact with simple cuboidal epithelial lining. The proximal capsule (blue arrow)and distal convoluted tubule (orange arrow) are also normal.

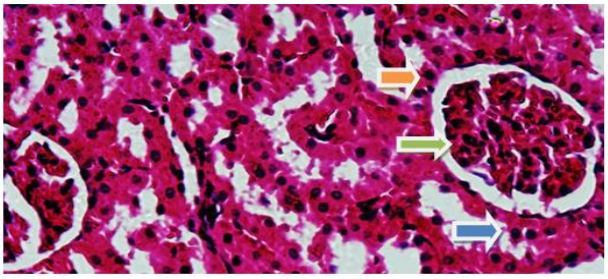

Plate1b (H&E X400) Photomicrograph of a normal histology of kidney of the control rat (group A) showing normal glomerulus and Bow's man capsule (green arrow) intact with simple cuboidal epithelial lining. The proximal capsule (blue arrow) and distal convoluted tubule (orange arrow) are also normal.

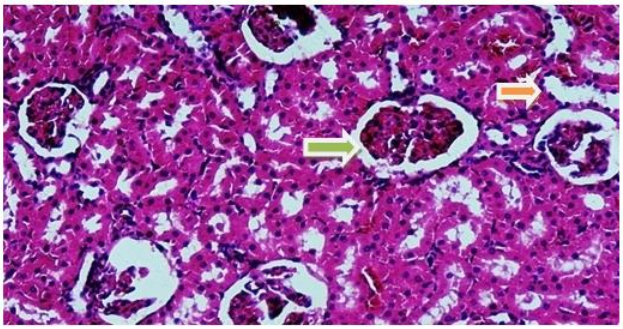

Plate2a (H&E X100) Photomircograph showing a section of kidney (Group B) given 4mg/kg of HgCl2 throughout the experiment with an enlargement of the glomerulus and some part of the Bow's man capsule (green arrow) are affected. There is also an enlargement of the collecting tubules(orange arrow).

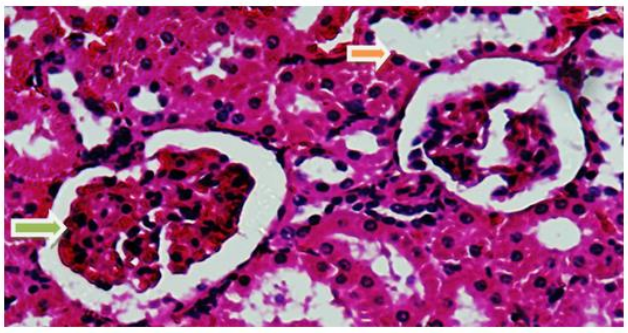

Plate2b (H&E X400)Photomircograph showing a section of kidney (Group B) given 4mg/kg of HgCl2 throughout the experiment with an enlargement of the glomerulus and some part of the Bow's man capsule(green arrow) are affected. There is also an enlargement of the collecting tubules (orange arrow).

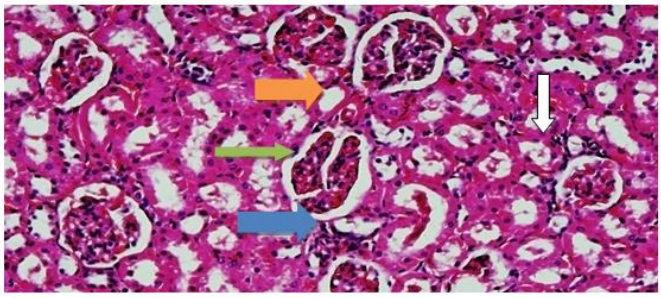

Plate3a (H&E X100)Photomicrograph showing a section of kidney (Group C) given 6mg/kg of HgCl2 throughout the experiment with increased lipid content(white arrow), enlargement of the collecting tubules. The cell is highly eosinophilic. There is haemorrhage throughout the surface of the tissue. There is no damage done to the glomerulus , Bow's man capsule (green arrow), proximal (blue arrow) and distal (orange arrow) convoluted tubules.

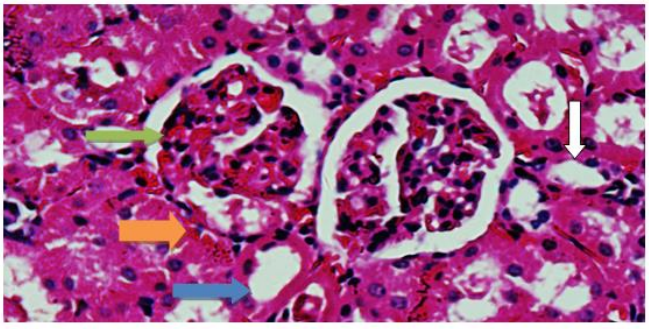

Plate3b (H&E X400) Photomicrograph showing a section of kidney (Group C) given 6mg/kg of HgCl2 throughout the experiment with increased lipid content(white arrow), enlargement of the collecting tubules. The cell is highly eosinophilic. There is haemorrhage throughout the surface of the tissue. There is no damage done to the glomerulus , Bow's man capsule (green arrow), proximal (blue arrow) and distal (orange arrow) convoluted tubules.

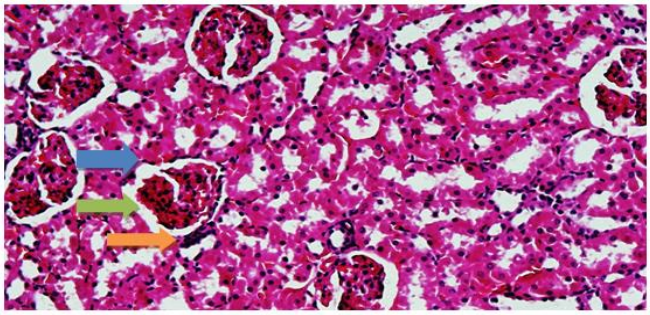

Plate4a (H&E X100) Photomicrograph showing a section of kidney (Group D) given 8mg/kg of HgCl2 throughout the experiment with the presence but reduce haemorrhage. There is presence of low adipose cell. The glomerulus, Bow's man capsule (green arrow), proximal (blue arrow) and distal (orange arrow) are intact.

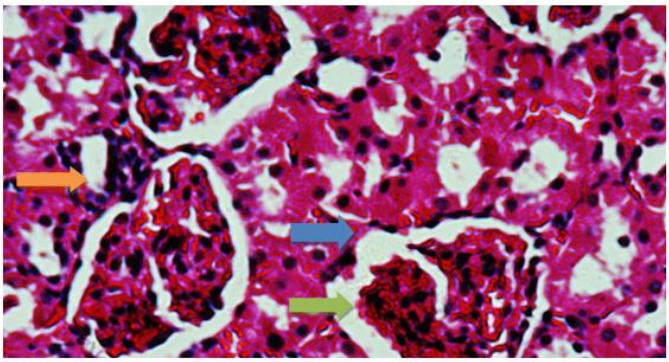

Plate4b (H&E X400) Photomicrograph showing a section of kidney (Group D) given 8mg/kg of HgCl2 throughout the experiment with the presence but reduce haemorrhage. There is presence of low adipose cell. The glomerulus, Bow's man capsule (green arrow), proximal (blue arrow) and distal (orange arrow) are intact.

DISCUSSION

Mercuric chloride (HgCl2), once absorbed is distributed in all tissues and low fractions have been shown to easily cross the brain-blood barrier and the placenta. However, the kidney was considered as the primary target organ, in which HgCl2 is intensively accumulated following chronic exposure (W.H.O.1991).

The present investigation was carried out in order to determine the biochemical repercussions of daily HgCl2 administration in Wistar rats. Indeed, It was revealed here that continuous oral administration of HgCl2 for 30 days, may have adversely affect specific biochemical parameters, by which mainly hepatotoxicity and nephrotoxicity are evaluated.

There was no observed significant difference in organ weights between the treated and controls after mercuric administration.

The alkaline phosphatase (ALP) is a well-known indicator of multiple toxicity cases, including those related to hepatic and renal dysfunctions. This enzymatic parameter is widely thought to be one of the most sensitive markers of Hg toxicity (Kumar, M. *et al.*, 2005). The study showed a significant increase in plasma ALP and ALT concentrations in mercury – treated rats.

In this respect, ALP activity alterations may result from Hg effect primarily on hepatic and renal tissues. Simultaneously, plasma urea concentration revealed a significant increase in this experiment. The photomicrographs of the treated rats compared with the control rats show an enlargement of the glomerulus, some part of the Bow's man capsule and the collecting tubules. The cell was highly eosinphilic. There was presence of haemorrhage throughout the surface of the tissue.

This result is undoubtedly related to acute and persistent renal injuries, thus confirming that the kidneys are very sensitive to Hg exposition (Agarwal, R., & Behari, J. R. 2007).

HgCl2-induced nephropathy has been reported to be associated with glucose reabsorption and urea excretion alterations, leading to hypoglycemia (due to glucosuria) and uremia as well as to hypoproteinemia (due to proteinuria) that indicates glomerular damage (Al-Madani, W. A. *et al.*, 2016).

Furthermore, acute administration of HgCl2 causes toxic effects on kidney and this damage was associated with the increase in serum ALP activity and urea (Lund, B. O. *et al.*, 1993).

In the present, the increase in creatinine might be due to renal damage. The observed increase in plasma and urine creatinine level is therefore a likely indication of glomerular dysfunction in rats exposed to mercuric chloride for 28 days which was similarly reported by previous investigators (Joshua, R.E. *et al.*, 2011; Gray, J.A. & Kavlock, R. J. 2012).

The study concluded that exposure of wistar rats to mercury chloride resulted in a dose-dependent histopathological changes with alteration of some biochemical parameters which ultimately may impair renal functions in the treated rats.

RFERENCES

1. WHO World Health Organization. (1991).Inorganic Mercury,Environmental Geneva, Health Criteria,-118.
2. Magos, L., & Clarkson, T.W .(2006). Overview of the clinical toxicity ofmercury. Ann. Clin. Biochem, (43), 257-268.
3. Schurz, F., Sabater-Vilar, M., & Fink-Gremmels, J. (2000). Mutagenicity ofmercury chloride and mechanisms of cellular defence: the role ofmetal-binding proteins. Mutagenesis, 15(6), 525-530.
4. Ghosh, A., & Sil, P.C. (2008). A protein from *Cajanusindicus*Spreng protectsliver and kidney against mercuric chloride-induced oxidative stress

5. Aleo, M. F., Morandini, F., Bettoni, F., Tanganelli, S., Vezzola, A., Giuliani, R., ... & Apostoli, P. (2002). In vitro study of the nephrotoxic mechanism of mercuric chloride. *La Medicina del lavoro*, *93*(3), 267-278.
6. Clarkson, T.W. (1997). The toxicology of mercury. Crit. Rev. Clin. Lab.Sci, (*34), 369-403*
7. Şener, G., Şehirli, A. Ö., & Ayanogˇlu-Dülger, G. (2003). Melatonin Protects against mercury (II)-induced oxidative tissue damage in rats. *Pharmacology & toxicology*, *93*(6), 290-296.
8. Wiggers GA, Peçanha FM, Briones AM, Pérez-Girón JV, Miguel M, 2002;110(Suppl. 5):851–4.
9. Lund, B. O., Miller, D. M., & Woods, J. S. (1993). Studies on Hg (II)-induced H2O2 formation and oxidative stress in vivo and in vitro in rat kidney mitochondria. *Biochemical pharmacology*, *45*(10), 2017-2024.
10. Wadaan, M. A. (2009). Effects of mercury exposure on blood chemistry and liver histopathology of male rats. *J Pharmacol Toxicol*, *4*(3), 126-131.
11. Bernardes, A. M., Espinosa, D. C. R., & Tenório, J. A. S. (2003). Collection and recycling of portable batteries: a worldwide overview compared to the Brazilian situation. *Journal of power sources*, *124*(2), 586-592.
12. Hursh, J. B., Clarkson, T. W., Cherian, M. G., Vostal, J. J., & Mallie, R. V. (1976). Clearance of mercury (Hg-197, Hg-203) vapor inhaled by human subjects. *Archives of Environmental Health: An International Journal*, *31*(6), 302-309.
13. Carleton, B., Foerster, V., Warren, L., & Smith, M. A. (2005). Post-Marketing Pharmacosurveillance in Canada: A Background Paper Prepared for the Working Conference on Strengthening the Evaluation of Real World Drug Safety and Effectiveness [Health Canada, 2005].
14. Kumar, M., Sharma, M. K., & Kumar, A. (2005). Spirulina fusiformis: a food supplement against mercury induced hepatic toxicity. *Journal of health science*, *51*(4), 424-430.
15. Agarwal, R., & Behari, J. R. (2007). Effect of selenium pretreatment in chronic mercury intoxication in rats. *Bulletin of environmental contamination and toxicology*, *79*(3), 306-310.
16. Al-Madani, W. A., Siddiqi, N. J., & Alhomida, A. S. (2009). Renal toxicity of mercuric chloride at different time intervals in rats. *Biochemistry Insights*, *2*, BCI-S2928.
17. Joshua, R.E., Evangelos, A.L., Jacob, D.P., Peter, C.L., & Walter, C.P. (2011). A novel method for the evaluation of proximal tubule epithelial cellular necrosis in the intact rat kidney *Journal of Applied Pharmaceutical Science, 1 (10),150-153*
18. Gray, J.A. & Kavlock, R. J. (2012). Toxic effect of mercury. *J.Pharmacol. Exp. Ther. 242 (1), 212-216.*