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Original Article

Deleterious effects of mercuric chloride on blood biochemistry, liver and kidney histology in female albino mice

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A B S T R A C T

Mercury is a widespread environmental and industrial pollutant, which induces severe effects on human and animal bio-systems. The experiment was designed to investigate the effects of different concentrations of mercuric chloride ($Hgcl_2$) on the hemato-biochemical and histological changes of liver and kidney in female Swiss Albino mice. A total of 24 numbers of mice of 28-30 days' age were randomly assigned into 4 equal groups (n=6) as A, B, C, D. Group A was kept as control. Mice of group B, C and D were provided $Hgcl_2$ at a dose rate of 5 mg, 10 mg and 15 mg per kg body weight in distilled water daily for 12 weeks respectively. At the end of the experimental period, blood and tissues were collected and processed for hematological, biochemical and histopathological examination. Results showed that $HgCl_2$ treated mice caused a significant decreased in weight gain even treated with low doses of $HgCl_2$. Total Erythrocyte Count (TEC), hemoglobin (Hb), Packed Cell Volume (PCV), Mean Corpuscular Volume (MCV) and Mean Corpuscular Hemoglobin (MCH) were significantly decreased in $HgCl_2$ treated mice than control one. Values of aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine, serum total cholesterol, low density lipoprotein (LDL), triglycerides (TG) glucose were significantly increased and alkaline phosphatase (ALP), high density lipoprotein (HDL) were decreased in mice treated with different concentrations of $HgCl_2$ compared with control group. Histo-pathological study showed that atrophy of the glomerulus was found in the kidney and presence of vacuoles and dilatation of sinusoidal spaces in the liver of $HgCl_2$ treated mice. In conclusion, this research suggested that $HgCl_2$ exerts deleterious impacts including association with hepatic and renal injuries.

Introduction

Mercury (Hg) is a toxic metal that is widely distributed in the environment and growing concern as a global pollutant because that causes potential health problems (Al-Othman *et al*., 2011). This non-biodegradable heavy metal induced potential effects at low doses and discharged by industries (Akinyeye and Okorie, 2012), agriculture and urban communities, reaches the environment and poses potential health hazard to livestock, wild life and human beings (Hounkpatin *et al.,* 2012). Mercury has been used in the manufacture of electrical equipment, scientific instruments, explosives, insecticides, batteries, antiseptic, disinfectant, preservative and as a photographic fixative. Moreover, previous studies revealed that common Indian food items like fish, prawn, cabbage and amaranthus have been found to contain high levels of Hg (Panda *et al*., 1992). Mercuric chloride ($HgCl_2$) is one of the most toxic forms of mercury because it easily forms organomercury complexes with proteins (Wargovich *et al.,* 2001). Exposure to inorganic mercury may occur by ingestion, inhalation and dermal contact that results in a variety of adverse neurological, respiratory, dermatological, reproductive, visual, metabolic, immunological, carcinogenicity and/or damage to kidney function and developmental disorders (Mergler *et al.,* 2007). Mercuric chloride is absorbed into blood stream: inorganic mercury combines with proteins in the plasma or enters the red blood cells (El-Shenawy and Hassan, 2008). The kidney, liver, gastrointestinal system, and central nervous system are the main target sites of mercury toxicity. The liver is a major site of metabolism for mercury and excreted by kidneys. It

accumulates in the liver and elevates liver malonaldehyde level resulting in hepatotoxicity (Lin *et al.*, 1996) that showed significant increase in liver enzymes and damage of liver cells (Sheikh *et al.*, 2011). $HgCl_2$ caused histopathological and ultra-structural lesions evidenced by fatty degeneration and cell necrosis in the liver and loss of brush border and cell loss in the cortex, tubular necrosis with casts in the kidney (Stacchiotti *et al.*, 2003). Mercury can cause biochemical damage to tissues through diverse mechanisms such as lipid peroxidation (Huang *et al.*, 1996), formation of reactive oxygen species (Woods *et al.*, 1990), altering protein synthesis and via binding to thiol groups (Zalups, 2000). However, biochemical parameters are still more indicative of early physiological changes following subchronic and chronic Hg exposure (Wadaan, 2009). The study was designed to evaluate some hematobiochemical markers with histological changes of liver and kidneys following oral exposure of varying concentrations of $HgCl_2$ in female albino mice.

Materials and Methods

Experimental animals

The mice used for this study were purchased from ICDDR'B, Dhaka. They were reared in a compartmentalized square wooden cages wrapped with wire mesh under controlled conditions of temperature (26-30) °C and relative humidity of 70-80% with natural day light.

Experimental chemical

Mercuric chloride was purchased from BDH Chemicals Ltd., Poole, England and was dissolved in distilled water (vehicle) as stock and the stock solution was made before adding to the mice.

Experimental design

The experiment was conducted in the Department of Physiology, Bangladesh Agricultural University, Mymensingh, from the 1st February to the 25th April, 2018 (12 weeks). A total of twenty-four Swiss Albino mice (*Mus musculus*), age of 25-28 days was used for this study. After 7 days, the mice were randomly assigned into 4 equal groups (n=6) as A, B, C, D. All groups were supplied with standard mice pellet. Group A was kept as untreated control. Mice of the group B, C and D were treated with mercuric chloride in drinking water at a dose rate of 5 mg, 10 mg and 15 mg /kg body weight respectively. Mercuric chloride was dissolved in distilled water.

Management practices

The mouse cages were kept on a well-ventilated experimental animal room. Feeds were kept in air tight poly packed sac to prevent spoilage. Mice cage were cleaned regularly and proper hygienic and sanitary measures were also adopted during the experimental period. Feces were removed regularly.

Body weight

Initial body weight of each mouse was measured with the help of an electric balance. Body weight was taken at 0 day (starting day of experiments) and then 3 weeks' intervals until end of experiments. Body weight gain was calculated as weight gain (g) = mean final body weight (g) minus mean initial weight (g). Percent body weight gain was calculated as percent weight gain (g) equal to mean final weight (g) minus mean initial weight (g) divide by mean initial weight X 100

Blood sample collection

Blood samples were collected directly from heart (Sarker *et al.*, 2019). The mice were kept fasting overnight. Then the mice were placed an airtight container one by one containing diethyl ether presoaked cotton. The unconscious mice were taken out and the blood was collected directly from heart by a sterile syringe. About 1.5 ml blood was collected and transferred half of blood into anticoagulant (sodium citrate) containing eppendorf tube and the remaining half of blood was transferred to another tube without anticoagulant for serum preparation. The blood containing tubes were placed in upright slanting position at room temperature for 6 hours. They were then incubated overnight in the refrigerator (4ºC). The serum samples were separated by centrifugation and collected by using 200 µl pipettes. Serum samples were stored in capped tube at -20ºC for biochemical analysis.

Hematological studies

Hemoglobin (Hb), Packed Cell Volume (PCV), Total Erythrocyte Count (TEC) and RBC Indices (MCV, MCHC and MCH) were performed as per standard method (Ghai, 2013). The RBC indices provides information about the hemoglobin content and size of erythrocytes that can be determined by calculating the values obtained from Total Count of RBC in million/cubic mm, Hb concentration in g% and PCV in %.

Serum biochemical studies

The serum biochemical profile like total serum cholesterol, triglycerides (TG), high density lipoprotein (HDL cholesterol), low density lipoprotein (LDL cholesterol), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), creatinine and glucose were performed at Professor Dr. Mohammad Hussain Central Laboratory in Bangladesh Agricultural University, Mymensingh-2200 colorimetrically using Humalyzer 2000 (Human type, Germany) following instructions provided by Sarker *et al.*, 2019.

Histopathology

The liver and kidney from each group of mice were collected after complete removal of blood by perfusion with phosphate buffered saline and kept in 10% neutral buffered formalin for 15 days. The well-fixed tissues were processed, sectioned and stained with Hematoxylin and Eosin (H & E) for histo-pathological study as per standard procedure (Robert *et al.*, 2014) in collaboration with the Department of Pathology, Mymensingh Medical College, Mymensingh. The stained slides were observed under Optka Vision Lite 21 and photographs of the characteristic findings were put down.

Statistical analysis

All data were subjected to statistical analysis using one-way ANOVA with post-hoc Tukey's test as per method (Steel and Torrie, 1980). Statistical analysis was performed using Graph Pad Prism 8 software.

Results and Discussion

This study was conducted on Swiss albino mice to observe the effects of mercuric chloride ($HgCl_2$) on body weight gain, hematological parameters, serum biochemistry and histopathological changes of liver and kidney in female mice.

Effect of $HgCl_2$ on body weight gain in mice

Average body weight and percentage of body weight gain in female mice are shown in table 1. It appears that there was significantly changed in the body weights of $HgCl_2$-treated female mice. The body weight was slowly increased in treated groups upon advancement of time. The percentage of the growth rate were decreased ($p<0.05$) in different treated groups compared to the values observed in control group**.** In toxicological studies, reduction in body weight is used as an indicator for the deterioration of general health status and changes of organ weight are one of the important criteria for evaluation of organ toxicity. In this study, HgCl2-treated mice exhibited significantly lower body weight gain than the control animals, as reported earlier by Mahboob *et al.* (2001). Weight loss is known to be the basic aspect of Hg toxicity and has been attributed to reduced food intake by animals (Jaiswal *et al.*, 2013).

Table 1. Comparison of average body weight in different treatment groups of female mice at 12th week.

Treated Group	Initial body weight (g)	Body weight (g) and percentage of growth rate			
		3rd week	6th week	9th week	12th week
Group A (Control)	36.40 ± 1.14	38.80 ± 1.31 (6.59%)	41.20 ± 1.31 (13.18 %)	43.60 ± 1.52 (19.78%)	46.20 ± 1.483 (26.92%)
Group B (5 mg Hg)	36.60 ± 1.67	37.60 ± 1.67 (2.73 %)	38.80 ± 1.78* (6.01 %)	39.80 ± 1.79* (8.74%)	40.80 ± 1.789* (11.47%)
Group C (10 mg Hg)	36.60 ± 1.14	37.40 ± 1.34 (2.18 %)	38.60 ± 1.14* (5.46 %)	39.60 ± 1.14* (8.19%)	40.40 ± 0.894** (9.28%)
Group D (15 mg Hg)	36.60 ± 1.10	37.20 ± 1.30 (1.63%)	38.20 ± 0.83* (4.37 %)	39.20 ± 0.87* (7.1%)	39.80 ± 0.837* (8.74%)

*significant at 5% level ($p<0.05$), ** significant at 1% level ($p<0.01$), NS=not significant

Effect of $HgCl_2$ on hematological parameters in mice

Effects of $HgCl_2$ on blood parameters in different groups of mice treated with $HgCl_2$ at the dose rate of 5 mg/kg, 10 mg/kg and 15 mg/kg of body weight are shown in Table 2. In this trial, the values of blood parameters altered significantly in the treated groups The hemoglobin concentration, TEC and PCV values were decreased in group B, C and D at $p<0.05$ compared to the value observed in control group. The present findings are supported by the findings of Guedenon *et al.* (2012) which demonstrated that the decrease in red blood cell can attributed to the decrease iron within erythrocytes or its content of hemoglobin and this causes decrease carrying capacity of oxygen by blood. According to Brandao *et al.* (2008) mercury exposure caused a reduction in the erythrocyte count. It is conceivable that heavy metals might have suppressed the activity of these hematopoietic tissues. The reduction in Hb can be probably due to the production of reactive oxygen species (ROS) under the influence of mercuric chloride. Hounkpatin *et al.* (2012) and Al-Salhen (2014) observed decrease Hb concentration could be due to either an increase in the rate at which Hb is destroyed or a decrease in the rate of Hb synthesis. This decrease in hemoglobin was also found in rabbits poisoned by lead (Bersenyi *et al.*, 2003) in rats exposed to cadmium chloride (Ognjanović *et al.*, 2003). Mercury could inhibit heme synthesis of red blood cells and cause anemia signs described by Bottomley and Muller-Eberhard (1998).

The values of MCV changed significantly in the treated groups. MCV was decreased in mice of B, C and D ($p<0.05$). In case of MCHC value, the amplitude of increase in MCHC value was found significantly ($p<0.05$) among mice of group C and D ($p<0.05$) compared to the value observed in control group (Table 2). The effects of $HgCl_2$ on MCH varied significantly in the treated groups. MCH showed insignificantly change in group B while decreased in group C and group D ($p<0.05$). The present findings are in agreement with previous reports (Al-Attar, 2011) which reported a significant increase in immature red blood cells in the fish Cyprinus carpio exposed to mercury. The decrease in MCH could also be due to the increased number of immature red blood cells with lower hemoglobin content released into blood circulation to compensate for the red blood cells depletion. A similar suggestion was proposed by Wepener, 1990 which explain the decrease in MCH of the fish Tilapia sparmanii treated with heavy metals.

Table 2. Effect of $HgCl_2$ treatment on hematological parameters in female mice at 12th week.

Parameters	Control	5 mg Hg	10 mg Hg	15 mg Hg
Hb (g %)	8.86 ± 0.261	8.00 ± 0.316	7.18 ± 0.228*	6.48 ± 0.228*
TEC (10^6/uL)	7.74 ± 0.105	7.14 ± 0.190	5.15 ± 0.096*	5.05 ± 0.088*
PCV (%)	32.80 ± 2.280	28.60 ± 2.408	23.20 ± 1.643*	21.20 ± 1.924*
MCV(fl)	34.59 ± 2.496	33.45 ± 2.439	30.41 ± 1.949	28.05 ± 2.364
MCHC (%)	30.06 ± 1.784	32.67 ± 1.865^{NS}	33.19 ± 1.600*	35.44± 2.118**
MCH (pg)	11.05 ± 0.195	10.89 ± 0.210^{NS}	10.73 ± 0.178*	10.61 ± 0.197**

*significant at 5% level ($p<0.05$), ** significant at 1% level ($p<0.01$), NS=not significant

Effect of $HgCl_2$ on biochemical parameters in mice

Lipid profile

Lipid profile e.g. Cholesterol, HDL-c, LDL-c and TG in different groups of mice treated with different concentration of $HgCl_2$ are presented in Table 3.

The effects of $HgCl_2$ on lipid profile varied significantly in the treated groups. $HgCl_2$ induced significant elevation of cholesterol, triglycerides and LDL-C. Moreover, HDL-C level decreased significantly in treated groups. The increased level of LDL-C and decreased HDL-C in Hg group reflected the abnormalities in lipid metabolism. The present findings are nearly similar with the previous findings (Bashandy *et al.*, 2011; Skoczynska *et al.*, 2009) which stated that mercury reflected the abnormalities in lipoprotein metabolism which may result in high level of cholesterol and development of atherosclerosis.

Table 3. Effect of $HgCl_2$ on serum lipid profile in female mice at 12th week.

Parameters	Cholesterol (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	TG (mg/dL)
Control	244.22 ± 2.449	48.26 ± 1.854	164.70 ± 1.166	156.29 ± 4.741
5mg Hg	288.96 ± 4.345*	44.61 ± 3.246^{NS}	207.00 ± 1.741*	186.67 ± 2.932*
10 mg Hg	307.10 ± 3.780*	42.33 ± 2.478*	222.76 ± 1.027*	210.03 ± 3.397*
15 mg Hg	329.79 ± 3.630*	40.26 ± 1.995*	242.81 ± 1.208*	233.52 ± 3.204*

*significant at 5% level ($p<0.05$), ** significant at 1% level ($p<0.01$), NS=not significant

Liver and kidney function tests along with serum glucose status

Liver and kidney function test including ALT, AST, ALP, creatinine and total protein in different groups of mice treated with different doses of HgCl2 are shown in Table 4. The values of ALT and AST altered significantly in the treated groups compared to that in control group. The values were significantly increased ($p<0.05$) among mice of group B, C and D whereas the values of ALP were decreased significantly ($p<0.05$). The deleterious effects of mercury on hepatocytes were clearly reflected in elevated levels of serum enzymes taken as indices for liver functions. AST and ALT are important and critical enzymes in the biological processes. As the elevation in the serum activity of ALT and AST, liver cytoplasmic enzyme, indicators of the hepatic dysfunction and damage (Dhu *et al.,* 2004) while the decrease in serum ALP level indicates that there was no congestion or cholestasis. The increase in AST and ALT in serum may be due to hepatocellular necrosis, which causes increase in the permeability of the cell membrane resulting in the release of transaminase in the blood stream (Deivanayagam *et al.,* 2010). Our results are in agreement with studies (Reus *et al.,* 2003; Sharma *et al.,* 2002 and El-Shenawy and Hassan, 2008). They reported that mice treated with $HgCl_2$ showed a significant elevation in serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) activities but significant decline in the alkaline phosphatase activity. Chatterjee *et al.* (1981) reported that alkaline phosphatase was involved in the synthesis of nuclear proteins, nucleic acids and phospholipids as well as in the cleavage of phosphate esters and in mobilizing carbohydrates and lipid metabolites to be utilized within the cells. Zhao *et al.* (2009) mentioned that mercury intoxication produced significant hepatic damage as evidenced by increase in the leakage of AST and ALT.

Serum creatinine values were significantly increased in Hgcl2 treated mice (Table 4)**.** The increased creatinine level clearly reflected progressing renal insufficiency in mice treated with mercuric chloride. It is reported that a significant increase in serum creatinine reflecting renal damage by inorganic mercury (Mesquita *et al.,* 2016). Serum creatinine concentration is one of the traditional screening indices for kidney function and renal structural integrity. (Novelli *et al.,* 1998). Renal functional impairment probably resulted from both vasoconstriction and a direct cytotoxic effect of mercury (Barregard *et al.,* 2010). The elevation of creatinine level was reported to be proportionate with the severity of renal insufficiency (Cid *et al.,* 2009). The values of total protein were increased significantly ($p<0.05$) in group B and ($p<0.01$) in group C & group D compared to the value observed in control group. Our study reported that treatment with $HgCl_2$ led to a significant increase in serum total protein level. The result is in agreement with (Necib *et al.,* 2013; Al-Othman *et al.,* 2011) and the increase in protein level may be due to inhibition of amino acid transporter (Brookes and Kristt, 1989) or acceleration of RNA synthesis (Sarafian and Verity, 1983). Urea augmentation could also come from protein catabolism acceleration because of oxidative stress provoked by mercury (Ismail *et al.,* 2014). The depletion of protein content may be due to degradation and the possible utilization of the degraded products for metabolic purposes (Tiwar and Singh, 2005).

Serum glucose values were also increased in the treated mice (Table 4). The values were differ significantly ($p<0.05$) The hyper-glycaemia has been linked to oxidative damage to cell (Mohamed *et al.,* 1999). One possible mechanism for hyper-glycaemic induced oxidative stress involves auto-oxidation of glucose, which can result in the production of O_2 and other ROS. Another way for hyper-glycaemia may be due to enhanced gluconeogenesis and glycolgenolysis and decreased glucose utilization under oxidative stress enzyme produced by mercury (Sheikh *et al.,* 2011).

Table 4. Effect of $HgCl_2$ on ALT, AST, ALP, Creatinine, Total protein and Glucose in female mice at 12th week.

Parameters	Control	5mg Hg	10 mg Hg	15 mg Hg
ALT (U/L)	2.89 ± 0.02	3.40 ± 0.04*	3.54 ± 0.024*	3.69 ± 0.03*
AST (U/L)	7.85 ± 0.17	9.01 ± 0.45*	11.19 ± 0.81*	13.22 ± 0.83*
ALP(U/L)	59.05 ± 2.01	48.20 ± 1.75*	44.03 ± 1.30*	42.27 ± 0.96*
Creatinine (U/L)	1.05 ± 0.05	2.27 ± 0.05*	2.37 ± 0.04*	2.43 ± 0.03*
Total protein (g/dL)	8.23±0.61	10.33±0.65*	11.78±0.74**	12.98±0.93**
Glucose (mg/dL)	123.48 ± 3.44	158.28 ± 2.48*	169.99 ± 3.42*	195.28 ± 3.18*

*Differ significantly at $p<0.05$ (control group versus $Hgcl_2$ treated group)

Effect of $HgCl_2$ on patho-physiological alterations in liver and kidney

Histopathological section of kidney and liver of $HgCl_2$ (group D) treated mice are shown in Figure 1 (A and B). Section of kidney of control group showed normal tissue structures and no detectable changes found in Bowman's capsule and renal tubules (Figure: 1.A). The degeneration of tubular cells with picnotic nuclei, loss of nuclei, edematous denaturation of the proximal tubule, atrophy of the glomerulus and enlarged Bowman's capsule were observed in the HE stained kidneys of $HgCl_2$ -exposed mice (Figure: 1.A, group D). Section of liver of control group showed a normal structure of hepatocytes with a granular cytoplasm, centrally placed nuclei and open sinusoidal spaces (Figure: 1.B). The liver of mercury-exposed mice showed degeneration of the cytoplasm, picnotic nuclei, presence of

vacuoles and dilatation of sinusoidal spaces (Figure: 1.B, group D). The present findings are agreement with the previous findings in rats (Agarwal *et al.*, 2007 and Patnaik *et al.*, 2010).

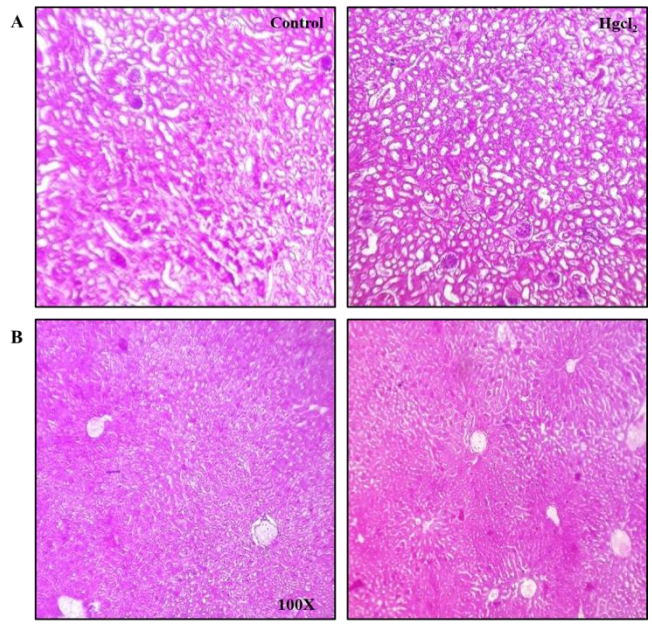

Figure 1. Effect of $HgCl_2$ on patho-physiological alterations in kidneys and liver. A, representative images of kidney section and B, representative images of liver section.

Conclusion

The study concluded that oral exposure to $HgCl_2$ causes hemato-biochemical alterations and hepatic and nephrotoxic effect in female mice. The result obtained from this study strengthens knowledge about the health hazards linked with $HgCl_2$.

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Authors' contribution

MA Miah designed the experiment, and MS Rakib performed the experiment. KM Sujan analyzed the data and wrote the draft. KM Sujan, and MA Miah critically revised the manuscript.

Conflict of interest

The author declares that no conflict of interest exists.

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