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A Sub-Chronic Toxicity Study of Mercuric Chloride in the Rat

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Abstract

Heavy metal accumulates mostly in mammalian liver and kidney as these organs involved in the detoxification and excretion of foreign materials. Chronic exposure of heavy metals leads to intoxication of these organs. In the present study sub-chronic effect of mercury (as mercuric chloride at a dose of 3.75 mg/kg body weight) on biochemical parameter was studied on the experimental animals. Results revealed that mercury increased the aspartate transpeptidase (AST), alanine transpeptidase (ALT), lactate dehydrogenase (LDH) and creatine phosphokinase (CPK) activities significantly than control group. This study also indicates that mercury increases the level of urea and creatinine; and decreases the iron level. Bioaccumulation of mercury was higher in kidney as compared to other organs.

Keywords: Albino rats, mercuric chloride, sub-chronic toxicity, LD50, AST, ALT, LDH, CPK.

1. Introduction

There is a growing problem of worldwide contamination of the environment with mercury. The fate and behavior of mercury in the environment depends on its chemical form. Inorganic mercury compounds enter water bodies by different ways and undergo a process of methylation (Gilmore and Henry, 1991). Mercury poisoning can result from inhalation, ingestion, or absorption through the skin and may be highly toxic and corrosive once absorbed into blood stream. High exposures to inorganic mercury may result in damage to the gastrointestinal tract, the nervous system, and the kidneys (US Environmental protection agency, 2010). Both inorganic and organic mercury compounds are absorbed through the gastrointestinal tract and affect other systems via this route. However, organic mercury compounds are more readily absorbed via ingestion than inorganic mercury compounds (US Environmental protection agency, 2010). Symptoms of high exposures to inorganic mercury include: skin rashes and dermatitis; mood swings; memory loss; mental disturbances; and muscle weakness (US Environmental protection agency, 2010).

Methyl mercury accumulates in lower organisms, and is enriched along the food chain (UNEP, 2002). Methyl and ethyl mercury compounds have been recognized as the cause of mercury poisoning and fatalities as a consequence of consuming contaminated foods. The toxicity signs and symptoms are non-specific at first; including parasthesias, malaise and blurred vision. These may develop later into visual field defects, deafness, dysarthria and ataxia followed by coma and death (Health Canada, 2008; Institoris *et al.*, 2002; and Fontaine *et al.*, 2008). Furthermore, mercury combines with proteins in the plasma or enters the red blood cells but does not readily pass into the brain or fetus and instead, may enter other body organs. The liver is a major site of metabolism for mercury and it can accumulate in the liver, resulting in severe hepatic damages (Wadaan, 2009). Studies have revealed that mercuric chloride caused histopathological and ultrastructural lesions in the liver evidenced by periportal fatty degeneration and cell necrosis (El-Shenawy and Hassan, 2008). Consumption of high quantity of mercury contaminated fish changes the blood pressure and cardiac autonomic activity (Valera *et al.*, 2011).

On the other hand, chronic exposure by inhalation, even at low concentrations in the range 0.7–42 μg/m^3, has been shown in case control studies to cause effects such as tremors, impaired cognitive skills, and sleep disturbance in workers. The serious consequences of chronic mercury toxicity make it important to understand their nature, in order to be able to design the most effective treatment modality (Ngim *et al*., 1992; Liang *et al*., 1993).

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The aim of the present work was to study the accumulation and the sub-chronic toxic effects of mercuric chloride on some biochemical parameters in different body organs, using the rat as the experimental model.

2. Materials and Methods

2.1. Animals

Male albino rats of a locally bred strain, each weighing 125-150 g, were used as the experimental animal model. Rats were obtained from the animal house of the Faculty of Medicine, Assiut University, Egypt. They were housed under constant environmental conditions of temperature (22 + 2 °C) and humidity, with a 12 hour light/dark cycle. All animals were fed *ad libitum* on a commercially available balanced ration.

2.2. Experimental Studies

2.2.1 Determination of The Median Lethal Dose (LD_{50})

These experiments were carried out according to Weil, (1952). One group of 5 animals received saline by gavages and served as control. Twenty rats were used for quantization of the LD_{50} of mercuric chloride. The rats used in this experiment were divided into four groups of 5 animals each. The four dosage levels used for the mercuric chloride experiment were 25, 50, 100 and 200 mg/kg body weight. All dosages were administered orally. The number of dead animals for each dose was recorded after 24 hours.

The oral LD_{50} for mercuric chloride was calculated to be 75 mg/kg. This value was taken as the basis for estimating the dosages used in further experiments where the animals used for the subchronic study for mercury received one twentieth ($1/20^{th}$) of the calculated LD_{50} [3.75 mg/kg] by the same route of administration.

2.2.2. Subchronic Toxicity Study of Mercuric Chloride

Each of twenty male albino rats received oral doses of 3.75 mg mercuric chloride/kg body weight by gavages twice weekly for 12 weeks. Each of a group of 10 rats received 1 ml normal saline twice weekly for the same duration by the same route and served as controls.

Every four weeks, blood samples were collected by orbital sinus bleeding from each rat for the duration of the experiment. The collected samples were left to clot at room temperature then centrifuged at 3000 rpm and used for the assays of the biochemical parameters.

At the end of the experimental period of 12 weeks, blood samples were obtained and the animals were then sacrificed by decapitation. Different internal organs and a sample of skeletal muscles were quickly dissected. The organs or tissues were washed with ice-cold normal saline and stored at -20 °C until assayed for mercury residue.

2.2.3. Biochemical Analyses

An autoanalyzer (Express Plus; Ciba Corning Diagnostics, Palo Alto, CA) was used for analysis of the biochemical parameters determined in the present study. Renal functions were assessed by urea and creatinine serum levels. Alanine transpeptidase (ALT), aspartate transpeptidase (AST) and lactate dehydrogenase (LH) were used to determine the extent of liver affection.

Other biochemical parameters assayed were uric acid, creatinine phosphokinase and alkaline phosphatase. Serum calcium, inorganic phosphate and iron were also assayed. Mercury levels in serum and in homogenized tissues were estimated using Thermo Scientific Graphite Furnace Atomic Absorption Spectrometer equipped with a vapor generation accessory (M-series Atomic absorption Spectrometry, Thermo scientific, USA) (Burger, 2004).

2.2.4. Statistical Analysis

All data are presented as mean ± SEM. One-way analysis of variance (ANOVA) was performed on each variable and the Bonferroni statistics employed to compare the mean values of the control and experimental groups. Differences were considered significant at $P<0.05$. All statistical analyses were performed using SPSS statistical software (version 10).

Results

The renal function of the rats (treated with mercury) was significantly inhibited following administration of mercuric chloride. The increases in urea and creatinine were apparent in the four week of the experimental period and increased steadily afterwards until week 12 (Table 1).

Table 1. Changes in renal function in rats treated with mercuric chloride (3.75 mg/kg) twice weekly for 12 weeks.
Data presented as mean ± standard error (n=10, each group).

Weeks	Urea mg/dl			Creatinine mg/dl		
	Control	Mercury treated	*P* value	Control	Mercury Treated	*P* value
4	20.9 ± 2.4	34.6 ± 2.1 (+65.6%)	0.001	0.45±0.01	0,63±0.01 (+40.0%)	0.001
8	21.4 ± 0.8	35.7 ± 2.7 (+66.8%)	0.001	0.44±0.01	0.66±0.04 (+50.0%)	0.001
12	22.7 ± 1.0	39.7 ± 2.6 (+74.9%)	0.001	0.45±0.01	0.73±0.11 (+62.2%)	0.02

*Number in parentheses represents percentage difference from the corresponding control value.
All values for treated animals were significantly higher than corresponding control values

Urea in the serum was elevated by 65.6% (34.6±2.1 mg/dl) in week 4 and reached 74.9% (39.7±2.6 mg/dl) above control value by week 12, while the corresponding increases in creatinine were 40.0% (0.63±0.01 mg/dl) and 62.2% (0.73±0.11 mg/dl) during the same period.

The liver functions were also affected by the administration of mercury. Liver affection was more apparent in the elevated activities of the serum enzymes which reflect the functions of hepatocytes. The activities of all three enzymes assessed; AST, ALT and LDH were all significantly elevated at week four. The level of AST, ALT and LDH were 223%, 282% and 108% higher than the respective control group after 4^{th} week. The statistical analysis indicates that there were no significant difference in the AST level in control and treated group after week 4 (table 2).

Table 2. Changes in liver functions in rats treated with mercuric chloride (3.75 mg/kg) twice weekly for 12 weeks

Time Weeks	AST U/L		ALT U/L		LDH U/L	
	Control	Mercury treated	Control	Mercury treated	Control	Mercury treated
4	73.1 ± 8.84	236.3 ± 30.77 (+223.3%, $p \leq 0.001$)	66.5 ± 10.08	254.0 ± 30.69 (+282.0%, $p \leq 0.001$)	85.5 ± 5.58	177.7 ±5.79 (+107.8%, $p \leq 0.001$)
8	73.7 ± 9.90	173.3 ± 67.37 (+135.1%, p=0.171, NS)	68.9 ± 10.14	160.7 ± 36.99 (+133.2%, $p \leq 0.038$)	82.8 ± 4.81	160.6 ± 8.02 (+107.1%, $p \leq 0.001$)
12	73.6 ± 7.65	197.9 ± 63.97 (+168.9%, p=0.082, NS)	65.6 ± 0.71	169.0 ±50.76 (+157.6%, p=0.074, NS)	83.3 ± 4.07	160.3 ± 6.98 (+92.4%, $p \leq 0.001$)

Data presented as mean ± standard error (n=10, each group).
*Number in parentheses represents percentage difference from the corresponding control value and p values. (NS, not significant)
AST: Aspartate transpeptidase, ALT: Alanine transpeptidase, LDH: Lactate dehydrogenase

The ALT level was significantly higher (133%) up to week 8. However the results indicate there were no significant differences between the ALT levels after 8th week. The LDH level was 92% (160.3 units/l) higher than the corresponding control group after 12th week.

Moreover, there was practically no change in the activity of ALP. The fluctuations in the activity of this enzyme did not exceed 6.9% (Table 3). The concentration of uric acid in the serum approximately followed the same pattern, with statistically insignificant increases between 5.8% (2.29 mg/dl) and 17.8% (2.36 mg/dl) above the corresponding controls. In the mean time, the activity of CPK was highest at the 4-week point reaching 203.7% (434.6±79.5 units/l) above control value, and started a steady, but slight decline afterwards reaching a mean value of 145% (367.2±50.5 units/l) above that of the corresponding control by 12th week. The result indicates that there was no significant difference in CPK level at 8th week.

Table 3. Changes in alkaline phosphatase, creatine phosphokinase and uric acid in the blood of rats treated with mercuric chloride (3.75 mg/kg) twice weekly for 12 weeks

Time Weeks	ALP (U/l)		CPK (U/l)		Uric Acid (mg/dl)	
	Control	Mercury treated	Control	Mercury treated	Control	Mercury treated
4	163.7 ± 8.41	156.7 ±6.26 (- 4.3%, p=0.522, NS)	143.1 ± 31.64	434.6 ± 79.50 (+203.7%, $p \leq 0.003$)	2.09 ± 0.12	2.29 ± .10 (+9.6%, p=0.221NS)
8	166.6 ± 7.53	158.6 ± 4.74 (- 4.8%, $p \leq 0.001$)	143.1 ± 30.45	326.1 ± 105.52 (+127.9%, p=0.115 NS)	2.09 ± o.12	2.46 ± 0.15 (+17.2%, p=0.404 NS)
12	167.8 ± 7.65	156.3 ± 5.16 (- 6.9% $p \leq 0.031$)	149.9 ± 33.52	367.2 ± 50.49 (+145.0%, $p \leq 0.002$)	2.02 ± 0.11	2.36 ± 0.16 (+16.8%, p=0.75 NS)

Data presented as mean ± standard error (n=10, each group).
*Number in parentheses represents percentage difference from the corresponding control value and P values (NS, no significant difference).
ALP: Alkaline phosphatase, CPK: Creatine phosphokinase

The changes in serum components extended to some inorganic elements (Table 4). The concentration of calcium increased in the early time points of the experimental period, reaching its highest values of 45.2% (14.51±0.23 mg/dl) above control in week 4. This was followed by fluctuations and tendency toward decline reaching a mean value higher by 13% (11.21±0.17 mg/dl) at 12th week. The changes in calcium concentration were not accompanied by changes in inorganic phosphorus, which showed no statistically significant changes throughout the treatment period. Iron in the sera of treated rats showed a moderate decrease of 29.0% (138.5±5.64 mg/dl) after four weeks and such decrease persisted throughout the treatment period, ranging between 25.1% and 29.0% below corresponding control values.

Table 4. Changes in the concentrations of calcium, inorganic phosphorus and iron in the sera and mercury in whole blood of rats treated with mercuric chloride (3.75 mg/kg, p.o) twice weekly for 12 weeks

Time Weeks	Calcium mg/dl		Inorganic Phosphorus mg/dl		Iron mg/dl		Mercury µg/dl	
	Control	Mercury Treated	Control	Mercury treated	Control	Mercury Treated	Control	Mercury treated
4	9.99 ± 0.20	14.51 ± 0.23 (+45.2%, ($p \leq 0.001$)	8.22 ±0.21	7,76 ±0.19 (- 5.6%, p=0.63NS)	195.2 ±2.65	138.5 ±5.64 (- 29.0%, $p \leq 0.001$)	0.99 ±0.06	5.21 ±0.23 (+426.3%, $p \leq 0.001$)
8	9.96 ± 0.21	12.49 ± 0.15 (+25.4%, p≤0.001)	8.38 ±0.21	8.22 ±0.21 (- 1.9%, p=0.60NS)	195.5 ±7.07	146.4 ±2.91 (- 25.1%, $p \leq 0.001$)	1.04 ±0.5	5.85 ±0.30 (+462.5%, $p \leq 0.001$)
12	9.91 ± 0.24	11.21 ± 0.17 (+13.1% $p \leq 0.001$)	8.24 ±0.21	7.80 ±0.12 (- 5.3%, p=0.095 NS)	196.2 ±6.27	145.3 ±2.29 (- 25.9%, $p \leq 0.001$)	1.04 ±0.06	6.85 ±0.26 (+558.6%, $p \leq 0.001$)

Data presented as mean ± standard error (n=10, each group).
*Number in parentheses represents percentage difference from the corresponding control value and p values (NS, no significant difference)

As a result of mercury administration, the concentration of mercury itself in whole blood of treated rats was highly elevated to a level 426.3% (5.21±0.23 µg/dl) above control within 4 weeks. The concentration of mercury steadily increased with further treatment to reach a final concentration of 558.6% (6.85±0.26 µg/dl) above control by the end of the experimental period (Table 4 and Figure 1).

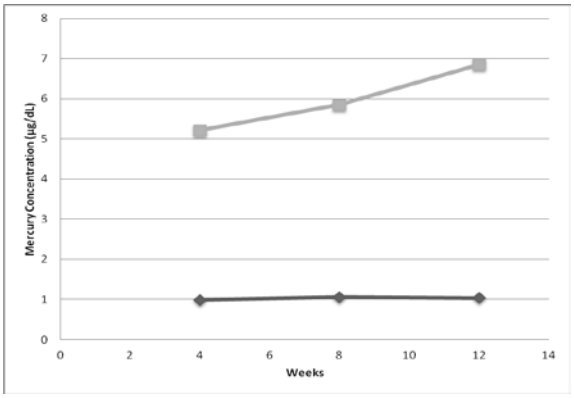
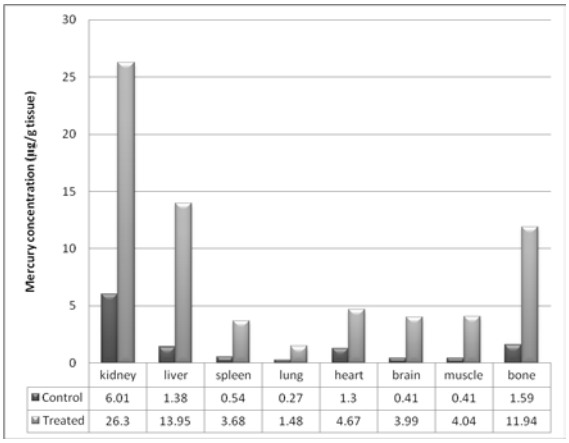


Figure 1. Changes in the concentration of mercury in the blood of rats treated with mercuric chloride (3.75 mg/kg, p.o.) twice weekly for 12 weeks (□- Treated, • - Control)

The distribution of mercury (µg/g) residues in different tissues and organs of treated rats is presented in Figure 2. The highest concentrations were found in the kidney, reaching a mean of 26.3 ± 4.93 µg/g tissues, followed by the liver, which contained an average of 13.95 ± 3.00 µg/g tissues. The level of mercury in the bone was unexpectedly high (11.94 ± 1.10 µg/g). Mercury concentrations in heart, muscle, brain and spleen were similar ranging from 4.67 to 3.68 µg/g tissue. The lowest concentration of mercury was found in lungs (1.48 ± 0.28 µg/g).



	kidney	liver	spleen	lung	heart	brain	muscle	bone
Control	6.01	1.38	0.54	0.27	1.3	0.41	0.41	1.59
Treated	26.3	13.95	3.68	1.48	4.67	3.99	4.04	11.94

Figure 2. Distribution of mercury in different organs and tissues of rats treated with mercuric chloride (3.75 mg/kg, p.o.) twice weekly for 12 weeks.

3. Discussion

Mercury is a widely used industrial chemical with serious health hazards resulting mainly from environmental pollution by industrial waste or accidental exposure. The serious nature of mercury toxicity necessitates full understanding of the underlying mechanisms involved in producing its harmful effects. Mercury toxicity is known to affect the redox status of the victims' tissues through increased production of free radicals leading to oxidative stress (Ercal *et al*., 2001). This causes disturbances in the functions of many body organs.

In the present study, the higher levels of urea and, in particular, creatinine clearly reflected progressing renal insufficiency in rats treated with mercuric chloride. Novelli *et al*. (1998) and Mahmoud (1999) reported higher urea and creatinine levels in rats administered mercury either in the form of inorganic salts or as a complex with metallothioneine. Such functional impairment probably resulted from both vasoconstriction and a direct cytotoxic effect of mercury (Girardi and Elias, 1993; and Barregard *et al*., 2010). Besides, the detrimental effect may be attributed mainly to the accumulation of this toxic metal in kidney. In the present work, renal concentration of mercury was highest among all tissues and organs tested, probably indicating the most serious organ affection.

Hepatic functions were also impaired by administration of mercury. Both the synthetic ability and the integrity of hepatocytes were affected. The deleterious effects of mercury on hepatocytes were clearly reflected in elevated levels of serum enzymes taken as indices for liver functions. Treatment with mercury significantly increased both serum ALT and AST activities. Mohmoud and Manal (1999) reported elevation in the activities of these enzymes following both acute and chronic exposure to mercury, which was attributed to its pathological effect in hepatic tissue. Moreover, the activity of LDH was also elevated as a result of mercury administration. The release of LDH into the serum is taken as an indication of hepatocyte damage and death.

The activity of CPK in the serum was significantly elevated, probably indicating multi-organ damage. Significant accumulation of mercury was shown in the present work to occur in all three organs, indicating widespread deleterious toxic effect of mercury in a multitude of body systems.

These findings are in agreement with those of Kuliczkowski *et al*. (2004) and Lim *et al*. (2010) who found marked elevation of serum CPK after mercury poisoning

The observed insignificant hyperuricemia associated with mercury toxicity may be attributed to its degenerative and necrotic effect upon many tissues including liver and kidney. As a result of necrosis, the broken down tissues caused accelerated catabolism of purines with subsequent increase in uric acid formation.

The degree of hyperuricaemia showed attenuation during the last four weeks, probably due to insufficiency of renal reabsorption of uric acid through degenerated renal tubules. The elevation of urea, creatinine and uric acid levels were reported to be proportionate with the severity of renal insufficiency (Kumar, 1994; Long *et al*., 1998; and Cid *et al*., 2009).

The disturbance in serum composition as a result of mercury toxicity was not limited to organic biochemicals, but extended to inorganic elements.

Serum calcium showed a significant increase starting early in the experiment. The accumulation of mercury in the bones may have affected the balance between the activities of osteoblasts and osteoclasts, leading to mobilization of calcium from the bones into the blood. Other mechanisms may have been involved, including effects on the parathyroid gland, calcitonin from the thyroid gland or disturbance in renal excretion of calcium as a result of renal damage (Shull *et al*., 1981). Contrary to the changes on calcium, inorganic phosphorus was not affected by administration of mercury. Plasma levels of phosphates are partly under the control of the parathyroid hormone, which controls its excretion by the kidney. The net effect is to increase the concentration of calcium and lower that of phosphate. This may explain the slight hypophosphatemia observed in the present study. The concentration of iron was also decreased, probably indicating iron deficiency anemia in mercury treated rats. Such deleterious effect of mercury started early after mercury administration and persisted throughout the whole experimental period. Lecavalier *et al*. (1994) and Institoris *et al*. (2002) reported that there was marked iron deficiency anemia in female rats after mercury administration, where as Grosicki and Kossakowski (1990), reported that mercuric chloride reduces the absorption of radiolabelled ferric chloride ($^{59}FeCl_3$) from stomach and intestine.

As may be expected, the concentration of mercury in the blood increased with the progress of the experiment. This means increased distribution of mercury into vital

organs with stronger toxic effects and metabolic disturbances. The same results were obtained by other researchers (Guglick *et al*., 1995; Harnly *et al*., 1997; and Schiawicke *et al*., 2008). Mercury residue was detected in all organs tested. The distribution of mercury into tissues and organs was not uniform. The kidney and liver showed the highest levels of mercury, which could be responsible for the abnormal functions of these organs. An unexpected result was the relatively high concentration of mercury in the bones. The combined effects in these organs probably represented a significant factor in the observed disturbance in calcium and phosphate homeostasis. Similar results were obtained by Pathak & Bhowmik (1998), Sundberg *et al*. (1999), and Yang *et al*. (1997) who found that mercury concentrations in liver, kidney and brain of young animals.

It is clear from the present work that mercury distributes in different body organs and tissues causing metabolic disturbances and deleterious effects.

The concentration of mercury residues depends on the particular organ and the extent of damage probably depends on both its concentration and the response and sensitivity of the organ.

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