Journal of Cell and Animal Biology Vol. 3 (12), pp.222-230, December, 2009
Available online at http://www.academicjournals.org/JCAB
ISSN 1996-0867 © 2009 Academic Journals

Full Length Research Paper

Effect of inorganic mercury on biochemical parameters in Wistar rat

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Accepted 10 November, 2009

The present study has been carried out to investigate the effect of inorganic mercury (mercuric chloride - $HgCl_2$) exposure on biochemical parameters in adult male rats. 48 rats whose average body weight was about 290 g were included in the experiment. $HgCl_2$, dissolved in distilled water, was administered *per os* at a dose of 0.25 mg/kg of body weight daily during 15, 30, 45 or 60 days. Rats receiving distilled water were considered as controls. At the end of each treatment period, the corresponding group of animals (both control and intoxicated groups) was euthanized in order to measure plasma glucose, cholesterol, triglycerides, total proteins and urea concentrations, alkaline phosphatase (ALP) activity and hepatic glutathione (GSH) level. Our results showed a significant disturbance of ALP activity and uremia throughout the experiment. Plasma triglycerides, cholesterol and total proteins levels were significantly deteriorated following $HgCl_2$ exposure by day 30. $HgCl_2$ also induced a hypoglycemia at days 45 and 60. Hepatic GSH content decreased significantly only at day 45 compared to controls. Our study suggests that $HgCl_2$ is mainly a nephrogenic pollutant and disturbs simultaneously the plasma biochemical profile and the hepatic GSH-related detoxifying system.

Key words: Mercuric chloride, biochemical profile, oxidative stress, nephrotoxicity, hepatotoxicity, 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 (Risher and Amler, 2005). Its wide industry-related effects on human and animal biosystems have been well documented (WHO, 1991) and general exposure to this biologically-active chemical agent has been shown to be exacerbated through contaminated water and food (Magos and Clarkson, 2006).

Nowadays, large populations worldwide are exposed to to relatively low levels of Hg, especially via the use of pesticides in agriculture and of fluorescent light bulbs as well (El-Shenawy and Hassan, 2008). In this context, Hg exists in a wide variety of physical and chemical states, each of which has specific characteristics for target organs (Aleo et al., 2002; Ghosh and Sil, 2008). For example, exposure to Hg vapor as well as to organic Hg compounds specifically affects the central nervous system (Vahter et al., 2000), while kidneys, liver and gastrointestinal tract are mainly targeted by inorganic Hg compounds, such as mercuric chloride ($HgCl_2$) (Schurz et al., 2000; Ghosh and Sil, 2008). In this respect, multiple mechanisms have been proposed to explain the biological toxicity of $HgCl_2$ by investigating the biochemical fate of various Hg forms (Gutierrez et al., 2006)

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2006). Indeed, the Hg^{2+} form has shown a great affinity for endogenous biomolecules-associated thiol (-SH) groups (Clarkson, 1997) and it is invariably found attached to SH-containing proteins, small-molecular weight peptides (such as glutathione) and amino acids (such as cysteine) (Perottoni et al., 2004b), leading to a profound deterioration of vital metabolic processes (Sener et al., 2003; Wiggers et al., 2008). Consequently, the oxidative stress was strongly suggested as one of the crucial mechanisms in Hg-induced pathological aspects (Lund et al., 1993; Clarkson, 1997; Perottoni et al., 2004a). However, biochemical parameters are still more indicative of early physiological changes following subchronic and chronic Hg exposure (Wadaan, 2009). Therefore, the toxicological assessment of the general health condition should take into account the biochemical modulations induced by this pollutant during the first stages of contamination.

Thus, the purpose of the present study was to evaluate some biochemical markers during chronic $HgCl_2$ intoxication and to examine how the rat organism responds chronically to this pollutant. Initially, $HgCl_2$-induced hepatotoxicity was estimated by determining the alkaline phosphatase (ALP) activity in plasma and the hepatic concentration of reduced glutathione (GSH). $HgCl_2$-induced nephrotoxicity in terms of urea concentration in plasma was also measured. In addition, some blood parameters (cholesterol, proteins, triglycerides and glucose), indicative of metabolic disturbances, were determined.

MATERIALS AND METHODS

Animals

Healthy adult male Wistar rats (*Rattus rattus*), weighing 290 ± 10 g, were obtained from Pasteur Institute (Algiers, Algeria) and used for the experiment. Animals were maintained on standard chow and water *ad libitum*. They were regrouped in large polyethylene cages (six rats per cage) in an air-conditioned animal house at a temperature of 25 ± 2°C with steady hygrometry (50%) on a 12:12 h light/dark cycle.

Treatment

The experimental protocol is presented in Table 1. Forty eight male rats were distributed into eight groups, 6 rats in each one. Four experimental groups were treated with $HgCl_2$ (MC) *per os* at a dose of 0.25 mg/kg of body weight daily during 15, 30, 45 or 60 days and four control groups (C) were treated with distilled water.

Collection of samples

At the end of each period of treatment, the animals were sacrificed by decapitation. The animal blood was then collected in heparinized tubes, cooled and then centrifuged. The plasma recovered thereafter was preserved at a temperature of -70°C for biochemical analysis. Liver was rapidly excised and then stored frozen at -70°C until estimation of reduced glutathione concentration was obtained. The investigation conformed to the Guide for the Care and Use of laboratory Animals.

Plasma biochemical analyses

Glucose was determined after enzymatic oxidation in the presence of glucose oxidase. The hydrogen peroxide formed reacts, under peroxidase activity, with phenol and 4-aminophenazone to form a red-violet quinoneimine dye, an indicator that was recorded at 500 nm (Barham and Trinder, 1972).

The cholesterol was determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine, measured at 500 nm, was formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxidase (Trinder, 1969).

The triglycerides were determined after enzymatic hydrolysis by lipases. The quinoneimine formed from hydrogen peroxide, 4-aminophenazone and 4-chlorophenol reactions under peroxidase activity was measured at 500 nm (Trinder, 1969).

The total proteins were determined based on the reaction, in an alkaline medium, of cupric ions with protein peptide bonds, resulting in the formation of a colored complex that indicates proteins concentration at 550 nm (Gornall et al., 1949).

Alkaline phosphatase (ALP) was determined by an optimized standard method according to Bowers and McComb's (1975) instructions.

Urea was measured based on the reaction of salicylate and hypochlorite with the ammonium ions to form a green complex (2.2 dicarboxylindophenol) which can be read at 600 nm (Patton and Crouch, 1977).

All these determinations were carried out using standard commercial test kits (RANDOX Laboratories Ltd, Co. Antrim, UK). The manufacturer's instructions on the assay procedures were strictly followed.

Hepatic reduced glutathione

Reduced glutathione (GSH) level was estimated in the deproteinized supernatant fraction of liver homogenate using 5,5 dithio-bis-nitrobenzoic acid (DTNB) at 412 nm (Weckbecker and Cory, 1988). The results were expressed in nmol GSH/mg of protein. Thus, liver total protein concentration was measured by the method of Lowry et al. (1951) using bovine serum albumin as standard.

Statistical analyses

The statistical analysis is realized using Mann-Whitney's test followed by Student-Fisher T-test to compare between paired groups, whereas Kruskal-Wallis non-parametric ANOVA followed by the one way analysis of variance (ANOVA) were used to compare between treated groups. The results are expressed as mean ± SEM and the statistical test was considered significant at $p \leq 0.05$ level. The data were processed and analyzed using the statistical software application package MINITAB 15.

RESULTS

Plasmatic glucose

There was no significant difference in plasmatic glucose concentration between the two groups during the second and the fourth week of treatment. However, hypoglycaemic

Table 1. Experimental protocol.

	Group	$HgCl_2$ dose (mg/kg/d)	Number of animals	Duration (day)	Decapitation day
1	Controls	0 (distilled water)	24	With other groups	With other groups
2	$HgCl_2$ treated	0.25	6	15	16th
3	$HgCl_2$ treated	0.25	6	30	31st
4	$HgCl_2$ treated	0.25	6	45	46th
5	$HgCl_2$ treated	0.25	6	60	61st

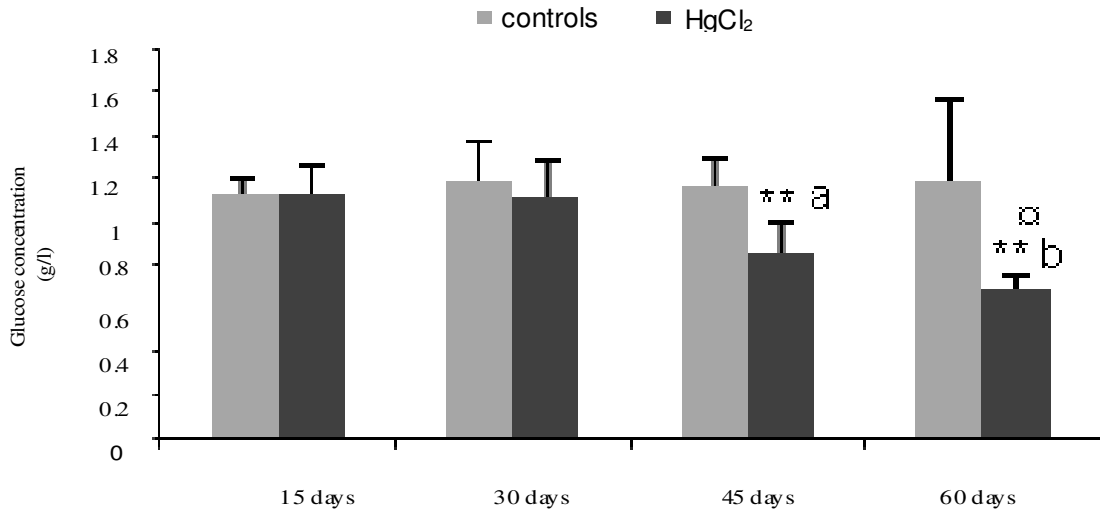

Figure 1. Variation in the level of glucose (g/l) in plasma of male rats treated with mercuric chloride and controls. Data are reported as mean ± SEM for 6 animals per group. a) $p \leq 0.05$; b) $p \leq 0.01$ (Mann-Whitney's test) and ** $p \leq 0.01$ (student t-test) compared to the control at the same time point. ¤ $p \leq 0.001$ compared to other treated groups (Kruskal-Wallis non-parametric ANOVA followed by ANOVA test).

effect of mercuric chloride was registered on day 45 and on day 60 of treatment compared with controls (Figure 1).

Plasmatic cholesterol

The administration of mercuric chloride with 0.25 mg/kg of body weight in the rats induced a significant reduction in the plasmatic cholesterol concentration on day 30, 45 and on day 60 of exposure (Figure 2).

Plasmatic triglycerides

Our results showed a significant decline of triglycerides in the treated animals at the second week, at the fourth week and at the last week of treatment in comparison with the controls (Figure 3).

Plasmatic total proteins

Evaluation of plasmatic total proteins revealed a significant decrease in treated groups on day 30, 45 and 60 compared to controls (Figure 4).

Plasmatic alkaline phosphatase

As shown in (Figure 5), a significant variation of the activity of plasmatic alkaline phosphatase in the rats treated with $HgCl_2$ was noted comparatively with control groups. In fact, this level increased on day 15 and 60 of treatment. However, the value of alkaline phosphatase decreased on day 30 and 45 of treatment.

Hepatic reduced glutathione

The findings exhibited that mercuric chloride treatment reduced the rate of hepatic glutathione in comparison with the control groups. However, this decrease was significant only in the 45th day of treatment (Figure 6).

Plasmatic urea

Simultaneously, the plasmatic urea concentration increased

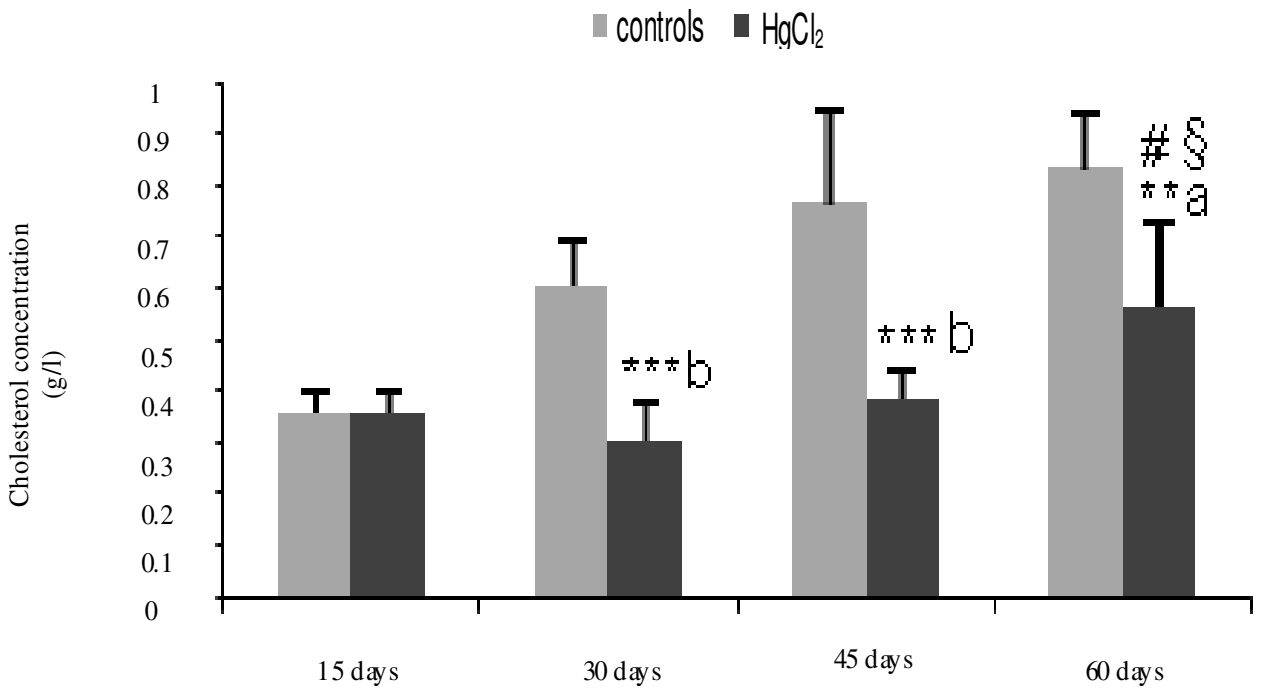

Figure 2. Variation in the level of cholesterol (g/l) in plasma of male rats treated with mercuric chloride and controls. Data are reported as mean ± SEM for 6 animals per group. a $p \leq 0.05$; b $p \leq 0.01$ (Mann-Whitney's test) and ** $p \leq 0.01$; *** $p \leq 0.001$ (Student T-test) compared to the control at the same time point. § $p \leq 0.01$ (Kruskal-Wallis non-parametric ANOVA) and # $p \leq 0.001$ (ANOVA test) compared to other treated groups.

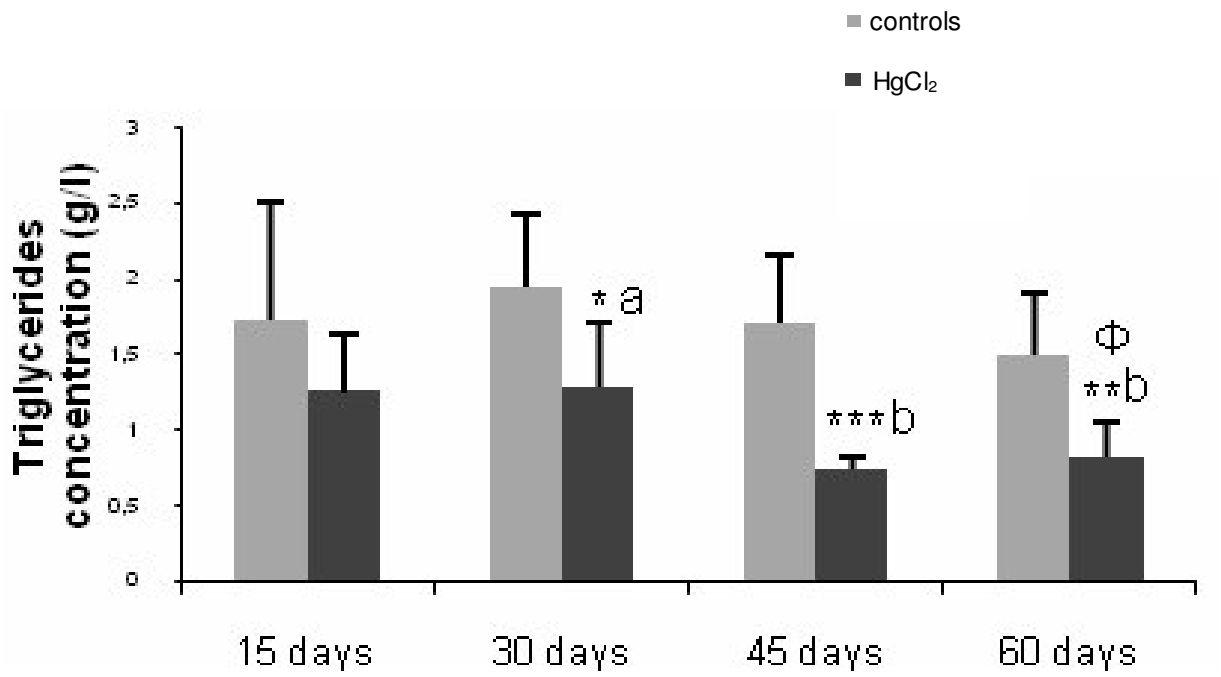

Figure 3. Variation in the level of triglycerides (g/l) in plasma of male rats treated with mercuric chloride and controls. Data are reported as mean ± SEM for 6 animals per group. a) $p \leq 0.05$; b) $p \leq 0.01$ (Mann-Whitney's test) and * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$ (Student T-test) compared to the control at the same time point. Φ $p \leq 0.01$ compared to other treated groups (Kruskal-Wallis non-parametric ANOVA followed by ANOVA test).

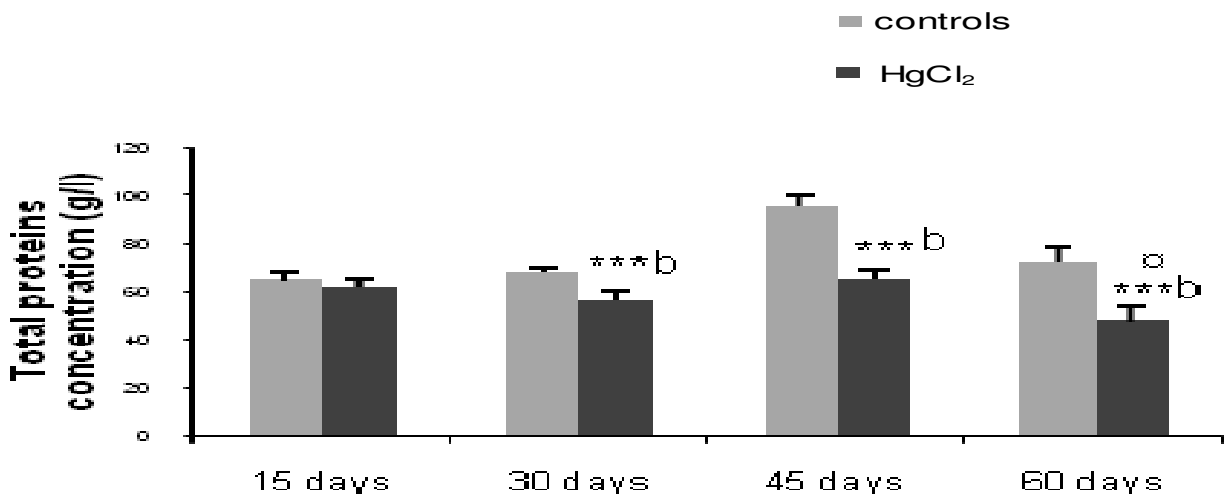

Figure 4. Variation in the level of total proteins (g/l) in plasma of male rats treated with mercuric chloride and controls. Data are reported as mean ± SEM for 6 animals per group. b) $p \leq 0.01$ (Mann-Whitney's test) and *** $p \leq 0.001$ (Student T-test) compared to the control at the same time point. ¤ $p \leq 0.001$ compared to other treated groups (Kruskal-Wallis non-parametric ANOVA followed by ANOVA test).

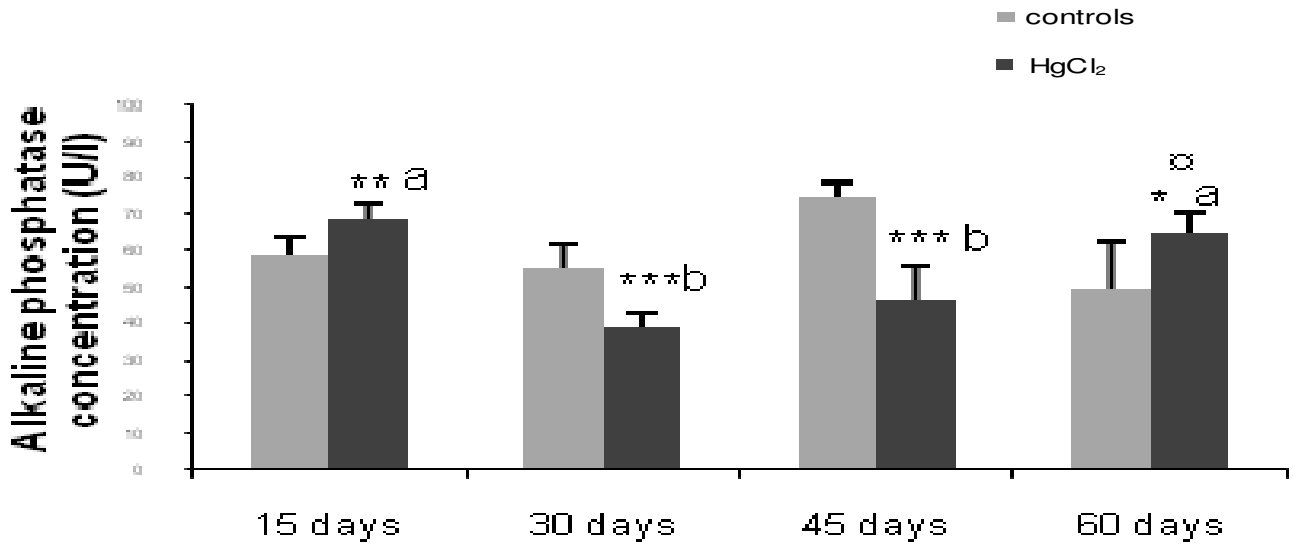

Figure 5. Variation in the activity of alkaline phosphatase (U/l) in plasma of male rats treated with mercuric chloride and controls. Data are reported as mean ± SEM for 6 animals per group. a) $p \leq 0.05$; b) $p \leq 0.01$ (Mann-Whitney's test) and * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$ (student's t-test) compared to the control at the same time point. ¤ $p \leq 0.001$ compared to other treated groups (Kruskal-Wallis non-parametric ANOVA followed by ANOVA test).

increased significantly in treated rats compared with controls during the second, the fourth, the sixth and the last week of exposure (Figure 7).

DISCUSSION

The toxicity of mercury depends on its chemical form. Various mercury compounds have different toxicities depending on physical and chemical properties that affect absorption, distribution, tissue affinities and stability within the biosystem. For instance, elemental mercury in the liquid state has unique toxic effects that differ from those of mercury vapor; likewise, organic mercury molecules are toxicologically different from inorganic forms (NTP, 1993).

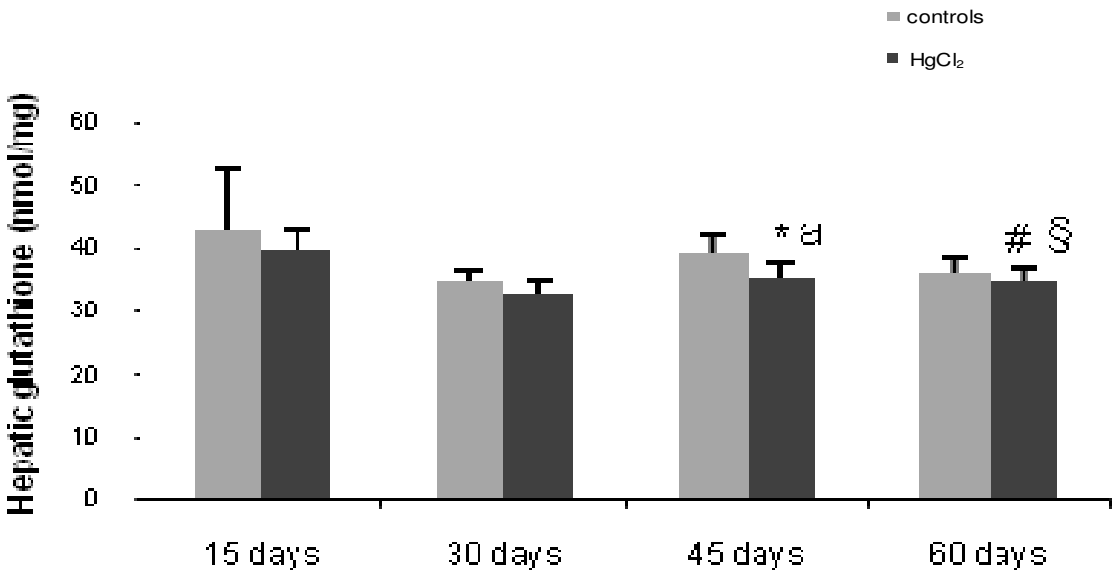

Figure 6. Variation in the level of reduced glutathione (nmol/mg proteins) in liver of male rats treated with mercuric chloride and controls. Data are reported as mean ± SEM for 6 animals per group. a $p \leq 0.05$ (Mann-Whitney's test) and * $p \leq 0.05$ (student's t-test) compared to the control at the same time point. § $p \leq 0.01$ (Kruskal-Wallis non-parametric ANOVA) and # $p \leq 0.001$ (ANOVA test) compared to other treated groups.

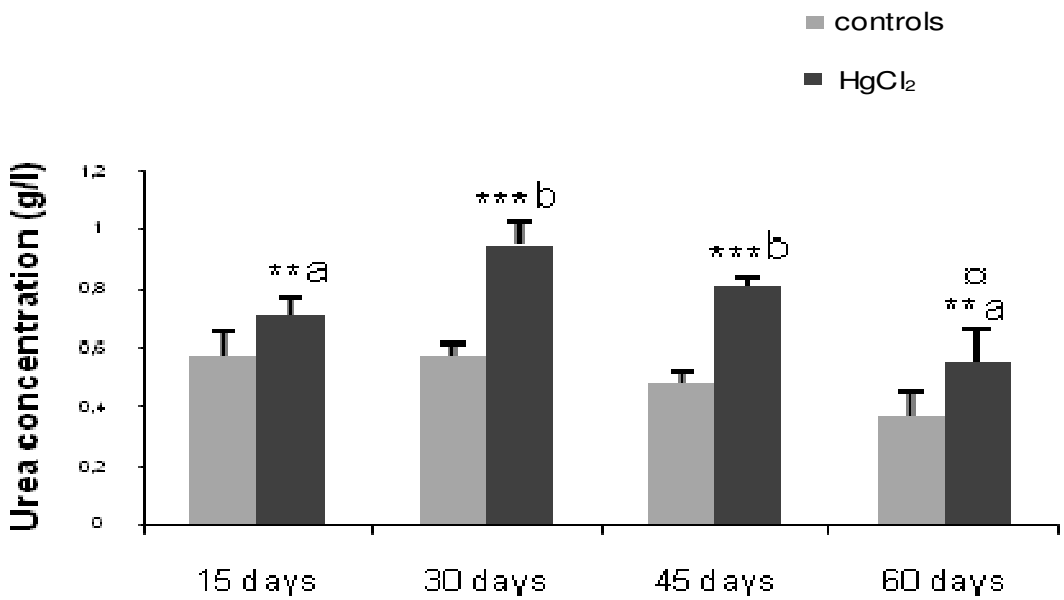

Figure 7. Variation in the level of urea (g/l) in plasma of male rats treated with mercuric chloride and controls. Data are reported as mean ± SEM for 6 animals per group. a $p \leq 0.05$; b $p \leq 0.01$ (Mann-Whitney's test) and ** $p \leq 0.01$; *** $p \leq 0.001$ (Student T-test) compared to the control at the same time point. ¤ $p \leq 0.001$ compared to other treated groups (Kruskal-Wallis non-parametric ANOVA followed by ANOVA test).

$HgCl_2$ is an inorganic compound used in various fields. Its numerous effects were evaluated in many toxicity and carcinogenicity studies because of its extensive use and its wide occurrence as an environmental pollutant (NTP, 1993; Langford and Ferner, 1999). Once absorbed, $HgCl_2$ 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 $HgCl_2$ is intensively accumulated following chronic exposure (WHO, 1991).

The present investigation was carried out in order to determine, in a chronological manner, the biochemical repercussions of daily $HgCl_2$ administration in male Wistar rats. Indeed, we revealed here that continuous oral administration of $HgCl_2$ during 15, 30, 45 or 60 days had a great impact on the measured biochemical parameters, by which mainly hepatotoxicity and nephrotoxicity are evaluated.

The plasma glucose concentration did not show any detectable variations between the two groups during the first month. By day 45, a considerable hypoglycemia was registered in intoxicated rats and this hypoglycemic impact of $HgCl_2$ was more obvious at day 60. Interestingly, Durczok et al. (2002) demonstrated that glucose uptake was deeply affected in several tissues of neonatal rats exposed chronically to methylmercury chloride (CH_3HgCl), confirming the existence of complex molecular mechanisms of Hg-induced dysglycaemia depending on the dose administered, the duration of exposure and the individual age. Plasma cholesterol levels indicated a relatively persistent elevation in untreated rats. Daily $HgCl_2$ administration caused a sudden acute decrease in cholesterolemia from day 30 until day 60 compared to controls, despite a continuous re-establishment of this parameter was registered with exposure progression. On the other hand, plasma triglycerides concentration showed a remarkable reduction only after two weeks of $HgCl_2$ exposure, with a significantly observable difference comparatively to controls from day 30 to the end of experiment. Accordingly, Wadaan (2009) have reported that serum cholesterol and triglycerides levels were significantly reduced following 8 weeks of $HgCl_2$ exposure in neonatal rats; however, serum glucose level did not show any significant change at the end of experiment. Recently, another report mentioned that Hg species promote cardiovascular disorders via metabolic changes, suggesting that blood cholesterol and triglycerides would consequently be involved in Hg-induced cardiovascular risks increase (Mozaffarian, 2009).

Plasma proteins play a prominent role in preventing increase of systemic metal-related free fractions, thus preserving the physiological functions by delaying metal accumulation and toxicity. In this study, plasma proteins levels were significantly reduced after one month of $HgCl_2$ exposure. 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 et al., 2005). We found a significant increase in plasma ALP concentration after 15 days of daily $HgCl_2$ exposure with a subsequent considerable drop at days 30 and 45. Unexpectedly, ALP levels have shown a recurrent increase at day 60 compared to controls. ALP is mainly a liver cytoplasmic enzyme; the elevation in its serum activity activity is generally related to necrotic lesions in this organ (El-Shenawy and Hassan, 2008). In this respect, ALP activity alterations may result from Hg effect primarily on hepatic and renal tissues. Furthermore, mercury may affect intestinal functions during absorption processes, thus inhibiting the enzyme synthesis and activity. Simultaneously, plasma urea concentration revealed a significant increase during the whole chronology of experiment. This result is undoubtedly related to acute and persistent renal injuries, thus confirming that the kidneys are very sensitive to Hg exposition (Agarwal and Behari, 2007). When attached together, our findings suggest that $HgCl_2$-exposed male rats had very serious renal disturbances that began only 15 days later. This $HgCl_2$-induced nephropathy would 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 et al., 2009). Additionally, this early nephrotoxicity being severe and irreversible, as revealed by highly persistent uremia, was accompanied by elevated ALP levels at day 15. Interestingly, subsequent plasma ALP level reductions at days 30 and 45 could reflect the advanced renal necrosis and dysfunction rather than liver lesions because hepatic GSH concentration were generally not disturbed compared to controls, except at day 45 when a significant difference was seen, demonstrating the liver resistance to the $HgCl_2$ insults. However, the ALP levels recurrence at day 60 may delineate the beginning of hepatotoxicity. Supporting the Hg-associated oxidative stress hypothesis, especially in liver injuries, it was previously mentioned that Hg reduces the GSH levels in the body (Krone et al., 2002), with this antioxidant decline being targeted by several mechanisms. Hg bind irreversibly to GSH, causing the loss of a great amount of GSH molecules through the bile, and a considerable loss of GSH is by far due to GSH reductase inhibition (Zalups and Lash, 1996). Hg also inhibits GSH synthetase so that GSH regeneration can not be made, and since it pro-motes hydrogen peroxide, lipid peroxides and hydroxyl radicals formation at the same time (Cheng et al., 2006; Jadhav et al., 2007), it is clear that its contamination underlie the serious imbalance in the oxidative/antioxidant ratio (Miller et al., 1991). Interestingly, because of the low antioxidant enzymes activity and decreased GSH content in the liver, this organ is thought to be highly susceptible to oxidative stress. Therefore, an increase in reactive oxygen species (ROS) formation by $HgCl_2$ may induce liver cell membrane structural and functional alterations, leading to noxious metabolic outcomes (Sharma et al., 2007).

In comparison to our findings, the treatment of rats by $HgCl_2$ induced a reduction in plasma triglycerides and glucose (Chowdhury et al., 1986). Moreover, chronic treatment of males and females rats by this mercuric compound at 5 mg/kg of body weight increased the plasma concentration of ALP and decreased the plasma urea and total proteins concentrations (NTP, 1993). Additionally,

Rao and Sharma (2001) announced a depletion of the testicular and epididymal ALP, cholesterol and proteins following the treatment of mice by $HgCl_2$ at 1.25 mg/kg/ day during 45 days. In contrast, another study indicated that exposure of mice to $HgCl_2$ low dose revealed no variation of the plasma concentrations of ALP, total proteins and glucose, but trend towards lower mean values of cholesterol in the 0.25 and 0.50 mg/kg/day groups was seen (Khan et al., 2004). Another report indicated that oral administration of $HgCl_2$ to rats with levels of 1 and 2 mg/kg of body weight during 30 days increased testicular ALP (Ramalingam and Vimaladevi, 2002). Furthermore, acute administration of $HgCl_2$ causes toxic effects on kidney and liver tissues and this damage was associated with the increase in serum ALP activity and urea (Ghosh and Sil, 2008). Consequently, it appears that the period of exposure to $HgCl_2$ could affect organs functions differently, with the kidneys being the most sensitive to such exposure because $HgCl_2$ has been specifically considered as a renal toxin (Duncan-Achanzar et al., 1996).

In conclusion, our findings suggest that exposure to $HgCl_2$ in male Wistar rats has differently and chronologically affected the plasma levels of glucose, triglycerides, cholesterol, proteins, ALP and urea. Interestingly, the accelerated increase in urea concentration and the remarkable stability in hepatic GSH concentration suggest that $HgCl_2$ is primarily a nephrogenic pollutant, thus highlighting the fact that general toxicological directives should basically focus on renal function during the early stages of $HgCl_2$ contamination, because early $HgCl_2$-induced nephrotoxicity could exacerbate the biochemical imbalance and accelerate hepatotoxicity.

REFERENCES

Agarwal R, Behari JR (2007). Effect of selenium pretreatment in chronic mercury intoxication in rats. Bull. Environ. Contam. Toxicol., 79: 306-310.

Aleo MF, Morandini F, Bettoni F, Tanganelli S, Vezzola A, Giuliani R, Steimberg N, Boniotti J, Bertasi B, Losio N, Apostoli P, Mazzoleni G (2002). In vitro study of the nephrotoxic mechanism of mercuric chloride. *La Medicina del lavoro*. 93(3): 267–278.

Al-Madani WA, Siddiqi NJ, Alhomida AS (2009). Renal toxicity of mercuric chloride at different time intervals in rats. Biochem. Insights 2: 37-45.

Barham D, Trinder P (1972). An improved colour reagent for the determination of blood glucose by the oxidase system. Analyst 97(151): 142-145.

Bowers GN, McComb RB (1975). Measurement of total alkaline phosphatase activity in human serum. Clin. Chem. 21(13): 1988-1995.

Cheng JP, Hu WX, Liu XJ, Zheng M, Shi W, Wang WH (2006). Expression of c-fos and oxidative stress on brain of rats reared on food from mercury–selenium coexisting mining area. J. Environ. Sci., (China) 18(4): 788-792.

Chowdhury AR, Vachhrajani KD, Makhija S, Kashyap SK (1986). Histophotometric and biochemical changes in the testicular tissues of rat treated with mercuric chloride. Biomed. Biochim. Acta 45: 949–956.

Clarkson TW (1997). The toxicology of mercury. Crit. Rev. Clin. Lab. Sci., 34: 369-403.

Duncan-Achanzar KB, Jones JT, Burke MF, Carter DE, Laird HE (1996). Inorganic mercury chloride-induced apoptosis in the cultured porcine renal cell line LLC-PK1.Pharmacol. Experim.Ther.,277(3): 1726-1732.

Durczok A, Szkilnik R, Brus R, Nowak P, Labus L, Konecki J, Drabek K, Kuballa G, Rycerski W, Mengel K (2002). Effect of organic mercury exposure during early stage of ontogenic development on the central dopaminergic system in adult rats. Polish J. Environ. Stud., 11(4): 307-314.

El-Shenawy SMA, Hassan NS (2008). Comparative evaluation of the protective effect of selenium and garlic against liver and kidney damage induced by mercury chloride in the rats. Pharmacol. Rep., 60: 199-208.

Ghosh A, Sil PC (2008). A protein from *Cajanus indicus* Spreng protects liver and kidney against mercuric chloride-induced oxidative stress. Biol. Pharm. Bull., 31(9): 1651-1658.

Gornall AC, Bardawill CJ, David MM (1949). Determination of serum proteins by means of the Biuret reaction. J. Biol. Chem., 177(2): 751-766.

Gutierrez LLP, Mazzotti NG, Araújo ASR, Klipel RB, Fernandes TRG, Llesuy SF, Belló-Klein A (2006). Peripheral markers of oxidative stress in chronic mercuric chloride intoxication. Braz. J. Med. Biol. Res., 39: 767-772.

Jadhav SH, Sarkar SN, Aggarwal M, Tripathi HC (2007). Induction of oxidative stress in erythrocytes of male rats subchronically exposed to a mixture of eight metals found as groundwater contaminants in different parts of India. Arch. Environ. Contam. Toxicol., 52(1): 145-151.

Khan AT, Atkinson A, Graham TC, Thompson SJ, Ali S, Shireen KF (2004). Effects of inorganic mercury on reproductive performance of mice. Food Chem. Toxicol., 42: 571-577.

Krone CA, Ely JT, Thoreson J (2002). Method for measuring mercury release from dental amalgam. Bull. Environ. Contam. Toxicol., 68: 180-186.

Kumar M, Sharma MK, Kumar A (2005). Spirulina fusiformis: A food supplement against mercury induced hepatic toxicity. J. Health Sci. 51(4): 424-430.

Langford NJ, Ferner RE (1999). Toxicity of mercury. J. Human hypertension, 13: 651-656.

Lowry OH, Rosebrough AL, Farr AL, Randall R (1951). Protein measurement with the Folin phenol reagent. J. Biol. Chem., 193: 265-275.

Lund BO, Miller MD, Woods JS (1993). Studies on Hg (II)-induced H_2O_2 formation and oxidative stress in vivo and in vitro in rat kidney mitochondria. Biochem. Pharmacol., 45: 2017-2024.

Magos L, Clarkson TW (2006). Overview of the clinical toxicity of mercury. Ann. Clin. Biochem., 43: 257-268.

Miller OM, Lund BO, Woods JS (1991). Reactivity of Hg (II) with superoxide: evidence for the catalytic dismutation of superoxide by Hg (II). J. Biochem. Toxicol., 6: 293-298.

Mozaffarian D (2009). Fish, mercury, selenium and cardiovascular risk: Current evidence and unanswered questions. Int. J. Environ. Res. Public Health, 6: 1894-1916.

NTP (1993). Toxicology and carcinogenesis studies of mercuric chloride (CAS no.7487-94) in F344/N rats and B6C3F1 mice (gavage studies). Research Triangle Park, NC: U.S. Department of Health and Human Services, National Institutes of Health; NIH publication no. 91-3139. (National Toxicology Program technical report no. 408). NTIS number: PB94-10649/XAB.

Patton CJ, Crouch SR (1977). Spectrophotometric and kinetic investigation of the Berthelot reaction for the determination of ammonia. Anal. Chem., 49: 464-469.

Perottoni J, Lobato LP, Silveira A, Rocha JBT, Emanuelli T (2004a). Effects of mercury and selenite on D-aminolevulinate dehydratase activity and on selected oxidative stress parameters in rats. Environ. Res., 95: 166-173.

Perottoni J, Rodrigues OED, Paixao MW, Zeni G, Lobato LP, Braga AL Rocha JBT, Emanuelli T (2004b). Renal and hepatic ALA-D activity and selected oxidative stress parameters of rats exposed to inorganic mercury and organoselenium compounds. Food Chem. Toxicol. 42: 17–28.

Ramalingam V, Vimaladevi V (2002). Effect of mercuric chloride on membrane-bound enzymes in rat testis. Asian J. Androl. 4: 309-311.

Rao MV, Sharma PSN (2001). Protective effect of vitamine E against mercuric chloride reproductive toxicity in male mice. Reprod. Toxicol.

15: 705-712.
Risher JF, Amler SN (2005). Mercury exposure: evaluation and intervention, the inappropriate use of chelating agents in diagnosis and treatment of putative mercury poisoning. Neurotoxicol. 26(4): 691–699.
Schurz F, Sabater-Vilar M, Fink-Gremmels J (2000). Mutagenicity of mercury chloride and mechanisms of cellular defence: the role of metal-binding proteins. Mutagenesis. 15(6): 525-530.
Sener G, Sehirli AO, Ayanoglu-Dülger G (2003). Melatonin protects against mercury(II)-induced oxidative tissue damage in rats. Pharmacol. Toxicol. 93(6): 290-296.
Sharma MK, Sharma A, Kumar A, Kumar M (2007). Spirulina fusiformis provides protection against mercuric chloride induced oxidative stress in Swiss albino mice. Food Chem. Toxicol. 45: 2412–2419.
Trinder P (1969). Ann. Clin. Biochem. 6: 24-27.
Vahter M, Åkesson A, Lind B, Björs U, Schütz A, Berglund M (2000). Longitudinal study of methylmercury and inorganic mercury in blood and urine of pregnant and lactating women, as well as in umbilical cord blood. Environ. Res. Section A. 84: 186-194.
Wadaan MAM (2009). Effect of mercury exposure on blood chemistry and liver histopathology of male rats. J. Pharmacol. Toxicol. 4(3): 126-131.
Weckbecker G, Cory JG (1988). Ribonucleotide reductase activity and growth of glutathione-depleted mouse leukemia L 1210 cells in vitro. Cancer Lett., 40: 257-264.
WHO (1991). World Health Organization. Inorganic Mercury, Environmental Health Criteria No 118, WHO, Geneva.
Wiggers GA, Peçanha FM, Briones AM, Pérez-Girón JV, Miguel M, Vassallo DV, Cachofeiro V, Alonso MJ, Salaices M (2008). Low mercury concentrations cause oxidative stress and endothelial dysfunction in conductance and resistance arteries. Am. J. Physiol. Heart Circ. Physiol., 295: 1033–1043.
Zalups RK, Lash LH (1996). Interactions between glutathione and mercury in the kidney, liver and blood. In: Chang0 LW, ed. Toxicology of Metals. Boca Raton: CRC Press; 145-163.