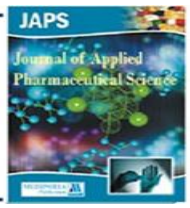
Journal of Applied Pharmaceutical Science

Available online at www.japsonline.com



ISSN: 2231-3354
Received on: 08-09-2011
Revised on: 13-09-2011
Accepted on: 19-09-2011

Effect of mercuric chloride on oxidative stress and target organ pathology in wistar rat

Sheikh T. J, Patel B. J and Joshi D.V

Sheikh T. J, Patel B. J and Joshi D.V
Regional Animal Disease Investigation Department, College of Veterinary Science and Animal Husbandry Sardarkrushinagar Dantiwada Agricultural University (SDAU) Banaskanth, Gujarat, India.

For Correspondence:
Sheikh T. J
Tel: +919052377854,
E-mail: drsheikh2 @yahoo.co.in

ABSTRACT

Mercury is the highly toxic metal that exerts its adverse effect on health of human and animals through air, soil water and food. For the present study, the toxic effect was observed by oral administration of mercuric chloride daily for 4 weeks at doses ranging from of 0 to 8 mg/kg/day. Histopathology was also carried out of both for kidney and liver. Significantl increased in lipid peroxidation and decreased Superoxide dismutase enzymes were observed in treated groups as compared to control . The gross changes were characterized by swellingand paleness of kidney and liver. Microscopic examination has revealed liver revealed hemorrhage, leukocytic infiltration, hepatocyte hypertrophy, necrosis and increased vacuolization incidence and the severity increased with increase treatment dose. In addition kidney showed proteineous cast in the lumen of tubules, narrowing of lumen, infiltration and necrosis.

Key words: Mercury, Lipid peroxidation, Superoxide dismutase, Liver, Kidney.

INTRODUCTION

Mercury is ubiquitous in the environment and is inevitable in both human and animals to avoid its exposure in some form or forms of mercury on a regular basis. Mercury occurs widely in the biosphere (Clarkson, 1987). In occupational and environmental settings, the most common cationic form of mercury encountered is mercuric form, which may have a valence of 1+ or 2+, depending on whether the mercuric ion is covalently bonded to a carbon atom of an organic side group, such as an alkyl group. Mammals and birds can be exposed to Hg pollution by two main routes *viz.*, inhalation of Hg vapor and ingestion of polluted food. The contribution of Hg inhaled from the air is negligible compared with intake from the food, except the occupational exposure. The water supply is also a relatively significant source of Hg. The toxicity of Hg is largely due to the high affinity to sulfahydral (SH) groups. Although the Hg compounds are highly specific for the sulfahydryl group, they are highly non-specific in their target because of the wide distribution of this group (Valko et al., 2005). One of the harmful effects of mercury action during its accumulation in a body in a region contaminated by mercury is the excessive release of reactive oxygen species and increased lipid peroxidation in the cells (Lund et al., 1993). Free radicals and intermediate products of peroxidation are capable of damaging the integrity and altering the function of biomembranes, which can lead to the development of many pathological process (Gutteridge, 1993). Various spesicific enzymes that limit free-radical formation, such as superoxide dismutase (SOD), catalase and glutation peroxidase, play an important role in the protection of cell membranes aganist oxidative damage (Faix et al., 2003).

In view of inadequate knowledge and vast dimension of the problem of environmental pollution and its animal and human related significance, the present study was undertaken to substantiate the renal toxicity of mercuric chloride in rat. In this context, the present study was designed to assess toxic effect of Mercuric Chloride in Wistar rats.

MATERIALS AND METHODS

Animal

Fourty Wistar rats of 5-7 weeks old were obtained from the Zydus Reseach centre, Zydus pharmaceutical company. Ahmedabad. The animals were housed in Polypropylene cages (5 animals /cage) and received water and pelleted food *ad libitum*. All rats were kept under controlled conditions of temperature (22±3ºC) and humidity (60±5%). They were given pellet food (Amrut feeds Ltd., Pune, India) and drinking water *ad libitum*. A twelve hour day and night cycle was maintained in the animal house. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC).

Mercuric chloride

Mercury was obtained in the form of Mercuric chloride from Merk India Ltd.(Mumbai India) and dissolved in the distilled water and administered through oral gavage.

Experimental Design

Experiment was conducted for four weeks.The animals were divided into following groups. Group I (n = 10) - Controlled animals (treated with vehicle alone), Group II (n = 10) - 2.0 mg/Kg $HgCl_2$, Group III (n = 10) - 4.0 mg/Kg $HgCl_2$, Group IV (n = 10) - 8.0 mg/Kg $HgCl_2$

Blood samples werecollected on 0 day, 14^{th} and 28^{th} day of experiment. Blood was collected similar time of day and by retro-orbital venous plexus puncture under ethyl- ether anesthesia.

OXIDATIVE STRESS ENZYMES:

LPO Estimation

Membrane peroxidative damage in erythrocytes was determined in terms of malondialdehyde (MDA) production by the method of Shafiq-U-Rehman (1984).

SOD Estimation

Superoxide dismutase was estimated as per the method described by Madesh and Balasubramanian (1998)

Histopathology

Tissue from the liver and kidney obtained from necropsied animals, and fixed in neutral buffer formalin and processed for hematoxylin and Eosin staining (Luna , 1968).

Statistical Analysis

Each value is expressed as mean and standard error (SE). One way analysis of variance (ANOVA) was used to compare each variable in the different studied groups. For all statistical comparisons a value of $p < 0.05$ was considered significant.

RESULT

Oxidative Stress Profile (See the Table – 1)

A significantly ($P < 0.05$) increase in lipid peroxidation was observed in Groups-III and IV post-treatment as compare with control animals, while on 28th day all mercuric chloride treated groups showed significantly ($P < 0.05$) higher values of lipid peroxidation as compared to control (TABLE No. 1). While A significant ($P < 0.05$) decrease in Superoxide dismutase levels were observed after 14 and 28 days of mercuric chloride treatment as compared to control.

Table 1 : Comparison of Mean (± S.E.) Lipid peroxidation and Superoxide dismutase in rats of different experimental groups at one to four weeks of post-treatment in rats of different experimental groups at one to four weeks of post-treatment.

ENZYMES	Group	DAYS INTERVAL		
		0 Days	14 Days	28 Days
LPO	I	4.64 ± 0.0	4.68 ± 0.01^{d}	4.73 ± 0.01^{d}
	II	4.67 ± 0.03	4.72 ± 0.0^{cd}	5.00 ± 0.04^{c}
	III	4.64 ± 0.0	4.82 ± 0.01^{b}	5.20 ± 0.05^{b}
	IV	4.64 ± 0.0	4.96 ± 0.03^{a}	5.72 ± 0.09^{a}
SOD	I	5.77 ± 0.01	5.75 ± 0.01^{a}	5.67 ± 0.01^{a}
	II	5.78 ± 0.01	5.26 ± 0.02^{b}	5.20 ± 0.03^{b}
	III	5.77 ± 0.0	5.12 ± 0.02^{c}	4.91 ± 0.06^{c}
	IV	5.79 ± 0.04	5.01 ± 0.05^{d}	4.25 ± 0.23^{d}

Lipid peroxidation- (nanomole (nM) of MDA formed per ml packed cells
Superoxide dismutase -1 unit of SOD is the amount (µg) of haemoglobin required to inhibit the MTT reduction by 50 %]

Grossly liver became pale and round border at the end of study. While liver from other groups *i.e.*, Groups-II and III revealed dark cherry color due to severe congestion. Microscopically, all treated groups liver revealed moderate to severe degrees of fatty changes (vacuolization) in hepatocytes (see image liver -II), sinusoidal dilatation, necrosis with congestion and hemorrhage. Severe congestion, hemorrhage along with moderate infiltration of mononuclear cells around central vein was also noticed (see image liver II). Karyorrhexis and scatted Red Blood Cells in hepatic cords were also present in treatment groups.

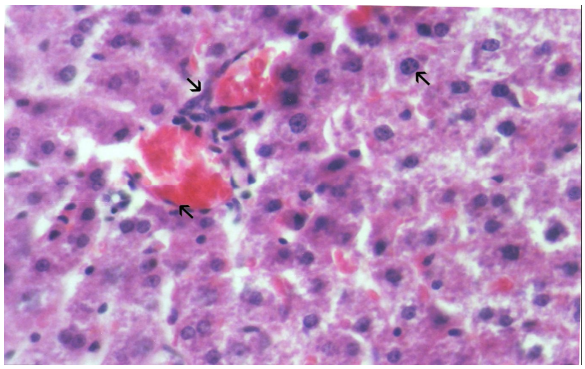


Fig 1 Histopathology of liver.

The gross changes of kidney were characterized by swelling, paleness of kidney, congestion and hemorrhage mostly in 4 and 8 mg/kg HgCl2 treated groups. Microscopic lesions with variable severity were noticed in kidney of all mercuric chloride treated rats. The lesions were characterized as various degrees of

haemorrhage, necrobiotic changes, and degenerative changes in tubular epithelium, hypercellularity of glomeruli, degeneration and intertabular haemorrhage, with prominent nuclei (see image Kidney -I), Congestion, edema, proteineous cast in lumen of tubules, and narrowing of lumen were also obsorved in different treated groups., while the medulla of kidney showed extensive haemorrhage with proteineous fluid filled lumen of tubuli (see image Kidney -I).

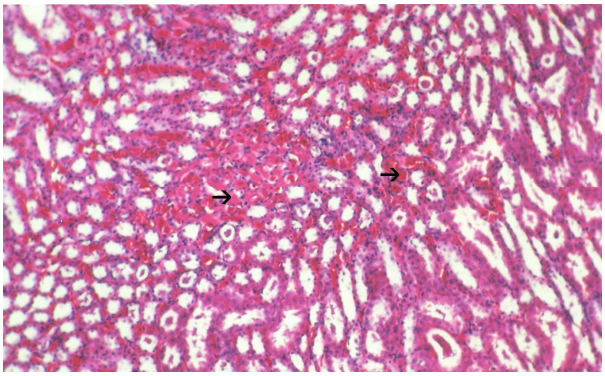


Fig 2 Histopathology of Kidney.

DISCUSSION

Oxidative stress is an important component of the mechanism of toxicity of metals. Acute exposure to mercury has been reported to increase LPO and decreases SOD (Gutierrez *et al.*, 2006) with 30 days experiment on Wistar rats.

The present study showed that acute exposure to mercury resulted in significantly ($P < 0.05$) increase in Lipid peroxidation in erythrocytes as evidenced by the increased production of malondialdehyde (MDA) in all treated groups. This was further associated with significant ($P < 0.05$) decreased in activity of antioxidant enzyme *i.e.*, Superoxide dismutase level in Red Blood Cell. Elevation of Lipid peroxidation in Red Blood Cell suggests formation of free radicals and participation of free radical induced oxidative cell injury in mediating the toxic effect of mercury. There is reduction in the antioxidant defense system (SOD) in mercury toxicity leading to disruption of pro-antioxidant balance in the body. The increase in Lipid peroxidation in Red Blood Cell might be due to peroxidation of unsaturated fatty acid in plasma membrane phospholipids of Red Blood Cell. Thus, increased Lipid peroxidation in Red Blood Cell is suggestive of progressive increase in cellular deformity, increase in membrane permeability and rigidity and disruption of structural and functional integrity of cell organelles.

Histopathology

Microscopic lesion in 28th day post-treatment includes, fatty changes, necrosis in hepatic cords. Scattered RBC in hepatic cords, along with degenerative changes, sinusoidal dilatation. In the present study, intense degenerative and necrobiotic changes among hepatocytes are suggestive of hepato-toxicity caused by mercuric chloride. These changes were due to consequence of inhibition of oxidative phosphorylation leading to ATP depletion and hepatocytes death.

Microscopically, haemorrhage, necrobiotic changes (Lindh and Johnsson, 1987), infiltration of mononuclear cells , Hypercellularity, degenerative, prominent nuclei (Dieter *et al.*, 1983), narrowing of lumen, low amount of proteineous cast in lumen of convoluted tubules, while medulla showed extensive haemorrhage, edematous fluid and dilatation of tubules were reported. The lesion observed in this study with higher intensity and necrosis only in second and third segment of proximal tubules and throughout medulla with 1 mg/kg HgCl2 dose in rats.

REFERENCES

Ahmed, K.E. Adam, S.E. Idris O.F. and Wahabi, A. A. Susceptibility of Nubian goats to mercury poisoning in Sudan. *Revue D-Elevage- et- de- Medicine –Veterinare-des Pays- Tropicaux*. 1999; 44 (2) : 123-129.

Carmignani, M.; Boscolo, P. and Preziosi, P. Renal ultrastructural alterations and cardiovascular functional changes in rats exposed to mercuric chloride. *Arch Toxiol.*, 1989 (Suppl. 13) : 353-356.

Clarkson, T. W (1987). Mercury. In: (Ed. Mertz, W.) Trace Elements in Human and Animal Nutrition. Academic Press, Inc., San Diego, Calif., 1:417-428.

Dieter, M.P.; Luster, M.I.; Boorman, G.A. *et al* . Immunological and biochemical response in mice treated with mercuric chloride. *Toxicol. Appl. Pharmacol.*, 1983; 68 : 218-228.

Faix, S.; Faixova, Z.; Michnova, E. and Varady, J. *et al.* Effect of per os administration of mercuric chloride on peroxidation processes in Japanese Quail. *Acta Vet. Brno.*, 2003;72:23-26.

Gutteridge JM. Free radicals in disease processes: a compilation of cause and consequence. *Free Radic. Res. Commun.* 1993;19: 141-158

Gutierrez, L.L.; Mazzotti, N.G.; Araujo, A.S.R.; Klipel, R.B.; Fernandes, T.R.G.; Llesuy, S.F. and Bello-Klein, A. Peripheral markers of oxidative stress in chronic mercuric chloride intoxication. *Journal of Medical and Biological Research.*, 2006;39(6):767-772.

Hall R.J.; Wilke, W.L. and Fettman, M.J. Renal resistance to mercuric chloride toxicity during prolonged exposure in rats. *Veterinary and Human Toxicology*. 1986;28(4):305-307.

Leecavlier, P.R.; Chu, I.; Villeneuve, D.; *et al*. Combined effects of mercury and hexachlorobenzene in the rat. Journal of Environmental Science and Health–Part-B. *Pesticide, Food Contaminants and Agricultural Waste*. 1994;29(5):951- 961.

Luna, L. G. (1968). Manual of histologic staining methods of the Armed Forces Institute of Pathology. 3rd Edition. McGraw-Hill Company, New York, New York, 258.

Lund BO, Miller DM, Woods JS. Studies on Hg (II)-induced H2O2 formation and oxidative stress in vivo and in vitro in rat kidney mitochondria. Biochem. Pharmacol. 1993;45: 2017- 2024.

Stoev, S. and Lazarova, S. Morphological investigation in experimental cases of mercuric poisoning in sheep. *Vet. Archiv.* 1998;68:163-171.

Valko M, Morris H and Cronin M T D. Metal Toxicity and oxidative stree. *Current Med c*hem, 2005;12:1161.