



Effect of Mercuric Chloride on Hepatic Phosphatases and Transaminases in Albino Rat

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Abstract: Mercuric chloride is a serious health hazard and produces various disorders. However, phosphatases and transaminases are marker enzymes of hepatic toxicity. Twenty four adult albino rats have taken and divided into 4 groups. Group one for acute study, while three for subacute studies with 3 rats in each. Control was also taken with similar references. Mercuric chloride gave orally administered ($LD_{50}=9.26\text{mg/kg b.w.}$) by gavage tube with distilled water. Rats were autopsized at predetermined time interval to assess hepatic toxicity. Phosphatases include alkaline phosphatase and acid phosphatase while transaminases include alanine transaminase and aspartate aminotransferase. Results revealed that ALP and ACP were significantly increased after acute and subacute treatment due to the destruction of the cell membrane of lysosomes. However, AST and ALT were also increased significantly due to toxic effect of mercuric chloride on hepatic cells. Hence, the present study demonstrates that mercuric chloride produces hepatic toxicity in the form of elevation of phosphatases and transaminases enzyme level.

Keywords: AST, ALT, ALP, ACP, *Rattus norvegicus*.

1. Introduction

Amongst known toxic heavy metals, “mercury” in any forms seems to be a ubiquitous environmental poison to any form of life. It is a serious pollutant (Environmental and occupational) with toxic effects in all living organisms (Berlin, 1987). Primary exposure occurs through environmental contamination as the result of mining, smelting, extensive industrial and agricultural usage, including inhalation and ingestion via the food chain (Hijova *et al.*, 2005). Mercury enters an organism in a variety of chemical forms (elemental, inorganic and organic), exhibiting its toxicologic characteristics, including neurotoxicity, nephrotoxicity as gastrointestinal toxicity with ulceration and haemorrhage (Clausen, 1993; Hua *et al.*, 1996; Longauer-Lewowicka and Zajac-Nedza, 1997; Deleu *et al.*, 1998; Gasso *et al.*, 2000). Most of inhaled vapour generated from metallic mercury is highly diffusible and lipid soluble and rapidly oxidizing to bivalent ionic mercury by complex catalase-hydrogen peroxide in the blood and is distributed through blood to various organs. It is well evident from the previous report

(Saxena and Mahour, 2006 and Mahour and Saxena, 2008).

The toxicity of mercury and its ability to react with and deplete free sulfhydryl groups are well known (Goyer, 1991). Depletion of protein-bound sulfhydryl groups results in the production of reactive oxygen species such as superoxide anion, hydrogen peroxide and hydroxyl radical. Although, mercury poisoning has been extensively studied, still little is known of the exact biochemical basis of various reported systems. Considering all these facts present investigations are carried out impact of mercury on, phosphatases and transaminases profile of *Rattus norvegicus*.

2. Materials and Methods

Albino rats (*Rattus norvegicus*) ranging in weight from 120-130gm with an average of $125\pm 2.36\text{gm}$, body size ranging from 15-16cm with an average of $15.5\pm 0.24\text{cm}$ and of 100 days of age from an inbred colony representing both the sexes were selected for experimentation. The rats were kept in polypropylene cages at the $20\pm 5^\circ\text{C}$ temperature, $50\pm 5\%$ humidity and

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10 hrs/day photoperiod. Rats were fed on pellet procured from M/s Lipton India Ltd., Kolkata and water was provided *ad libitum*.

Mercuric chloride was obtained from Bayer India Ltd., Mumbai. The acute oral LD₅₀ was determined on albino rats. The mercuric chloride was dissolved in distilled water and introduced orally by gavage tube @ 10ml/kg body weight. The data were analyzed by probit analysis (Finney, 1971) for LD₅₀ determination. Rats form the control set was given distilled water only.

Twenty four albino rats were divided into two sets of 12 each. The first set of 12 rats included four treatment groups, one for acute (1 day) and three for subacute (7, 14 and 21 days) studies for mercuric chloride with 3 rats in each group. The second set of 12 rats served as control having four groups viz. acute (1 day) and subacute (7, 14 and 21 days) with 3 rats in each group. The doses were introduced orally through a gavage tube for 1, 7, 14 and 21 days. The doses were selected on the basis of LD₅₀. The selected sublethal dose of 1/10 LD₅₀ was given to rats. The acute and subacute doses of mercuric chloride were 0.926mg/kg b. wt. and 0.044mg/kg b.wt. respectively.

In the present studies, animals received human care in compliance with the *Guide for the Care and Use of Laboratory Animals* prepared by the National Academy of Sciences and published by the National Institutes of Health (National, 1985).

All the experimental rats were taken out after a predetermined time interval and anaesthetized by chloroform. The liver was taken out for various hepatic enzymes like phosphatases (Kind and King, 1954) and transaminases (Reitman and Frankel, 1957). Phosphatases include alkaline phosphate (ALP) and acid phosphatase (ACP) while transaminases include Aspartate aminotransferase (AST) and alanine aminotransferase (ALT).

Statistical significance between experimental and control values were calculated according to Fischer's student 't' test (Fischer and Yates, 1951).

3. Results

Mercuric chloride showed a dose-response relationship pattern in the experimental animal. This dose-response relationship has been marked in the form of elevation in phosphatases and transaminases enzyme activities.

Acid phosphatase (ACP) and alkaline phosphatase (ALP) were significantly increased after acute (1d) and subacute (7, 14 and 21d) treatment of mercuric chloride in albino rat due to a toxic effect on hepatic tissues.

Similar observations have obtained in case of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) after acutely and subacutely intoxication of mercuric chloride in experimental animals.

4. Discussion

Phosphatase is a complex enzyme, which performs multiple cellular and metabolic functions such as growth differentiation, protein synthesis of certain enzymes and transport of phosphorylated intermediates across cell membranes and bone mineralization. Phosphatases are present in most tissues; the richest sources being osteoblasts in the bone, the bile canaliculi in the liver, the small intestinal epithelium etc. in all these sites it seems to be involved in the transport of phosphatase across cell membranes.

The elevation in activity of acid phosphatases in the liver of mercuric chloride treated rats suggested the increase in the secretion of hydrolytic enzymes from lysosomes. The lysosomal system has been shown to be very sensitive to changes in the intra and extracellular environment and subsequently many physiological and pathological processes. At the cellular level, lysosomes are important in the uptake, sequestration and bioaccumulation of various heavy metals.

In the present investigation elevation in alkaline phosphatase activity shows significant increase after acute treatment and highly significant increase after sub-acute (7, 14 and 21 d) treatment. The toxic effect of mercuric chloride enhances with the time duration of intoxication and it may be due to the presence of low molecular weight protein like metallothionein in the liver because Hg⁺⁺ readily reacts and forms complexes with organic ligands notably sulphhydryl groups. Metallothionein is an important intracellular sequestration site for toxic elements such as mercury, particularly in the liver. Continuous intoxication of mercuric chloride elevates, the concentration of metallothionein in the liver and for release of this effect, the alkaline phosphatase, a hydrolytic enzyme gets increased.

The increased enzyme activity of alkaline phosphatase in liver of mercuric chloride treated rats could be due to damage to the cell membrane of tissues, where this enzyme is firmly attached to the cell membrane joining the binary canaliculus and sinusoidal border of parenchyma cells (Mitra and Sur, 1997; Janbaz and Gilani, 2000; Hukkeri *et al.*, 2003; Nair, 2006 and Saxena and Mahour, 2006). Present findings gain support by Mehra and Kanwar (1986) and Dikshith *et al.*, (1989) who observed activity of liver alkaline phosphatase in rats after cadmium chloride intoxication.

Again, increase in liver alkaline phosphatase is an affirmation to and Johri *et al.*, (2004) following chromium VI and beryllium toxicity in albino rats respectively. Janbaz (2003), Biswas *et al.*, (2004), Kumar and Kumar (2004), Manjusha *et al.*, (2004), Zaman and Ahmed (2004) and Rathore and Varghese (2006) have also found similar responses following carbon tetrachloride, thioacetamide, allyl alcohol, mercuric chloride and copper respectively.

Table 1. Effect of sublethal doses of mercuric chloride on enzymological parameters of albino rat after acute (1 day) and sub-acute (7, 14 and 21 days) treatment.

Parameters	Treatment set	Mercuric chloride treatment (Mean±S.E.)			
		Acute		Sub-acute	
		1 day	7 days	14 days	21 days
Alkaline phosphatase (ALP) KA	Control	4.00±0.815	6.00±0.61	8.50±0.21	11.0±0.54
	Treated	7.00±0.408*	10.66±0.678**	13.50±0.61**	16.00±0.408***
Acid phosphatase (ACP) KA	Control	20.00±0.815	28.33±1.225	36.66±0.815	40.0±0.408
	Treated	25.00±0.815*	37.0±0.815**	40.83±0.613**	45.83±0.339**
Aspartate aminotransferase (AST) U/L	Control	46.4±0.76	46.9±0.86	42.36±0.88	40.33±0.54
	Treated	102.4±1.14**	103.5±0.78**	95.10±0.15**	91.6±0.65**
Alanine aminotransferase (ALT) U/L	Control	31.90±0.64	33.40±0.47	34.90±0.78	43.80±0.44
	Treated	70.80±0.92**	123.8±1.50**	86.50±0.84**	75.16±0.89**

***=<0.001; **=<0.01; *=<0.05

Hepatic hydrolytic enzymes are towards increase after acute and subacute studies reflecting hyper lysosomal activity. Further, destruction of the cell membrane of lysosomes under the stress of mercuric chloride could be considered as a possible reason for elevation in hepatic hydrolytic enzymes. Thus, evaluating acid and alkaline phosphatase activity useful information on the mode of action of mercuric chloride is ascertained which highlights its toxicity in albino rat.

On the other hand, transaminases play vital role in metabolism of non-essential amino acids. These enzymes commonly employed as diagnostic tools in the assessment of liver damage in clinical practice (Adolph and Lorenz, 1978; Goetz, 1980) and cellular damage of vital organs following exposure to toxic agents (Moss *et al.*, 1986). During cellular damage, these enzymes are leaked into serum and hence the elevation of the activities of these enzymes in serum is considered as a sensitive indicator of even minor cell damage because, the levels of these enzymes exceed those of extracellular fluid by more than three orders of magnitude (Moss *et al.*, 1986).

The intoxication of mercuric chloride in rats significantly increased the AST activity. Raised activity of serum transaminase in intoxicated rats as found in the present study can be attributed to the damaged structural integrity of the liver because these are cytoplasmic in location and are released into circulation after cellular damage. Sublethal doses of mercuric chloride result in cell lysis and cytoplasmic hepatic enzymes are released into blood circulation. The present findings are in affirmation to Nair (2006) and Saxena and Mahour (2006) who observed enhancement in the AST level in albino rats after cadmium chloride and mercuric chloride intoxication respectively.

The increase in AST level after metallic compound treatment is in accordance with Despande *et al.*, (1998); Janbaz *et al.*, (2003); Johri *et al.*, (2004); Manjusha *et al.*, (2004); Zaman and Ahmed (2004) and Kumar *et al.*, (2005) following carbon tetrachloride, chromium, paracetamol, beryllium, thioacetamide, cadmium chloride and mercuric chloride intoxication respectively.

Aminotransferase transfer an amino group from an alpha-amino acid to an alpha-keto acid. As a result of which different alpha-amino and different alpha-keto

acids are formed. Further, aminotransferase requires pyridoxal-5-phosphate as a cofactor which is present in adequate amounts normally, but it may be deficient in some pathological states leading to a reduced enzyme activity under stressful conditions. In the present investigation, this stress is mercuric chloride treatment.

Intoxication of mercuric chloride leads to significant increase in ALT activity after acute and subacute treatment and are in affirmation to Despande *et al.*, (1998); Janbaz *et al.*, (2003); Johri *et al.*, (2004); Manjusha *et al.*, (2004); Zaman and Ahmed (2004) and Kumar *et al.*, (2005) who also observed enhancement in ALT activity after carbon tetrachloride, chromium IV, paracetamol, beryllium, thioacetamide, cadmium chloride, mercuric chloride intoxication respectively.

The increase in ALT activity in experimental rats may be due to leakage of enzymes from the cytosol of liver, which gets entry into the bloodstream, results in high levels of enzyme activity which is reflected by pathogenicity in hepatic cells.

The present findings are in accordance to Nair (2006) and Saxena and Mahour (2006) who observed enhanced activity of ALT in rats after cadmium chloride and mercuric chloride intoxication respectively. ALT activity becomes an excellent indicator of mercuric chloride induced hepatocellular necrosis.

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