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Hepatoprotective effects of taurine against mercury induced toxicity in rats

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Abstract: *An attempt has been made to study the influence of taurine on mercury intoxicated rats. The animals were treated with sublethal dose of mercuric chloride (2 mg/kg body wt.) for 30 days. During the mercury treatment, the level of Aspartate transaminase(AST), Alanine transaminase(ALT) and Alkaline phosphatase(ALP) in serum and lipid peroxidation(LPO) in liver tissue significantly increased whereas Glutathione (GSH), Glutathione peroxidase(GPx), Catalase (CAT) and Superoxide dismutase (SOD) were simultaneously decreased in the liver tissue. Present results indicate that the liver tissue was completely damaged, after mercury treatment. In another group of animals, taurine (5 mg/kg body wt.) was administrated for another 15 days. Taurine administration was observed to improve the liver function in mercury intoxicated animal as indicated by the decline in increased levels of AST, ALT and ALP in serum and LPO content in liver tissue. The decreased level of antioxidant system (GSH, GPx, CAT and SOD) has been promoted. Results suggested that taurine played a vital role in reducing the mercury toxicity in intoxicated animals.*

Key words: *Mercury, Taurine, Rat, Antioxidants, Toxicity*
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Introduction

Human activities play a major role in polluting the environment by toxic and carcinogenic metal compounds. There are evidences that these metals by accumulating contaminate water sources and food chain with their compounds. Hence, industrial pollution of the environment with metal compounds is becoming a serious problem (Foulkes, 1990; Chougule *et al.,* 2005; Sarath Babu *et al.,* 2007). Unlike most organic pollutants, heavy metals are not degraded rather accumulate in the environment (Migliore *et al*., 1999) and food chain.

Mercuric chloride is an inorganic compound that is used in agriculture as fungicide in medicine as topical antiseptic and disinfectant and in chemistry as an intermediate in the production of other mercury compounds (NTP, 1993). Mercury and its compounds are widely used in industries and their hazards to animals have been well documented (Margarat *et al*., 2001; Kavitha and Jagadeesan, 2003; Jagadeesan, 2004; Sankar Samipillai and Jagadeesan, 2004, 2005). It comes from weathering process of earth's crust, industrial discharge, pest or disease control agent applied to plants, urbanization, surface runoff, mining, soil erosion, sewage effluent (Mitchel, 1972). Although, people know the adverse effects of mercury, they use mercury in electric apparatus, choloro-alkali plants, caustic soda and caustic potash industries *etc*. as well as in ayurvedic medicines, antiseptics, parasiticidal, fungicidal chemicals and also in the dentistry for amalgam fillings (Margarat *et al*., 2001; Clarkson, 2002; Jagadeesan, 2004; Sankar Samipillai and Jagadeesan, 2004, 2005). The toxic effect of mercury varies according to the chemical composition.

Taurine (2-aminoethane sulphonic acid) is the major free intra cellular amino acid found in millimolar concentration in many animal tissues especially muscle, brain, liver, heart *etc.* (Wright *et al*., 1986). It is an essential sulfonated beta amino acid derived from methionine and cysteine metabolism. Taurine is present in high concentration in most tissues particularly in proinflammatory cells such as polymorphonuclear phagocytes and in the retina (Droge and Breitkreutz, 1999). Metabolic action of taurine includes bile acid conjugation, detoxification, membrane stabilization, osmoregulation and modulation of cellular calcium level (Chesney, 1985; Birdsall, 1998; Huxtable, 1992; Redmond *et al*., 1996). The beneficial effect of taurine as an antioxidant in biological system have been attributed to its ability to stabilize biomembranes (Wright *et al*., 1986) scavanging reactive oxygen species (Wright *et al*., 1985).

Taurine may have protective effect on the tissue damage that results from oxygen free radicals in mercury induced toxicity. Within this point of view, the present study has been aimed to find out the ability of taurine to protect the liver tissue against mercury induced toxicity.

Materials and Methods

Normal adult female rats, *Rattus norvegicus*, of the wistar strain weighing ranging from 200 ± 5 g were used in the experiments. All the animals were fed on a standard rat feed (Hindustan Lever Ltd., Mumbai) and water *ad Libitum*. Experimental protocol was approved by the Institutional Animals Ethics Committee (IAEC) of RMMCH, Annamalai University.

Table - 1: Levels of AST, ALT and ALP in the serum of rat treated with mercuric chloride followed by taurine treatment

Parameters	Control	$HgCl_2$	$HgCl_2$ + taurine	Taurine
AST U/L	40.16±0.07	110.76±0.24*	44.52±0.51**	38.22±0.05
ALT U/L	18.25±0.08	53.64±0.04*	20.18±0.09**	18.58±0.16
ALP U/L	111.76±0.38	390.64±0.27*	121.64±0.37**	110.91±0.39

Mean ± S.D. of six individual observations, Significance *($p<0.05$) Group I compared with group II,
Significance **($p<0.05$) Group II compared with group III

Group I	Untreated control	Provided standard diet and clear water *ad libitum* and observed for 30 days
Group II	Mercuric chloride treatment	2 mg / kg body weight. Oral administration (dietary exposure) daily upto 30 days
Group III	Mercuric chloride	followed by taurine 2 mg/kg body wt. of mercuric chloride for 30 days followed by 5 mg / kg body wt. of taurine for another 15 days (dietary exposure).
Group IV	Taurine alone treatment	5 mg / kg body weight. Oral administration daily upto 15 days (dietary exposure)

Total weight of the diet was kept constant throughout the experimental period. After the scheduled treatments, the blood sample was taken from the tail vein and serum was trapped and then used for various enzymatic assays (AST, ALT and ALP) by adopting the method of King (1965) and then the animals were sacrificed by cervical dislocation. The whole liver tissue was isolated immediately from the animals in the cold room and then used for estimation of lipid peroxidation by the method of Nichans and Samuelson (1968), reduced glutathione by the method of Beutler and Kelley (1963), glutathione peroxidase by the method of Rotruck *et al*. (1973),catalase by the method of Sinha (1972) and superoxide dismutase by the method of Kakkar *et al.* (1984).

Statistical significance was evaluated using ANOVA followed by Duncan multiple range test (DMRT)(Duncan,1957).

Results and Discussion

The present work showed the increased levels of AST, ALT, ALP in the serum of rat when treated with mercuric chloride (Table 1). During the recovery period, the levels of AST,ALT,ALP activities were found close to normal (Table 1). In the liver tissue of mercuric chloride treated mice, the level of LPO was significantly enhanced and simultaneously GSH, GPx, CAT and SOD were significantly decreased. During recovery period, the levels of GSH,GPx,CAT and SOD were observed to be close to normal level (Table 2). These results suggested the mercury induced hepatotoxicity and oxidative stress in animals.

Mercury is a transition metal and it promotes the formation of reactive oxygen species (ROS) such as hydrogen peroxides. These ROS enhance the peroxides and reactive hydroxyl radicals (Miller *et al.*, 1999; Hussain *et al.,* 1999). These lipid peroxides and hydroxyl radical may cause cell membrane damage and thus destroy the cell. Mercury also inhibits the activities of free radical quenching enzymes such as catalase, superoxide dismutase and glutathione peroxidase (Benov *et al.*, 1990). AST and ALT also serve as biomarkers for liver function.

Mercury intoxication showed a significant increase in AST, ALT and ALP activities. These results may be due to hepatocellular necrosis which causes increase in the permeability of cell membrane resulting in the release of these enzyme in the blood stream (Rana *et al.*, 1996; Sharma *et al.*, 2002). Hwang *et al.* (2000), have also observed similar type of results in rat serum when treated with cadmium. Other studies also observed that the liver damage in Cd treated mice was mainly due to the elevation of AST ALT and ALP levels in serum (Dudley *et al.*, 1985; Hu *et al.*, 1991).

Lipid peroxidation is a chemical mechanism capable of disrupting the structure and function of the biological membranes, that occurs as a result of free radical attack on lipids. The ability of mercury to produce ROS was indicated in the present study by increased amount of hepatic lipid peroxides (LPO). Other studies have reported that intracellular generation of hydrogen peroxides (H_2O_2) could be involved in the initiation of mercury hepatotoxicity in mice (Kavitha and Jagadeesan, 2004; Durak *et al.*, 2002). Mercury causes cell membrane damage like lipid peroxidation which leads to the imbalance between synthesis and degradation of enzyme protein (Padi and Chopra, 2002). The excess production of ROS by mercury may be explained by its ability to produce alteration in mitochondria by blocking the permeability transition pore (Nicolli *et al.*, 1995; Kavitha and Jagadeesan, 2004).

Reactive oxygen metabolites (ROMs) are generated by a specialized phagocytic cells (neutrophils) as cytotoxic agents to fight invading micro-organism, a process known as the respiratory or oxidative burst. For this purpose, phagocytes use the membrane bound NADPH oxidase complex which catalyzes one electron, reduction of O_2 into O^-_2. The ROMs are generated in biological system via several enzymatic and non-enzymatic pathways (Morel *et al.*,1991). A variety of mammalian cell types are able to produce ROMs after specific stimulation (Gamaley and Klyubin, 1999). The

Table - 2: Level of lipid peroxidation and antioxidants in the liver tissue of rat treated with mercuric chloride followed by taurine treatment

Parameters	Control	$HgCl_2$	$HgCl_2$ + taurine	Taurine
Lipid peroxidation (nmoles/g wet wt.of tissue)	0.400±0.36	2.250±0.36*	0.403±0.09**	0.403±0.01
Reduced glutathione (μmoles/g wet wt.of tissue)	45.153±0.43	27.488±0.92*	45.642±0.51**	46.791±0.54
Glutathione peroxidase (μmoles/mg protein/min)	0.187±0.01	0.104±0.01*	0.205±0.02**	0.187±0.02
Catalase (μmoles/mg protein/min)	76.491±0.64	41.398±0.47*	80.865±0.80**	82.735±0.37
Super oxide dismutase (Units/mg protein)	16.622±0.87	9.401±0.46*	13.262±0.02**	17.313±0.35

Mean ± S.D. of six individual observations, Significance *($p<0.05$) Group I compared with group II,
Significance* *($p<0.05$) Group II compared with group III

ROMs are also produced by electron leakage from the transport chain in mitochondria and endoplasmic reticulam where molecular O_2 is sequentially reduced to O_2 and H_2O_2 (Chessman and Slater, 1993) when ROS begins to accumulate, hepatic cells exhibit a defensive mechanism by using various antioxidant enzymes. The major detoxifying system for peroxides are GSH and catalase (Meister, 1983). Catalase is an antioxidant enzyme which destroys H_2O_2 that can form a highly reactive radical in the presence of iron as catalyst (Gutteridge, 1995). Mercury leads to increased lipid peroxidation, oxidative stress and hepatotoxicity due to reduced antioxidant system (Kavitha and Jagadeesan, 2004; Wolf *et al.*, 1994).

In the present study, depletion of GSH content can account for the inhibition of GPx activity. In addition, high level of peroxides may cause the inhibition of catalase activity in liver tissue(Ghadermarzi and Moosavi- Movahedi, 1996; Mary and Reddy, 1999).

GSH plays a vital role in the liver in detoxification and regulating the thiosulphide status of the cell. Liver is the pool of glutathione generating factor which supplies to other organs. The liable pools of glutathione function as reservoir of cysteine. Glutathione may be consumed by conjugation reaction, which mainly involve metabolism of xenobiotic agent. However, the principle mechanism of hepatocyte glutathione turn over to be cellular efflux (Sies *et al.*, 1978; Horiuch *et al.*, 1978).

Glutathine peroxidase is well known to defence against oxidative stress, which in turn needs glutathione as co factors. GPx catalyzes the oxidation of GSH to GSSG, this oxidation reaction occurs at the expense of H_2O_2. SOD are family of metallo enzyme, which is considered to be a stress protein which is synthesized in response to oxidative stress (McCord, 1990). It has been detected in a large number of tissues and organism and is present to protect the cell from damage caused by O_2 (Fridovich, 1972).

In the present study, taurine supplementation significantly reduced mercury induced hepatotoxicity and oxidative stress. The reduced level of mercury toxicity in mercury toxicated animals manifested by the improvement in antioxidants and decreased level of LPO content (Table 2). Taurine has been demonstrated to protect against the hepatotoxicity induced by free radicals generating liver tissues (Margarat *et al.*, 2001; Sharma *et al.*, 2002; Koch *et al.*, 2004; Kavitha and Jagadeesan, 2004) . Taurine has been demonstrated to act as a direct antioxidant that scavenges oxygen free radicals, thus inhibiting lipid peroxidation and also as an indirect antioxidant that controls the increase in membrane permeability resulting from oxidative stress in liver (Koch *et al.*, 2004).

As an indirect antioxdidant, taurine has been proposed as a membrane stabilizer that can manage membrane organisation, prevent ion leakage and water influx and subsequently avoid cell swelling. Therefore, it is reasonable to assume that taurine may act as a good scavanger in reducing the production of lipid peroxidation induced by heavy metal (Hwang *et al.*, 1998; Koch *et al.*, 2004).

From this studyit can be concluded that taurine reduces the oxidative stress through inhibition of lipid peroxidation and also through increased GPx, CAT and SOD which replenish GSH stores and allows for correct cell defense against ROS by taurine. Hence, a dietary taurine play a vital role in reducing mercury toxicity in mercury intoxicated rats.

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