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Tribulus terrestris extract protects against mercury-induced oxidative tissue damage in mice

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Abstract

Mercury is a highly toxic metal which induces oxidative stress in the body. In this study we aimed to investigate the possible protective effect of *Tribulus terrestris*, an antioxidant agent, against experimental mercury toxicity in rat model. Following a single dose of 2 mg/kg mercuric chloride (HgCl2; Hg group) either saline or *Tribulus terrestris* (50 mg/kg) was administered for 15 days. After decapitation of the rats trunk blood was obtained and the tissue samples from the liver and kidney were taken for the determination of Lipid peroxidation and glutathione (GSH) levels. AST, ALT, BUN and Creatinine levels were assayed in serum samples. The results revealed that $HgCl_2$ induced oxidative damage caused significant decrease in GSH level, significant increase in LPO activity content of the tissues. Treatment of rats with *Tribulus terrestris* significantly increased the GSH level and decreased the LPO. Similarly, serum ALT, AST and BUN and Creatinine levels were elevated in the mercury group as compared to control group. On the other hand, *Tribulus terrestris* treatment reversed all these biochemical indices. Our results implicate that mercury-induced oxidative damage in liver, and kidney tissues protected by *Tribulus terrestris* extract, with its antioxidant effects.

1. Introduction

Mercury is a widespread environmental and industrial pollutant, which induces severe alterations in the tissues of both animals [1,2,3]. Various mechanisms, including lipid peroxidation have been proposed for the biological toxicity of mercuric chloride ($HgCl_2$),and it has been demonstrated that lipid peroxidation occurs in the kidney, liver and other tissues of the rats and mice following administration of $HgCl_2$ [4,]. Mercury is one of the oldest chemical elements used in human applications. In its elemental state, mercury is a silver-white liquid, being also known as metallic mercury (Hg^o). However, mercury may also be present in two oxidized forms [mercurous ion (Hg^{2+})and mercuric ion (Hg^{2+})] and as different organ metallic species(alkyl mercury, alkoxy mercury and phenylmercury), being thes hort chain alkyl mercury species, as methylmercury (CH_3Hg) and dimethylmercury ($(CH_3)2Hg$), the most dangerous compounds in terms of their toxicological effects. These organo metallic compounds have a higher solubility in lipids when compared to inorganic species, making it easier to diffuse through the lipidicmatrix of the cellular membrane, therefore increasing its toxicity potential [5]. Over the last decade evidence has accumulated for a role of reactive oxygen metabolites as a mediator of tissue injury in several animal models of toxicity including $HgCl_2$.The prooxidant properties of mercury are well established [6]. Woods *et al.* [7] investigated the etiology of mercury-induced porphyrinuria under in vitro conditions; their findings support the view that Hg(II) ions both compromise the antioxidant potential of GSH and promote formation of reactive species via thiol complexation. Stacey and Kappas [3] reported induction of lipid peroxidation associated with mercury treatment of isolated rat hepatocytes, and suggested a causative role of oxidative stress in mercury cytotoxicity.Mercury versatility as a metal explains its numerous applications in areas as different as industry, odontology, pharmacology,primary gold mining and agriculture. Many of these applications are based on the unusual ability of mercury to bind other metals making amalgams. In odontology, for example, mercury–silver amalgams are used to fill dental cavities. Whenever the filling is involved in the chewing of food, a tiny amount of mercury is vaporized reaching olfactory bulbs and brain [8]. Thus, mercury levels in brain may be proportional to the number of mercury containing amalgams present in tooth fillings. In fact, the study of Kingman *et al.* [9] carried out among a military

population from United States demonstrated that individuals with amalgams show four–five-fold higher concentrations of mercury in urine and blood. Although people know the adverse effect of mercury, they used mercury in electric apparatus, chloro-alkali plants, caustic soda, and caustic potash industry etc. a well as in ayurvedic medicines, antiseptics, parasiticidal, fungicidal effects and also in the dentistry for fillings[10].The cellular mechanisms by which mercury compounds exert their neurotoxic action were obtained from in vitro studies for which high concentrations were used in acute conditions. It has been proposed by Sorg *et al.* [6] that the mechanism of toxicity of mercury could be via binding to thiol groups. Since GSH is an important molecule in the cellular defense against chemically reactive toxic compounds or oxidative stress, a significant reduction in GSH levels as a consequence of mercury treatment leads to a reduction of effectiveness of the antioxidant enzyme defense system.

Tribulus terrestris L. is a member of the *Zygophyllaceae* family. It is an annual herb about 30–70 cm high and has pinnate leaves (of unequal length), yellow flowers and characteristic satellite shaped carpel fruits. Extracts from this plant have been used traditionally in treating a variety of diseases including hypertension and coronary heart disease, ocular inflammation and infertility in both sexes. The extracts have also been used as diuretics. Recent pharmacological studies tend to support these uses. Al-Ali *et al.* [11] have demonstrated diuretic activity in rats while Adaikan *et al.* [12] have shown that crude extract of *Tribulus terrestris* enhanced electrically- and nitroglycerine induced relaxation of the rabbit corpus cavernous consistent with a pro-erectile function. The fruits of *Tribulus terrestris* is a famous traditional Chinese medicine. In the Shern Nong Pharmacopoeia "the oldest known pharmacological work in China it is described as a highly valuable drug used to restore the depressed liver for the treatment of fullness in the chest and mastitis and also used to dispel the wind and clear the eyes for the treatment of acute conjunctivitis,headache[14]. *Tribulus terrestris* is also reported to have antimicrobial antihypertension diuretic antiacetylcholine and haemolyticactivity andantioxidant [13,14]. Based on these reports, this study was designed to determine the possible protective effect of *Tribulus terrestris* against oxidative damage of liver and kidney following oral administration of $HgCl_2$, by determining biochemical parameters.

2. Materials and Methods

Preparation of plant extract

The fruit of *Tribulus terrestris* were collected from the local areas of Chidambaram, Cuddalore District,Tamilnadu India. Then collected fruit were cleaned and shade-dried. The dried leaves were pulverized by a mechanical grinder and passed through a 20-mesh sieve. A powdered leaf (500 g) was successively extracted with petroleum ether, Chloroform and ethanol using a Soxhlet apparatus and water extracted by cold maceration. The extraction was carried out for 24 hrs at room temperature with mild shaking. The extracts were filtered and concentrated at 35° C, and the weight of each residue was recorded and percentage yield was calculated.

The wister strain mice weighing ranging from 20±5g were used in this experiments. They were divided at random into four groups (each of six rats). All the animals were fed on a standard mice feed and water *ad Libitum*. Experimental protocol was approved by the Institutional Animals Ethics Committee (IAEC) of RMMCH, Annamalai University. Wistar albino rats were divided into four groups each consisting of six animals: (1) saline (0.9% NaCl)-treated control group (C); (2) mercuric chloride (2 mg/kg orally for 30 days., single dose)-treated group (Hg); (3) mercuric chloride (2 mg/kg orally single dose) + *Tribulus terrestris*(5.0 mg/kg daily orally. for 15 days) treated group (Hg + *Tribulus terrestris*), (4) *Tribulus terrestris* (5.0 mg/kg daily for 15 days)-treated control group (*Tribulus terrestris*). After decapitation, trunk blood was collected; the serum was separated and measured the aspartate aminotransferase (AST), alanine aminotransferase(ALT) levels by the method of King,[20] and creatinine, by the method of Skeggs,[21] blood urea nitrogen (BUN)Bonseses and Taussly[22] and then the tissues were isolated and used for the lipid peroxidation by the method of Nichens and Samuelson [23] and glutathione by the method of Beutler and Kelley [24]. Statistical significance was evaluated using ANOVA followed by Duncan Multible Range Test (DMRT) (Duncan[25].

3. Results

Level of AST and ALT

The effects of *Tribulus terrestris* and mercury on the activities of AST and ALT in the plasma are shown in Tables1. It was found that the activities of AST and ALT in the plasma of rats fed with the supplement of mercury were gradually increased. The activities of AST and ALT in the plasma of rats are increased with the increasing level of mercury. The activities of AST and ALT in those rats fed diet with supplement of taurine were significantly to reduce the toxicity of

cadmium(*P*_0.05), indicating taurine might play protective effect on cadmium toxicity in rats.

Level of BUN and Creatinine

BUN and creatinine concentrations were studied to assess the renal functions, Mercury chloride induced significant elevations in BUN and Creatinine levels when compared to control values while *Tribulus terrestris* treatment to the mercury group kidney functions were found to be decreased. Similarly *Tribulus terrestris* treatment reduced the elevated serum BUN and Creaatine levels due to mercury administration(Table 1).

Table 1.Level of Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), blood urea nitrogen (BUN), and creatinine in serum of mice treated with mercury followed by *Tribulus terrestris*

Parameters	control	Mercuri chloride	Mercuric chloride+ *Tribulus terrestris*	*Tribulus terrestris*
ALT(U/L)	46.3±1.3	38.452.3±1.4*	52.3±1.4**	49.152.3±1.4
AST(U/L)	186.3±1.6	275.6±1.12*	196.5±1.0**	187.6±1.8
BUN(U/L)	24.6±0.6	48.9±0.37*	33.4±.98**	25.1±0.68
Creatinine(U/L)	0.48±0.65	1.27±0.35*	33.5±0.57**	0.52±0.72

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

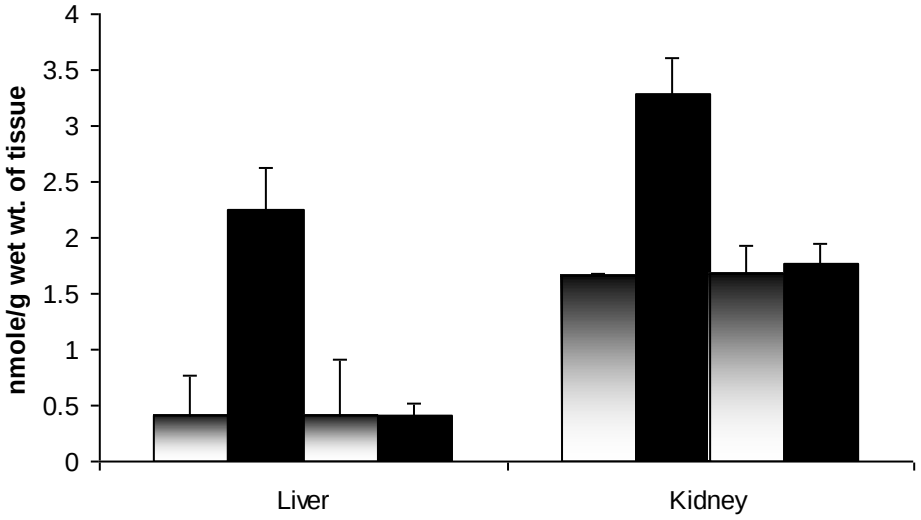

Fig.2 Level of Lipid peroxidation in liver and kidney tissues of mice treated with mercury followed by *Tribulus terrestris* Mean ± S.D of six individual observations; Significance *($p<0.05$) Group II compared with group I; Significance **($p<0.05$) group III compared with group II

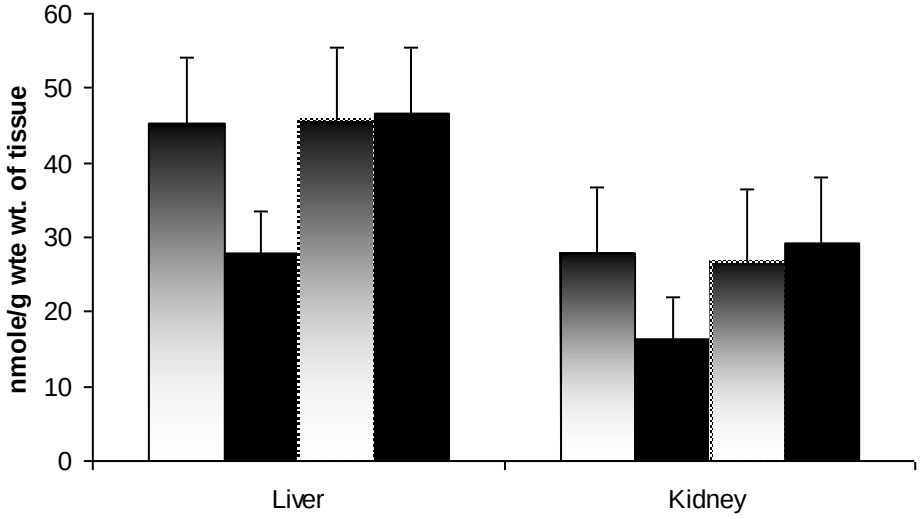

Fig.2 Level of Glutathione in liver and kidney tissues of mice treated with mercury followed by *Tribulus terrestris* Mean ± S.D of six individual observations ;Significance *($p<0.05$) Group II compared with group I; Significance **($p<0.05$) group III compared with group II

Level of lipid peroxidation,and Glutathione

As shown in content of MDA, an end product of lipid peroxidation,in the rats treated with mercury was significantly increased in liver and kidney($p < 0.05$) when compared to the vehicle control group. The treatment with *Tribulus terrestris* resulted in a significant decrease in LPO in liver and kidney tissues($p<0.05$) when compared to the mercury group. GSH levels in the liver and kidney tissues of saline-treated mercury group were significantly decreased ($p < 0.05$), in all the studied tissues were significantly increased when compared with the control group ($p< 0.05$). However, treatment with *Tribulus terrestris* significantly reversed the GSH and levels back to the control levels in all the tissues ($p < 0.05$).

4. Discussion

AST is responsible for transferring amino group from aspartate to α-β-glutaric acid forming glutamate and oxaloacetate. The rise in AST level is virtually responsible for all types of hepatic disease. Its peak concentration and ratio to other enzymes reflect the type of hepatic damage .ALT is responsible for transferring an amino group from alanine to α-ketaglutaric acid forming glutamate and pyruvate. It is well known that AST is very specific enzyme for hepatic tissue. It is more sensitive to hepatic damage and its level rises faster and higher in most types of hepato cellular damage.Blood urea nitrogen measures the amount of urea nitrogen, a waste product of protein metabolism in the blood. Urea is formed by the liver and carried out by the blood to the kidneys for excretion, because urea is cleared from the blood stream by the kidneys. Quantitative test of BUN is measuring the amount of urea nitrogen occurred in the blood. It is also useful to detect the function of kidney tissue. BUN is typically measured to assess kidney function. Creatinine is also one of the waste products of protein metabolism, which is excreted by the kidney in the urine. Creatinine is also used to measure the filtration rate of the kidney. The measurement of BUN and creatinine can be used to determine kidney function. BUN and creatinine are the indicators for the function of kidney.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 .GSH plays a vital role in the liver in detoxification reaction and regulating the thio sulphide status of the cell. Liver, is viewed as a glutathione generating factor which supplies to other organs. Liver is the pool of glutathione content. 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 (Jagadeesan and Sankarsamipillai,2007).In the present study, increases in lipid peroxidation, glutathione content due to toxic effects of $HgCl_2$ were accompanied by significant reductions in glutathione levels of the liver and kidney tissues, implicating the presence of oxidative tissue damage. Furthermore, these tissue

injuries caused functional impairment as evidenced with renal and hepatic function tests. *Tribulus terrestris* extract, as an antioxidant agent, ameliorated oxidative injury in the tissues and functional deteriorations. The prooxidant properties of mercury are well established. An imbalance in the antioxidant protective mechanisms leading to oxidative stress in the cells is being identified as a common factor in $HgCl_2$ exposure. A drastic decrease in the antioxidant enzymes, catalase and glutathioneperoxidase, accompanies a drastic increase in reactive oxygen species production. This could be due either to a loss of the cells expressing these enzymes, to a direct effect of reactive oxygen species on the enzymes, or due to a direct inhibition from mercury causing impairment of the antioxidant function and hence, increased reactive oxygen species production[6]. It has been reported that mercury can inactivate a number of enzymes by blocking the functional sites through binding to sulfhydryl groups, which are part of catalytic or binding domains[26]. Thus, it was suggested that in addition to depletion of intracellular thiol pools, the oxidant pathway may be a primary mechanism of induction of the response for Hg to induce oxygen free radicals or promote formation of lipid peroxides. Although it is difficult to quantitative reactive oxygen metabolites because of their reactive nature. As a free radical generating system, lipid peroxidation has been suggested to be closely related with Hg-induced tissue damage[27,28]. In fact, Kavitha and jagadeesan[23] reported that acute treatment with mercury induced the dramatic increase in reactive oxygen species accumulating in mice liver tissue leading to lipid peroxidation, protein degradation, and finally to cell death. Hg(II) applied chronically at low concentrations also seems to induce reactive oxygen species release as well as inhibiting the enzymes that neutralize reactive oxygen species, but during chronic exposure reactive oxygen species seems to be neutralized by antioxidant defense mechanisms of the cell. In the present study we observed a significant increase in LPO content, during mercury toxicity, which is in agreement with the previous studies, where lipid peroxidation products were increased[29]. Furthermore, since tissue damage causes functional impairments, in the saline-treated mercuric chloride group, increases in lipid peroxidation cause significant increases in both renal and liver function tests (AST,ALT,BUN,and creatinine respectively). Our results show that *Tribulus terrestris* treatment significantly inhibits MDA production, implying a reduction in lipid peroxidation and cellular injury that protect the tissues against mercury induced oxidative damage accordingly functional parameters were also improved. The beneficial effects of the traditional medicine of *Tribulus terrestris* was evident from the significanincrease in the body weight of the animal [30]. Different mechanism of action seem to underlie the pharmacological activity of *Tribulus terrestris*, and inhibition of biogenic amine uptake[12]. *Tribulus terrestris* extract contains many different flavone glycosides and terpenoids. Flavonoids seem to be responsible for the antioxidant activity that is considered one of the main mechanisms involved in the pharmacological effects of the extract. The protective activity of *Tribulus terrestris* against myocardialischemia/reperfusion injury is usually associated with the free radical scavenging activity of flavonoid components [31]. On the other hand, more recently Pietri *et al.*[32] demonstrated that terpenic components of the extract could be involved in the cardioprotective activity, probably by inhibiting free radical formation[32]. In the study of Margarat *et al*., [33], the protective effect of *Tribulus terrestris* on hepatic tissue in mice with chronic liver injury induced by mercury was attributed to the inhibition of lipid peroxidation. Furthermore, *Tribulus terrestris* was also shown to enhance the activity of antioxidant enzymes, superoxidedismutase and glutathione peroxidase, and protected liver tissues against ischemia/reperfusion injury[6].It has been demonstrated that the principal toxic effects of mercury arise from alterations in the structural integrity of mitochondrial inner membrane, resulting in loss of the normal cation selectivity which permits it to participate effectively in oxidative metabolism[1].The mitochondrial electron transport chain is the principal site of cellular production of reactive oxidants, superoxide(O_2^-) and hydrogen peroxide (H_2O_2). Action of mercury to perturb mitochondrial inner membrane function results in depletion of mitochondrial reduced glutathione content and increased formation of H_2O_2 by the mitochondrial electron transport chain in vitro[34]. It was suggested that such reactions occur in vivo following treatment with $HgCl_2$, and moreover, that increased H_2O_2 formation may be accompanied by increased peroxidation of mitochondrial lipids, consistent with an oxidative stress [1, 35]. In our study, glutathione levels of liver and kidney tissues were significantly decreased by Hg toxicity, and due to its antioxidant activity, *Tribulus terrestris* treatment reduced the Hg-induced oxidative injury and restored the GSH levels significantly. Thus, it seems likely that the alleviation of Hg-induced oxidative tissue damage by *Tribulus terrestris* involves the suppression of a variety of pro-inflammatory mediators produced by leukocytes and macrophages. Besides their direct damaging effects on tissues, ROS trigger the accumulation of leukocytes, which further enhance

the tissue injury when activated[36]. Reactive oxygen species can generate HOCl in the presence of neutrophil-derived MPO, and initiate the deactivation of antiproteases and activation of latent proteases,which lead to tissue damage[37]). In many diseases and acute inflammatory disorders, the important components of the pathologic process are linked to neutrophils' ability to release a complex assortment of agents that can destroy normal cells, and dissolve connective tissue. Increasing evidence suggests that mesengial cells and neutrophils release chemotactic substances, which further promote neutrophil migration to tissues, activating neutrophils and increasing injury[36]. Thus, observations of the present study demonstrate that $HgCl_2$ induces tissue injury both directly by promoting oxidative damage and by binding to tissue proteins, as well as indirectly by stimulating neutrophil infiltration. In conclusion, the present study demonstrates that acute$HgCl_2$ induced toxicity involves oxidative damage in various tissues. In this study it was also demonstrated first time that *Tribulus terrestris*, may afford the protection against acute $HgCl_2$ toxicity, by reduction of free radical accumulation and preventing neutrophil infiltration and GSH depletion. Therefore, its therapeutic role as a "tissue injury-limiting agent" must be further elucidated in oxidant induced tissue damage.

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