April 2010 ISSN : 0974 - 7427 Volume 4 Issue 1

Trade Science Inc.

BIOCHEMISTRY
An Indian Journal

Regular Paper

BCAIJ, 4(1), 2010 [49-53]

Hepatoprotective effect of *Solanum trilobatum* against mercury induced toxicity in rats

P.Muthukumaran*[1], S.Prabhu[1], K.Pattabiraman John Mohd[2]

[1]Department of Siddha Medicine, Faculty of Science, Tamil University, Thanjavur - 613 010, Tamilnadu, (INDIA)

[2]Kashmir Tibya College Hospital and Research Centre, Saidakadal- Srinagar, (INDIA)

E-mail: muthubabi_p@yahoo.co.in; dr.johnsoporee@yahoo.com

Received: 30th January, 2010 ; Accepted: 9th February, 2010

ABSTRACT

An attempt has been made to study the influence of *Solanum trilobatum* extract on mercury intoxicated rats. The animals were treated with sublethal dose of mercuric chloride (2mg/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 and Reduced glutathione (GSH), Glutathione peroxidase (GPx), Catalase (CAT) and Superoxide dismutase (SOD) were simultaneously decreased in the respective tissue. This result indicates that the liver tissue was completely damaged. After mercury treatment, *solanum trilobatum* extract (150mg/kg body wt.) was administrated in mercury intoxicated animals for another 15 days. *Solanum trilobatum* extract administration has improved the liver function in mercury intoxicated animal as indicated by declined in the increased level 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. This result suggested that *Solanum trilobatum* extract has play a vital role at the time of reducing the mercury toxicity in intoxicated animals.

KEYWORDS

Mercury;
Solanum trilobatum;
Rat;
Antioxidants;
LPO;
AST;
ALT;
ALP.

INTRODUCTION

Human activities play a major role in the pollution of environment with toxic and carcinogenic metal compound. There is an accumulating contamination of water sources and food chain with these compounds. Hence, industrial pollution of the environment with metal compounds is becoming a significant problem[8]. Unlike most organic pollutants, heavymetals are not degraded and accumulate in the environment[28] and food chain.

Mercuric chloride is an inorganic compound that has been used in agriculture as fungicides, in medicine as topical antiseptical and disinfectants, and in chemistry as an intermediate in the production of other mercury compounds[35]. Mercury and its compound are used widely in industries and their hazards to animal beings have been well documented[17,19,41,42]. Mercury

Regular Paper

and its compounds are used in a variety of products and manufacturing process. It comes from weathering process of earth's crust, industrial discharge, pest or disease control agent applied to plants, urbanization, surface run off, mining, soil erosion, sewage effluent[30]. Although people know the adverse effect of mercury, they used mercury in electric apparatus, choloro-alkali plants, caustic soda, and caustic potash industry etc. as well as in ayurvedic medicines, antiseptics, parasiticidal, fungicidal effects and also in the denstistry for amalgam fillings[3,17,41,42]. The toxic effect of mercury varies according to the chemical composition.

Indian medicinal plants belonging to about 49 families were investigated as liver protective drugs[13] *Solanum trilobatum* Linn (Family : Solanaceae) is used in siddha system of medicine as an expectorant and and in the treatment of respiratory diseases, asthama chronic febrile infections, tuberclosis, cardic and liver diseases[32] Sobatum, beta-solamarine, solaine, solasodine, glycoalkaloid and diosogenin and tomatidine are the constituents isolated from this plants[46] This plant possesses a broad spectrum of antibiotic antibacterial, antimitotic and anticancer activity[23] No detailed study has been conducted on the hepatoprotective activity of *Solanum trilobatum* .Therefore, the present study was carried out to explore hepatoprotective efficacy of *Solanum trilobatum* extract against mercury induced hepatotoxicity in animal models.

MATERIALS AND METHODS

Preparation of plant extract

One kg of plant materials (whole plant) was shade dried, coarsely powdered and allowed to soak in 2 l of 90 per cent alcohol for 48h at room temperature The extract was filtered and concentrated on a water bath to 20 ml. The inorganic material was precipitated and filtered off. The filterate was again concentrated and dried in vaccum. The yield of the extract was w/w of powderded methanol extract, which was stored in refrigerator for further use.

The doses of Solanum trilobatum extract were selected on the basis of acute toxicity study and the LD 50 of the extract was found to be 5g/ kg bodyweight. Solanum trilobatum extract administration did not produce any abnormalities such as atoxic, circling, lacrimation, labowed breathing etc., inthe animals throughout the experimental period. The present dose levelselected for the present study was non –toxic and safe.

Animals

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

Group I	Untreated control	Provided standard diet and clear water *ad libitum* and observed for 30 days
Group II	Mercuric Chloride treatment	2mg / kg body weight. Oral administration daily up to 30 days
Group III	Mercuric chloride followed by *Solanum trilobatum*	2mg/kg body wt. of mercuric chloride for 30 days followed by 150mg / kg body wt. of *Solanum trilobatum* for another 15 days. Oral administration daily up to 30 and 15 days respectively.
Group IV	*Solanum trilobatum* alone treatmentl	150mg / kg body weight. Oral administration daily up to 15days

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 enzyme assays (AST, ALT, ALP by adopting the method of King[21] 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[33], Reduced glutathione by the method of Beutler and Kelley[1], Glutathione Peroxidase by the method of Rotruck *et al*.,[40], Catalase by the method of Sinha[45], Superoxide dismutase by the method of Kakkar *et al*.,[18]

Statistical significance was evaluated using ANOVA followed by Duncan Multiple Range Test (DMRT)[6]

RESULT AND DISCUSSION

Mercury is a transition metal and it promotes the formation of reactive oxygen species (ROS) such as hydrogen peroxides. These ROS enhances the peroxides and reactive hydroxyl radicals[16,29]. 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 catalase, superoxide dismutase and glutathine peroxidase. AST and ALT also serve as biomarkers for liver function.

The present work showed the increased level of AST, ALT, and ALP in the serum of rat when treated with mercuric chloride (TABLE 1). During the recovery period, the level of AST, ALT, ALP activities were reached to near normal (TABLE 1). In the liver tissue, the level of LPO was significantly enhanced and simultaneously GSH, GPX, CAT and SOD were significantly decreased when treated with mercuric chloride. During recovery period, the level of GSH, GPX, CAT and SOD were reached to near normal level (TABLE 2)

TABLE 1 : Level of AST, ALT and ALP in the serum of rats treated with mercuric chloride followed by *Solanum trilobatum* treatment

Parameters	Control	$HgCl_2$	$HgCl_2$ + *Solanum trilobatum*	*Solanum trilobatum*
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

In the present work, AST, ALT and ALP in serum were significantly increased (TABLE 1) and enhancement of LPO content and simultaneously decreased level of GSH, CAT, GPx and SOD were observed in mercury intoxicated animals (TABLE 2). These results suggested that the mercury induced hepatotoxicity and oxidative stress in animals.

Mercury intoxication showed a significant increase in AST, ALT and ALP activities. This 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[37,43]. Hwang *et al*., (2000) have also observed similar type of results in rat serum when treated with cadmium. They are also observed that the liver damage is mainly responsible for elevating the AST ALT, and ALP level in Cd intoxicated animals serum.[5,15]

TABLE 2 : Level of lipid peroxidation and antioxidants in the liver tissue of rats treated with mercuric chloride followed by *Solanum trilobatum* treatment

Parameters	Control	$HgCl_2$	$HgCl_2$ + *Solanum trilobatum*	*Solanum trilobatum*
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

Lipid peroxidation is a chemical mechanism capabe 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[7,20]. Mercury causes cell membrane damage like lipid peroxidation which leads to the imbalance between synthesis and degratation of enzyme protein[36]. The excess production of ROS by mercury may be explained by its ability to produce alteration in mitochondria by blocking the permeability transition pore[20,34].

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

Regular Paper

NADPH oxidase complex which catalyses one electrons reduction of O_2 into O. The ROMs are generated in biological system via several enzymatic and non-enzymatic pathways[31]. A variety of mammalian cell types are able to produce ROMs after specific stimulation[10]. The ROMs are also produced by electrom leakage from the transport chain in mitochondria and endoplasmic reticulam where molecular O_2 is sequentially reduced to O_2 and H_2O_2[2] when ROS beginning to accumulate, hepatic cells exhibit a defensive mechanism by using various antioxidant enzymes. The major detoxifying systems for peroxides are GSH and catalase[27]. Catalase is an antioxidant enzyme which destroys H_2O_2 that can from a highly reactive radical in the presence of iron as catalyst[12]. Mercury leads to increased lipid peroxidation, oxidative stress and hepatotoxicity due to reduced antioxidant system[20,47].

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[11,25].

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 turns over to be cellular efflux[14,44].

Glutathine peroxidase is well known to defense against oxidative stress, which in turn needs glutathione as co factors. GPx catalyses 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[26]. 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[9].

Mohanan et al[24] suggested that sobatum, an active constituent of *Solanum trilobatum extract* possesses freeradicals scavenging activity. The phytoconstituents of *Solanum trilobatum* extract such as solasodine exert antioxidative property, supporting the present findings.

In the present study, *Solanum trilobatum* extract 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).

From these study we conclude that 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 *Solanum trilobatum* extract . Hence, a diatary *Solanum trilobatum* extract play a vital role in reducing mercury toxicity in mercury intoxicated rats.

ACKNOWLEDGEMENT

The authors are thankful to Professor and Head, Department of Siddha medicine, Faculty of sciences, Tamil university for providing necessary lab facilities to carry out the work successfully.

REFERENCES

[1] E.Beutler, B.M.Kelley; Experimental, **29**, 97 **(1963)**.
[2] K.H.Chessman, T.F.Slater; British.Med.Bull., **49**, 481 **(1993)**.
[3] T.W.Clarkson; Environ.Health.Perspect., **110**, 11-23 **(2002)**.
[4] W.Droge, R.Breitkreutz; Curr.Opin.Nut.Metab. Care., **2**, 493-498 **(1999)**.
[5] R.E.Dudley, L.M.Gammel, C.D.Klasson; Toxicl. Appl.Pharmacol., **74**, 299 **(1985)**.
[6] B.D.Duncan; Biometrics, **13**, 359-364 **(1957)**.
[7] I.Durak, M.Kacmaz, B.Cimen, S.Bayakkocak, S.Elagum, S.Zturk; Transplant.Immunol., **10**, 255-258 **(2002)**.
[8] E.C.Foulkes; Biological Effects of Heavy Metal. In: 'Metal Carcinogenesis'. CRC Press. Florida, USA, **2**, **(1990)**.
[9] I.Fridovich; Acc.Chem.Res., **5**, 321 **(1972)**.
[10] I.A.Gamaley, I.V.Klyubin; Int.Rev.Cytol., **188**, 203 **(1999)**.
[11] M.Ghadermarzi, A.A.Moosavi-Movahedi; J.Enzyme Inhi., **10**, 167-175 **(1996)**.
[12] J.M.C.Gutteridge; Clin.Chem., **14**, 1819-1828 **(1995)**.

[13] S.S.Handa, A.Sharma, K.K.Chakraborti; Fitoterapia, **57**, 307-45 **(1986)**.

[14] S.Horiuch, M.Inoue, Y.Morino; Eur.J.Biohcme., **87**, 429 **(1978)**.

[15] C.C.Hu, C.J.Yem, M.L.Jang, C.B.Liu, W.K.Chen, C.Chung; Chung.Shan.Med.J., **2**, 97 **(1991)**.

[16] S.Hussain, A.Atkinson, S.J.Thompson, A.T.Khan; J.Environ.Sci.Health B, **34**, 645 **(1999)**.

[17] G.Jagadeesan; Biochem.Cell.Arch., **4**, 51-60 **(2004)**.

[18] P.Kakkar, B.Das, P.N.Visvanathan; J.Biochemistry and Biophysics, **211**, 131-132 **(1984)**.

[19] A.V.Kavitha, G.Jagadeesan; J.Expl.Zool., **6**, 213-219 **(2003)**.

[20] A.V.Kavitha, G.Jagadeesan; Tropical Biomed., **21**, 1-7 **(2004)**.

[21] J.King; In: 'Practical Clinical Enzymology', D.Van Nostrand, Co., London, 106-107 **(1965)**.

[22] O.R.Koch, G.Pani, S.Borrello, R.Colavitti, A.Cravero, S.Farre, T.Galeotti; Mol.Asp.Med., **25**, 191-198 **(2004)**.

[23] P.V.Mohanan, K.S.Devi; Cancer Lett.; **112**, 219-23 **(1997)**.

[24] P.V.Mohanan, K.Rathinam, K.S.Devi; Indian J.Pharmacol., **29**, 129-31 **(1997)**.

[25] C.V.Mary, S.L.N.Reddy; Mus Musculus J.Environ.Biol., **20**, 103-106 **(1999)**.

[26] J.M.McCord; Is Superoxide Dismutase a Stress Protein? In 'Stress Proteins in Inflammation' Edited by R.Barbon, C.Rice-Evans, D.Blake, C.Winrow. (Richelien, London), 125 **(1990)**.

[27] A.Meister; Selective Modification of Glutathione Metabolism Science, **22**, 472-478 **(1983)**.

[28] L.Migliore, L.Cocchi, C.Nesti, E.Sabbioni; Environ.Mol.Mutagen., **34**, 279-284 **(1999)**.

[29] O.M.Miller, B.O.Lund, J.S.Woods; J.Biochem. Toxicol., **6**, 293 **(1999)**.

[30] R.Mitchel; 'Water Pollution Microbiology', John Wiley & Sons, New York, 1-65, 205-288, **(1972)**.

[31] F.Morel, J.Doussiere, P.V.Vignais; Eur.J.Biochem., **201**, 523 **(1991)**.

[32] K.M.Nadkarni; 'Indian Material Medica', 3rd Ed., Bombay: Popular Prakasan Pvt. Ltd., **1**, 1153-4 **(1976)**.

[33] W.G.Nichans, B.Samuelson; E.J.Biochemistry, **6**, 126-130 **(1968)**.

[34] A.Nilcolli, E.Basso, V.Petronilli, R.M.Wenger, P.Bernard; J.Biol.Chem., **271**, 2185-2192 **(1995)**.

[35] NTP; Toxicology and Carcinogenesis Studies of Mercuric Chloride in F_{344} Rats and $B_6C_3F_1$ Mice (Gavage Studies) National Toxicolopgy Programme Technical Reports Series 408, 265 **(1993)**.

[36] S.S.Padi, K.Chopra; Nephron., **92**, 685-692 **(2002)**.

[37] S.V.S.Rana, R.Singh, S.Verma; I.J.Exp.Biol., **34**, 177 **(1996)**.

[38] H.P.Redmond, J.H.Wang, D.Bouchier-Hayes; Arch.Surg., **131**, 1280-1287 **(1996)**.

[39] C.A.Rice-Evans, C.Diplock; Free.Rad.Biol.Med., **15**, 77 **(1993)**.

[40] J.T.Rotruck, A.L.Pope, H.E.Ganther, A.B.Swanson, D.G.Hafteman, W.G.Hoekstra; Science, **179**, 588-590 **(1973)**.

[41] S.Sankar Samipillai, G.Jagadeesan; Ind.J.Environ. Toxicol., **14**, 29-33 **(2004)**.

[42] S.Sankar Samipillai, G.Jagadeesan; I.J.Expl.Zool., **8**, 295-300 **(2005)**.

[43] M.K.Sharma, M.Kumar, A.Kumar; I.J.Expl.Biol., **40**, 1079-1082 **(2002)**.

[44] H.Sies, G.M.Bartoli, R.F.Burk, Waydhas; Eur.J. Biochem., **89**, 113 **(1978)**.

[45] K.A.Sinha; Analytical Biochemistry, **47**, 389-394 **(1972)**.

[46] J.Subraramani, P.C.Josekutty, A.R.Metha, P.N.Bhatt; Indian J.Exp.Biol., **27**, 189 **(1989)**.

[47] A.Wolf, C.F.Trendelenburg, C.Diez-Fernandez, P.Prieto, A.Cordier; J.Pharm.Exp.Ther., **280**, 1328-1334 **(1994)**.