Available online at www.journalijmrr.com

INTERNATIONAL JOURNAL OF MODERN RESEARCH AND REVIEWS

ISSN: 2347-8314

International Journal of Modern Research and Reviews
Volume1, Issue 1, pp 20-23 December,2013

ORIGINAL ARTICLE

HEPATOPROTECTIVE ACTIVITY OF *TRIBULUS TERRESTRIS* ON EXPERIMENTAL LIVER DAMAGE IN MICE

T.Sugunavarman and *G. Jagadeesan

Department of Zoology, Annamalai University, Annamalai nagar,608002 Tamilnadu

Article History: *Received 18th Nov,2013, Accepted 24th Nov,2013, Published 16th December2013*

ABSTRACT

The aqueous extract of *Tribulus terrestris* was tested for hepatoprotective activity against mercury-induced hepatotoxicities in mice. *Tribulus terrestris* exhibited significant hepatoprotective activity by reducing mercury induced change in bio-chemical parameters that was evident by enzymatic examination. The present study shows the activity of Aspartate transaminase(AST), Alanine transaminase(ALT), Alkaline phosphatase(ALP), and Bilirubin were increased and Glutathione(GSH) content was decreased in mercury treatment. During the *Tribulus terrestris* extract treatment these biochemical parameters reached near normal level. The plant extract may interfere with free-radical formation, which may conclude in hepatoprotective action against mercury toxicity.

Keywords: *Tribulus terrestris*, Mercury, Biochemical study, Mice

1. INTRODUCTION

Heavy metals are known for their strong action on biological tissues (More *et al*., 2005). Metal ions once absorbed into the body are capable of reacting with a variety of active binding sites and then disturbing the normal physiology of an organism which may lead to the death of organism. Harper *et al* (1978) have reported that the toxic effect of heavy metals on enzyme system depends on the capacity of toxicants to react with ligands, which is essential for the normal functioning of that system. The heavy metals may cause injury to organism and the damaged tissues shall dysfunction, which results in altered enzyme activity. Thus enzyme bioassay can provide diagnostic tool to assess a change or damage caused to organism due to administration of heavy metals. In clinical medicine, serum enzyme analysis has been used for decades to diagnose, both the site and extent of organ injury. 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◦). 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 the short chain alkyl mercury species, as methylmercury (CH_3Hg) and dimethylmercury (CH_32Hg), 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 lipidic matrix of the cellular membrane, therefore increasing its toxicity potential (USEPA,1997).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 (Tiwari and Srivastava, 2001). 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 (Tiwari and Srivastava, 2001).Bilirubin is a bile pigment and is formed from haemoglobin in the reticulo endothelial system and then it circulates, attached to the albumin, and its accumulation is a measure of hepatocyte formation and the rate of erythrocyte degradation (Rahman *et al*., 1999; Margarat 2001; Kavitha, 2001).

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

Corresponding author:
Dr.G.Jagadeesan, Department of Zoology, Annamalai University,Annamalai Nagar-608002

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.,(2003) have demonstrated diuretic activity in rats while Adaikan *et al.,* (2000) 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(Kavitha and Jagadeesan,2003;Margarat and Jagadeesan,2000)

Tribulus terrestris is also reported to have antimicrobial antihypertension diuretic antiacetylcholine and haemolyticactivity and antioxidant (Kavitha and Jagadeesan,2003;Margarat and Jagadeesan,2000). 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. The present study was carried out to determine the effect of the aqueous extract of the herb on experimental liver damage induced by mercury.

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.

Animals

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 mice). 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 RMMCH, Annamalai University.

Experimental design

The dosage of mercuric chloride has been determined from our previous study as sufficient to elicit mild or moderate oxidative stress for mercuric chloride (Kavitha and Jagadeesan,2004), while *Tribulus terrestris*, is effective for antioxidant effect(Kavitha and Jagadeesan,2006). The plant *Tribulus terrestris* (TT) popularly known as puncture vine is a perennial creeping herb with a worldwide distribution. Since ancient times it is regarded as an aphrodisiac in addition to its beneficial claims on various ailments such as urinary infections, inflammations, leucorrhoea, oedema and ascites(Chemexcil,1992). The extract (TT) (obtained from Sopharma, Bulgaria & Tegushindo,Indonesia) from the air-dried aerial parts of the plantcontains steroidal glycosides (saponins) of furostanoltype, the predominant furostanol being protodiosc in protodioscin which constitutes about 5% of the extract(Dicova and Ogayanova,1993). The aqueous extracts of *Tribulus terrestris* was prepared by boiling 95-g fine powder of fruits of *Tribulus terrestris* in 400 ml distilled water for 30 min and then filtering it with a Whatman filter paper twice. Then removal of solvent and drying yield the extract. Wistar albino mice 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., single dose for 30 days)-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) and Alkaline phosphatase levels The liver tissue was used for the estimation of Reduced glutathione(GSH).

Estimation of serum Aspartate amino transferase (AST) and Alanine amino transferase (ALT)

The activity of AST and ALT was determined by adopting the method of King (1965). 1 ml of substrate (AST-1.33g of L.aspartic acid and 15 mg of α- Ketoglutaric acid were dissolved in 20.5 ml of phosphate buffer and 1N sodium hydroxide to adjust pH 7.5 and made upto 50 ml with phosphate buffer; ALT – 1.78g of DL-alanine and 30mg of α-ketoglutaric acid were dissolved in 20 ml of buffer. The pH was adjusted to 7.5 with 1N sodium hydroxide and made upto 100 ml with buffer. A few drops of chloroform was added) was taken in a clean test tube and it was incubated for 5 minutes at 37°C. Then 0.2 ml of serum was added in the test tube and incubation was maintained for an hour in the case of AST and 30 minutes for ALT. The reaction was arrested by adding1.0 ml of DNPH reagent and then the tubes were kept at room temperature for 20 minutes. Then 10 ml of 0.4N sodium hydroxide solution was added and the colour developed was read at 520 nm against a reagent blank in UV spectrophotometer. Pyruvic acid was also treated in similar manner for the standard. The activities of serum AST and ALT are expressed as U/L of serum.

Assay of alkaline phosphatase (ALP) in serum

Serum ALP was assayed by the method of King, (1965). 1.0ml of buffer substrate was added to 0.2 mL of serum and incubated at 37°C for one hour. The tubes were removed and 1.0 mL of 10% TCA was added, mixed and centrifuged to 10 minutes 1.0 mL of super natent was treated with 1.0 mL ammonium molybdate and 0.4 mL of ANSA. A system devoid of enzyme served as control. A series of potassium dihydrogen phosphate standards in the concentration of 2-8 μg were also processed

similarly. The absorbance was measured in spectrophotometer at 620 nm.

Estimation of serum bilirubin

Serum bilirubin content was estimated by the method of Malloy and Evelyn (1937).0.2 ml of serum was taken in a clean dry test tube. The following reagent mixture consisting of 1.8 ml of distilled water, 0.5 ml Diazo reagent and 2.5 ml of methanol was added and then kept in the room temperature for 30 minutes. The colour developed was read at 540 nm against the reagent blank in the UV-spectrophotometer. Bilirubin was used to construct the standard graph. The values are expressed as mg/dl of serum.

Estimation of reduced glutathione (GSH)

The level of reduced glutathione was determined by the method of Beutler and Kellay, (1963).The isolated tissue was homogenized in phosphate buffer and centrifuged at 2500 rpm for 5 minutes. 0.2 ml of the supernatant was taken in a clean test tube and 1.8 ml of EDTA solution was added. To this 3.0 ml of precipitating reagent was added and mixed thoroughly and kept for 5 minutes before centrifugation at 3000 rpm for 10 minutes. After centrifugation the aliquot was filtered. 2.0 ml of the filterate was taken in a clean test tube and then 4.0 ml of 0.3M disodium hydrogen phosphate solutions and 1.0 ml of DTNB reagent were added. The appearance of yellow colour was read at 412 nm in UV spectrophotometer. Reduced glutathione was used to construct the standard graph.The values are expressed as µmole/mg wet wt. of tissues

Statistical analysis

Statistical significance was evaluated using ANOVA followed by Duncan Multible Range Test (DMRT) (Duncan,1957).

3. RESULTS

The aqueous leaf extract of *Tribulus terrestris* was found to e practically nontoxic when administered orally to mice and its LD_{50} value was found to be 2g/kgbody wt. Administration of mercury caused significant liver damage, as evidenced by the altered serum biochemical parameters. post-treatment of mice with *Tribulus terrestris* aqueous extract exhibited marked protection against mercury-induced hepatotoxicity, which is shown in Tables 1. The aqueous extract of *Tribulus terrestris* showed significant hepatoprotective activity against mercury.

Table 1. Effect of an aqueous extract of *Tribulus trrestris* extract on mercury induced hepatotoxicity in mice

Parameters	control	Mercuri chloride	Mercuric chloride+ *Tribulus terrestris*	*Tribulus terrestris*
ALT(U/L)	126.3±1.3	823.4±1.4*	119.8±1.4**	129.1±1.4
AST(U/L)	62.3±1.6	190.27±1.1*2	59.5±1.0**	65.6±1.82
ALP(U/L)	139.6±0.6	249.9±0.37*	128.4±.98**	143.1±0.68
Bilirubin(U/L)	1.01±0.65	3.45±0.35*	0.99±0.57**	0.97±0.72
GSH(nmole/mg wet wt. of tissue)	10.65±1.02	6.74±1.04*	9.81±1.65**	11.54±0.98

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

4. DISCUSSION

Hepatic cells appear to participate in a variety of enzymatic metabolic activities and mercury produced marked liver damage at the given doses as expected (Roderick etal., 1989; Kenneth et al., 1992). Administration of mercury elevated the serum levels of AST,ALT ALP and bilirubin significantly, due to its enzymatic activation of mercury free radical, which in turn alters the structure and function of liver cells (Singh etal., 1998) The changes in AST and ALT activities could be expected in association with a pathology involving necrosis of the liver tissues of the animal, when the plasma AST increases in such cases and escapes to the plasma from the injured hepatic cells. In addition, plasma ALT level is also useful in indicating the existence of liver diseases, as this enzyme is present in large quantity in the liver. It increases in serum when cellular degeneration or destruction occurs in this organ (Hassoun and Stohs, 1995). During the recovery period (Mercuric chloride followed by *Tribulus terrestris*), the increased level of bilirubin decreased to reach near normal level. This result suggests that *Tribulus terrestris* prevent the disintegration of bile pigments in the blood. The increased number of hepatocytes and uniformed shape of the hepatocytes were observed in the liver of mice when treated with *Tribulus terrestris*. This may be due to membrane stablization function of *Tribulus terrestris* in the liver tissue of rats. El-Demerdash (2004) reported that selenium proved to be beneficial in decreasing the level of bilirubin in the serum of heavy metal intoxicated rats. Roy *et al*., (2006) also suggested that *Psidium guajava* leaf extract reduced the level of bilirubin in the CCl_4 intoxicated rat serum. Posttreatment with *Tribulus terrestris* aqueous extract showed a dose-dependent protection against the injurious effects of mercury that may result from the interference with cytochrome P450, resulting in the hindrance of the formation of hepatotoxic free radicals(Sharma et al., 1994; Nadeem et al., 1997). Posttreatment with aqueous v extract restored the depleted GSH concentration near normalcy and also brought down the elevated levels of SGOT, SGPT, ALKP and bilirubin. These biochemical restorations may be due to the inhibitory effects on cytochrome P450 or/and promotion of its glucuronidation(Wesley et al., 1992; Gilman et al., 1992). Further studies are in progress to isolate the active constituents and also to evaluate the exact mechanism of action.

5. ACKNOWLEDGEMENT

The authors are grateful to Professor and Head, Department of Zoloogy, Annamalai University to complete the work successfully.

6. REFERENCES

Adaikan, P.G., Gauthamman,K., Prasad,R.N. and Ng,S.S.2000. Protractile pharmacological effects of tribulus terrestris on the rabbit corpuscavernosum. Annal of the Medicine Singapore,29:22-26.

Ak-Ali,M., Wabbi,S., Twaij,H. and Al-Badr,A. 2003. Tribulus terrestris. Preliminary study of its diuretic and contractile effects and composition with Zea mays.*J.Ethanopharmacol*.85:257-260.

Beutler, E. and Kelley, B. M. 1963. The effect of disodium nitrate on RBC glutathione. *Experientia* 29: 97.

Chemexcil,1992. Tribulus terrestris Linn. (N.O, Zygophyllaceae). In: Selected Medicinal plants of India(A Monpgraph of identity, safety and clinical usage). Complied by Bharatiya Vidya Bhavan's Swamy prakashnanda Ayurveda Research centre for Chemexcil. Tata Press, Bombay.pp. 323-326.

Dikova,N. and Ognyanova,P.1993. Pharmacokinetic studies of Tribesten. Anniversary Scientific Session'35. Chemica.

Duncan, B.D. 1957. Duncan's multiple range test for correlated and hetenoscedastic mean. *Biometrics*. 13: 359-364.

El-Demerdash, F.M. 2004. Antioxidant effect of vitamin E and selenium on lipid peroxidation, enzyme activities and biochemical parameters in rats exposed to aluminium. *J. Trace. Element Med. Biol*. 18: 113-121.

Gilman, A.G., Rall, T.W., Nies, A.S. andTaylor, P. 1992. The Pharmacological Basis of Therapeutics:13. Mc Graw Hill International Edition, London.

Harper, H.A., Rodwell, V.W. and Mayes, P.A. 1978. Review of physiological chemistry. 19th ed. Large Medical Publication. California.

Hassoun, E.A. and Stohs, S.J. 1995. Comparative studies on oxidative stress as a mechanism for the fetotoxicity of TCDD, endrin and linadane in C57 BL/6J and DBA/2J mice. *Teratology* 51 : 186.

Kavitha, A.V. 2001. Effect of mercuric chloride and the influence of *Tribulus terrestirs* on the digestive system of mice, *Mus musculus* (Linn) M.Phil. Thesis, Annamalai University, Tamil nadu, India.

Kavitha, A.V. and Jagadeesan, G. 2003. Invitro studies on the role of Tribulus terrestris extract on the mercury intoxicated mice, Mus musculus large intestine. A histological survey. *J.Exp.Zool. India*. 6:213-219

Kavitha, A.V. and Jagadeesan, G. 2006. Role of *Tribulus terrestris* (Linn.) (zygophyllacea) against mercuric chloride induced nephrotoxicity in mice, *Mus musculus* (Linn.) *J. Environ. Biol*. 27: 397-400.

Kenneth, L.M., Howard, F.M., Brain, B.H., David, W.N.1992. Melmon and Morrelli's clinical pharmacology. In:Basic Principles in Therapeutics, vol. 233. McGraw-Hill,London, p. 799.

King, J. 1965. In: Practical clinical enzymology var Nortant, D. Company. London. 106-107.

Mally, H.T., Evelyn, K.A. 1937. Estimation of serumbilirubin level. *J. Biol. Chem*. 191, 481.

Margarat A. and Jagadeesan, G. 2000. Effect of *Tribulus terrestris* extract on mercuric chloride poisoning in mice,*Mus musculus*-a biochemical study. *Ind. J. Environ. Toxicol*. 10: 14-15.

Margarat, A. 2001. Effect of *Tribulus terrestris* (zygophyllaceae) extract on mice, *Mus musculus* (Linn) exposed to sub-lethal dose of mercuric chloride Ph.D. Thesis, Annamalainagar. Tamil nadu, India.

More, T.G., Rajput, R.A. and Bandela, N.N. 2005. Effect of heavy metal on enzyme succinic dehydrogenase of freshwater bivalve, *Lamellidenus marginalis*. *Poll. Res*. 24: 675-679.

Nadeem, M., Dandiya, P.C., Pasha, K.V., Imran, M., Balani,D.K.and Vohora, S.B. 1997. Hepatoprotective activity ofSolanum nigrum fruits. *Fitoterapia* 3, 245.

Rahman, M.F., Siddiqui, M.K. J. and Gamil, K. 1999. Biochemical alterations induced by a new phosphorthionate (RPR-II) in tissues of male and female rats. *Ind. J. Exp. Boil*. 37: 546-552.

Roderick, N.M.M., Peter, P.A., Peter, J.S., 1989. . Pathologyof Liver, vol. 534. Livingstone, Great Britain, Churchill.

Roy, C.K., Kamath, U.K. and Sad, M. 2006. Hepatotoxicity of *Psidium guajava* Linn. leaf extract. *Ind. J. exp. Biol.*. 44: 305-311.

Sharma, A., Mathur, R. and Shukla, S. 1994. Hepatoprotectiveaction of a propriety herbal preparation against carbontetrachloride intoxication. *Indian Drugs* 32, 120.

Singh, B., Saxena, A.K., Chandan, B.K., Suri, O.P., Suri,K.A. and Sathi, N.K. 1998. Hepatoprotective activity ofverbenalin on experimental liver damage in rodents. *Fitoterapia*, 60, 135

Tiwari, A. and Srivastava, V.K. 2001. Acetaminophen poisoning induced enzymological and biochemical changes in liver and kidney of male mice, *Mus musculus Biochem. Cell. Arch*. 1: 71-76.

United States Protection Agency.1997. Mercury study reports to congress: health effects of mercury and mercury compounds. EPA.4562/R-97-007. Washington, DC,USA

Wesley, G.C., Brater, C.C. and Alice, R.J. 1992. Cloth's Medical Pharmacology, vol. 41. Mosby Year Book, US.