

Amelorative action of different solvent extractions of *Tribulus terrestris* (Linn.) extract on blood transaminase activities of mercuric chloride poisoned mice, *Mus musculus* (Linn.)

G. Jagadeesan* and A. Margarat[‡]

Toxicological division, Department of Zoology, Faculty of Science, Annamalai University, Annamalainagar-608002.

[‡] Government Arts and Science College, Villupuram

*Corresponding Author, Email: jaga_200@yahoo.co.in; sakipillai_200@yahoo.co.in

Keywords

Mercuric chloride
AST
ALT
Bilirubin
Tribulus terrestris

Abstract

Haematological parameters are considered as promising indicators of the physiological status of the animal. The present study was carried out on the influence of different solvent extractions of *Tribulus terrestris* extract on mercuric chloride (1.2 mg/kg body weight) intoxicated mice. An enhanced level of aspartate transaminase (AST), alanine transaminase (ALT) activities and bilirubin content were noticed in mercury intoxicated animal. The increased in the enzymatic activity may be due to the damage to the cells of liver caused by the heavy metal. But, the mercury intoxicated mice were treated with different solvent extraction of *Tribulus terrestris* showed the restoration of haematological parameters.

1. Introduction

The major route of absorption of environmental pollutant (heavy metal) is blood. It is the only medium of internal transporter, which directly contacts with various organs of the body and transport to the target tissues. A pathophysiological reflector of the whole body is blood and it is also play vital role for the regulation of life processes (1). Its' composition and constituents undergo changes due to the toxic effect of various toxicants (2-6). Vatal and Aiyar (7) observed changes in AST and ALT level in the blood of heavy metal treated rats. Haematoenzymological reports on mammalian system due to heavy metal treatments are scanty.

In the present study, showed how far mercury treatment has interfered with haematoenzymology. Various parameters such as AST, ALT and bilirubin of the blood reveal the physiological status of the animal. It is worth pointing out that there has not been any attempt to study the effect of herbal, *Tribulus terrestris* on mercuric chloride intoxicated mice. In this context, the present work was carried out on mice with reference to the effects of sub-lethal dose of mercuric chloride followed by different solvent extractions of *T. terrestris*.

2. Materials and Methods

Selection of plant

Tribulus terrestris L. (Zygophyllaceae) commonly known as caltrop or devil's thorn, Gokshura in

Sanskrit and Nerunji in Tamil, is an annual or perennial plant found trailing in sandy soils throughout India. It is used as Ayurvedic medicine in 52 to 141 herbal formulations. The beneficial effects are diuretic, tonic and curing the kidney diseases and also removal of stone from the kidney tissues (8-10).

Preparation of plant extract

Fresh leaves, fruits and flowers were collected and dried in shade in room temperature (25+-2°C) and powdered in an electric blender. Then, 250g powder of *Tribulus terrestris* was kept in the soxhlet apparatus. Soxhlation was done for the different solvents (pet-ether, benzene, ethyl acetate, chloroform and methanol) upto 24 hours for separating the contents, which were present in it.

Range finding test

Acute toxicity studies of *Tribulus terrestris* extract of different solvents were carried out on female Swiss albino mice. The solutions were prepared over a wide range of concentrations for *Tribulus terrestris* extract of different solvents (5,10,15,20 and 25mg/kg body weight of the animal). The range finding tests were conducted by allowing 6 mice in each dose after the oral administration of the extract. The rate of mortality was observed in each dose at different periods. The dose at which 100% survival was observed at maximum concentration for 15 days was considered as sub-lethal dose. Based on the range finding test, the sub-lethal dose for albino mice was

found to be 6mg /kg body weight of the animal for *Tribulus terrestris* extract of different solvents.

Experimental design

Sixty laboratories breed white mice, *M. musculus* (Linn.), 45 days old and weighing 25+ 0.5 gram were procured from the Department of Experimental Science, RMMCH, Annamalai University. After acclimatization, the mice were

divided into 4 batches (III and IV batches divided into 5 groups of six animals each) of required animals each. They were housed separately in suitable cage and fed on standard laboratory diet supplied by Hindustan laboratory Linmited, Mumbai and Tap water *ad-libitum*. Experimental protocol was approved by the Institutional Animals Ethics Committee (IAEC) of RMMCH, Annamalai University, Annamalai nagar – 608 002 India.

Batch I	Untreated control	Kept on standard diet and clean water <i>ad libitum</i> and observed for 45 days.
Batch II	Mercury treated	1.2 mg of mercuric chloride/kg body weight, orally, every day upto 45 days.
Batch III	Extract treatment on 45 days mercury intoxicated mice	
Group 1	Pet- ether extraction of <i>Tribulus terrestris</i> treated on mercury intoxicated mice.	6 mg of <i>Tribulus terrestris</i> extract /kg body weight orally, every day upto 15 days.
Group 2	Benzene extraction of <i>Tribulus terrestris</i> treated on mercury intoxicated mice.	6 mg of <i>Tribulus terrestris</i> extract /kg body weight orally, every day upto 15 days.
Group 3	Ethyl acetate extraction of <i>Tribulus terrestris</i> treated on mercury intoxicated mice.	6 mg of <i>Tribulus terrestris</i> extract /kg body weight orally, every day upto 15 days.
Group 4	Chloroform extraction of <i>Tribulus terrestris</i> treated on mercury intoxicated mice.	6 mg of <i>Tribulus terrestris</i> extract /kg body weight orally, every day upto 15 days.
Group 5	Methanol extraction of <i>Tribulus terrestris</i> treated on mercury intoxicated mice.	6 mg of <i>Tribulus terrestris</i> extract /kg body weight orally, every day upto 15 days.
Batch IV	Extract alone on untreated control mice	
Group 6	Pet- ether extraction of <i>Tribulus terrestris</i> alone	6 mg of <i>Tribulus terrestris</i> extract /kg body weight orally, every day upto 15 days.
Group 7	Benzene extraction of <i>Tribulus terrestris</i> alone	6 mg of <i>Tribulus terrestris</i> extract /kg body weight orally, every day upto 15 days.
Group 8	Ethyl acetate extraction of <i>Tribulus terrestris</i> alone	6 mg of <i>Tribulus terrestris</i> extract /kg body weight orally, every day upto 15 days.
Group 9	Chloroform extraction of <i>Tribulus terrestris</i> alone	6 mg of <i>Tribulus terrestris</i> extract /kg body weight orally, every day upto 15 days.
Group 10	Methanol extraction of <i>Tribulus terrestris</i> alone	6 mg of <i>Tribulus terrestris</i> extract /kg body weight orally, every day upto 15 days.

After the scheduled treatment, the animals were sacrificed by cervical dislocation and the blood was collected immediately and then used for following estimations.

Estimation of Serum Aspartate Transaminase (AST) and Serum Alanine Transaminase (ALT)

AST and ALT was determined by the method of king (11).

1ml of AST and ALT substrates (**AST substrate**– 1.33g of L-aspartic acid and 15 mg of α -ketoglutaric acid were dissolved in 20.5 ml of buffer and 1N sodium hydroxide to adjust P^H 7.5 and made up to 50 ml with the phosphate buffer, **ALT substrate** - 1.78g of D L- alanine and 30 mg of α - ketoglutaric acid were dissolved in 20 ml of buffer. The P^H was adjusted to 7.5 with 1N sodium hydroxide and made up to 100 ml with buffer. A few drops of chloroform were added to the substrates.) was taken into a clean test tubes and it was incubated for 5 minutes at 37°C. Then 0.2ml of serum was added in the test tubes and incubation was continued for an hour in the case of AST and 30 minutes for ALT. The reaction was arrested by adding 1.0 ml of colour reagent (200 mg of 2,4, dinitrophenyl hydrazine (DNPH) was dissolved in hot 1N hydrochloric acid and made up to 100 ml with hydrochloric acid). And then the tubes were kept at room temperature for 20 minutes. Then 10ml of 0.4N sodium hydroxide solution was added and the color developed was read at 520 nm against the reagent blank in the UV-spectrophotometer. A set of pyruvic acid was also treated in the similar manner for the standard.

The activities of serum AST and ALT are expressed as U/L of serum.

Estimation of Serum Bilirubin

Serum bilirubin was estimated by the method of Malloy and Evelyn (12).

0.2ml of serum was taken in a clean dry test tube. The following reagent mixture was added to the test tube. The reagent mixture, consisting of 1.8ml of distilled water, 0.5 ml of diazo-reagent (prepared freshly before use by mixing 10 ml of a 1% solution of sulphanic acid in 0.2N hydrochloric acid and 0.3 ml of 0.5% sodium nitrite solution) and 2.5 ml of methanol was added and then allowed to stand in the room temperature for 30 minutes. The color developed was read at 540 nm against the reagent blank in the UV-spectrophotometer. Bilirubin was used to construct the standard graph.

The serum bilirubin level is expressed as mg/dl of serum.

3. Results

Changes in Serum Parameters

The level of AST, ALT and bilirubin values noticed in the untreated control mice were 137.08 ± 4.469 U/L, 38.03 ± 2.953 U/L and 0.31 ± 0.068 mg/dl in the respective factors (Table- 1).

Serum Aspartate Transaminase (AST) level

The mercury intoxicated mice showed an increase in AST level. The percentage change over control was +67.296. During the recovery period, both increase and decrease trend of AST levels were observed in the serum of mercury intoxicated mice again treated with different solvent (Pet-ether, Benzene, Ethyl acetate, chloroform and Methanol) extractions of *Tribulus terrestris* respectively. The percentage change over mercury treated were +1.746, +64.628, -28.384, -18.777 and -22.270 respectively. The extract alone treated groups were showed +3.649, +55.474, +1.459, -8.759 and -20.437 percentage change over untreated control in the respective groups (Table- 1).

Serum Alanine Transaminase (ALT) level

An enhanced level of ALT activity was noticed in the mercury treated mice. The percentage change over untreated control was +20.457. During the recovery period, the mercury intoxicated mice were again treated with different solvent extractions of *Tribulus terrestris* for 15 days, the mice were showed both decreased and increased level of ALT activity in the respective groups. The percentage change over mercury treated were -5.631, +152.988, -10.973, +0.851 and +30.102 in the group 1 to group 5. The respective solvent extract alone treated mice also showed an enhanced level of ALT activity (+40.967, +53.983, +20.851, +29.108 and +22.008) (Table- 1).

Total Bilirubin

Treatment of mercury on untreated control mice showed considerable increase in the level of total bilirubin content in the serum. The percentage change over untreated control was +70.967. During the recovery period, the respective solvent extractions of *T. terrestris* treated mice were shows the decreased and an increased level (-15.094, +13.207, -37.735, -15.094 and +13.207) of total bilirubin content in the serum. Different solvents of *Tribulus terrestris* extract alone treated groups animals showed +6.451, +29.032, -3.225, +6.451 and +12.903 percentage change over untreated control of the respective groups (Table- 1).

Table 1. Changes in the hepatic marker enzymes and bilirubin level in mice, *Mus musculus*, treated for 45 days at sub-lethal concentration of mercuric chloride and mercuric chloride followed by 15 days of exposure to *Tribulus terrestris* extract of different solvents (Values expressed as AST – U/L; ALT – U/L; Bilirubin - mg / dl).

Name of the Parameters	Batch I	Batch II	<i>Tribulus terrestris</i> extract of different solvents (15 days)									
			Batch III					Batch IV				
			Group1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8	Group 9	Group 10
AST	137.08± 4.469	229.33± 5.878	233.33± 4.229	377.50± 5.057	164.33± 4.887	186.00± 3.829	178.16± 4.336	142.28± 4.783	213.18± 3.657	139.51± 2.363	125.08± 3.295	109.53± 6.481
% COUTC % COHgT		+67.296* +1.746	+70.072* +1.746	+175.182* +64.628*	+19.708* -28.384*	+35.766* -18.777*	+29.927* -22.270*	+3.649 -37.990*	+55.474* -6.986*	+1.459 -39.301*	-8.759* -45.414*	-20.437* -52.401*
ALT	38.03± 2.953	45.81± 3.294	43.23± 2.851	115.90± 3.263	40.78± 2.873	46.20± 3.546	59.60± 2.422	53.61± 2.117	58.56± 3.765	45.96± 3.250	49.10± 4.251	46.40± 3.874
% COUTC % COHgT		+20.457* -5.631	+13.673* -5.631	+204.759* +152.988*	+7.239 -10.973*	+21.483* +0.851	+56.718* +30.102*	+40.967* +17.026	+53.983* +27.832*	+20.851* +0.327	+29.108* +8.491	+22.008* +1.274
Bilirubin	0.31± 0.068	0.53± 0.094	0.45± 0.095	0.60± 0.129	0.33± 0.094	0.45± 0.095	0.60± 0.129	0.33± 0.074	0.40± 0.129	0.30± 0.081	0.33± 0.094	0.35± 0.076
% COUTC % COHgT		+70.967* -15.094	+45.161* -15.094	+93.548* +13.207	+6.451 -37.735*	+45.161* -15.094	+93.548* +13.207	+6.451* -37.735*	+29.032* -24.528	-3.225 -43.396*	+6.451 -37.735*	+12.903* -33.962*

Mean ± S.D (mean of six individual observations)

* Significance at 5 percent level of 't' test

% COUTC – percentage change over untreated control

% COHgT – percentage change over mercury treated

Batch I - Untreated control

Batch II – Mercury treated (45 days)

Batch III- Treatment on 45 days mercury intoxicated mice

Group 1 – Pet-ether; Group 2 – Benzene, Group 3– Ethyl acetate

Group 4– Chloroform; Group 5 – Methanol

Batch IV – Extract alone on untreated control mice

Group 6 - Pet-ether; Group 7 – Benzene; Group 8 – Ethyl acetate

Group 9 – Chloroform; Group10 -Methanol

4. Discussion

Aminotransferases are the enzymes having wide tissue distribution with the highest concentrations found in the liver and kidney tissues. Any alteration in the level of these enzymes in the serum/plasma indicates hepatocellular damage (13). Adolph and Lorenz (14) also pointed out that severe hepatocellular lesions with hepatic parenchymal cells necrosis are usually accompanied by a marked elevation of serum transaminases. Liver functions have been estimated by transaminase activities of serum. AST and ALT, which has been extensively studied during acute and chronic intoxication of the liver (15). Changes in metabolic activities of liver enzymes are related to the metabolic activation or detoxification of hepatotoxic compounds (16-19). Bilirubin is a product of hemoglobin degradation and its accumulation is a measure of the hepatocyte formation and the rate of erythrocyte degradation. Although it is not a sensitive indicator, bilirubin levels may raise in disease of hepatocytes (necrosis), or excretion (duct obstruction), defects in conjugating enzymes and in haemolysis (20). There may be a reduction in the number of functioning liver cells as in chronic hepatitis. So, that all liver functions are impaired. Bilirubin estimation is

reliable sensitive in the diagnosis of hepatic diseases (21).

In the present investigation, the increased level of AST, ALT, and bilirubin level in the serum were observed at sub-lethal concentration of mercuric chloride treatment for 45days. Normally, the blood serum contains higher level of AST than ALT. During the mercury treatment, the condition of the respective enzymes level was maintained, but, the increasing level of AST was higher than the ALT. Accumulation of toxicants beyond a tolerable level in the liver may cause histopathological and enzymatic changes (18 & 22). Mercury significantly raised the AST and ALT level and they serve as reliable markers of liver cell damage. Antioxidants succeeded in restricting the efflux of transaminases from liver to blood. Rana *et al.* (15) also reported that mercury inhibits key enzymes in the blood and also in several tissues like liver, kidney, brain etc. The increased AST and ALT were due to tissue damage and subsequent leakage or due to increased synthesis of aminotransferases (23 & 19). The liver damage indicated as the increase in ALT activity, which is known to be the most sensitive parameter of acute hepatotoxicity caused by the heavy metals (24 & 17). The elevation of bilirubin in mice mainly suggested that the cellular damage occurred in the liver tissue leading to the failure of excretion of

bilirubin into the bile. Blockage and damage of bile duct was also one of the reasons for the increased bilirubin level in the serum. The increased bilirubin may be due to the conditions such as toxic metal in which there is an extensive damage to liver cells but also a considerable degree of intrahepatic obstruction, resulting in appreciable increase (25). The another possible reason for elevated level of bilirubin was due to enhanced hemolysis.

During the recovery span, the 45 days mercury intoxicated mice, treated with *Tribulus terrestris* extract of different solvents were showed decreased level of AST in all groups except group1 and group2 than mercury intoxicated mice. These observations confirm improved liver functions in mice and group3 showed drastic recovery and also showed decreased level of bilirubin in all groups except group2 and group 5 than the mercury intoxicated mice. Decrease in serum bilirubin level after treatment with the extract indicates the effectiveness of the extract in normal functional status of the liver. This might be the possible reason for reduced bilirubin level in serum. These observations also suggested the protective action of extract against the damage of the liver. *Tribulus terrestris* is being used against various diseases for a long time for the strengthening of the body's resistance, restoring normal function of the body to consolidate the constitution and promoting blood circulation(26-28). In the present study, among the all solvent extraction, Benzene solvent extraction of *Tribulus terrestris* was not promote the liver function. Because the AST, ALT and Bilurubin levels were not restored. But in Pet-ether solvent extractions of *T. terrestris* administration was drastically restore the transaminase activities and bilurubin level to near normal level in the serum of mercury intoxicated mice. The present study suggest that the pet ethar solvant extractions of *T. terrestris* having more efficacy than the other solvant extractions to promote the elimination/nullify the mercury toxicity in mice. The elemination of metal led to decrease the free radicals level and the cell membrane damage was decreased. These might cause the less leak out of the enzymes such as AST and ALT into plasma. Similar type of results were observed by Rathore and Nandi (29) in cadmium intoxicated rabbits when administrated with Liv 52. Cadmium treatment increases AST and ALT activities, while Liv. 52 therapy restores them to normal values.

Acknowledgement

The authors are thankful to Professor and Head, Department of Zoology, Annamalai University for providing necessary facilities to carried out this experimental works Successfully.

References

- Jagadeesan, G. 2004. Mercury poisoning and its antidotes. *Biochem. Cell. Arch.* 4: 61-83.
- Harting, T., J. Steinpack and D. Smidt, 1981. Investigations on blood of European pigs raised under tropical conditions. *Anim. Res. Develop.*, 14: 101-118.
- Jagadeesan, G. and S. Vijayalakshmi, 1999. Effects of mercury followed by imercaprol treatment on protein metabolism in different tissues of *Labeo rohita* (Ham) fingerlings. *Ind. J. Environ. Toxicol.* 9: 33-35.
- Mohmadsadick, M.A. and Lalita Kaul, 2000. Induced selenium toxicity in broiler chicks: A haemato – biochemical study. *Indian J. Environ. Toxicol.*, 10 (1): 19-22.
- Sankarsami Pillai, S. and G. Jagadeesan, 2006. Anti Haematoprotective effect of Taurine against mercury induced toxicity in mice. *J. Haematol. & Ecotoxicol.* 1 (1): 21-28.
- Sankarsami Pillai, S. and G. Jagadeesan, 2009. Protective role of Taurine against mercuric chloride intoxicated rats. *Recent Res. Sci. and Technol.*, 1 (2): 81-87.
- Vatal, M. and A.S. Aiyar, 1988. Effect of lithium carbonate on serum glutamate oxaloacetate transaminase, glutamate pyruvate transaminase and lactate dehydrogenase of rats. *Indian J. Exp. Biol.*, 26(10): 801-803.
- *Anand, R., G. K. Patnaik, D. K. Kulshreshtha and B. N. Dhawan, 1994. *Indian J. Exp. Biol.*, 32(8): 548- 552.
- Gupta, S. K., R. Zafar and D. Pathak, 1997. Review on phytochemical and pharmacological aspects of *Tribulus terrestris* Linn. *Indian Drugs*, 34(6): 422- 426.
- Sangeeta, D., H.Sidhu, S.K.Thind and R.Nath, 1994. Effect of *Tribulus terrestris* on oxalate metabolism in rats. *J. Ethnopharmacol.*, 44: 61-66.
- King, J., 1965. In: *Practical Clinical Enzymology*. Van Nartand, D. Company, London, 106-107.
- Malloy, E. and K. Evelyn, 1937. The determination of bilirubin with the photoelectric colorimeter. *J. Biol. Chem.*, 119: 481-485.
- *Hess, B., 1962. *Enzymes in blood plasma*. Gorge Thieme Verlag, Stuttgart., 71-90.
- Adolph, L. and R. Lorenz, 1982. Enzyme diagnosis in disease of the heart, liver and pancreas. Salzweg passau, Germany, 83.
- Rana, S.V.S., Rekha Singh and Seema Verma, 1996. Protective effects of few antioxidants on liver function in rats treated with cadmium and mercury. *Indian J. Exp. Biol.*, 34(2): 177-179.

16. Rikans, L.E., 1984. Influence of aging on the susceptibility of rats to hepatotoxic injury. *Toxicol. Appl. Pharmacol.*, 73: 243-249.
 17. Yamano, T., Mitsuru Shimizu and Tsutomu Noda, 1998. Age related change in cadmium – induced hepatotoxicity in wistar rats: Role of kupffer cells and Neutrophils. *Toxicol. Appl. Pharmacol.*, 151(1): 9-15.
 18. Jagadeesan, G., A.V. Kavitha and J. Subashini, 2005. FT-IR Study of the influence of *Tribulus terrestris* on mercury intoxicated mice, *Mus musculus*, liver. *Tropical Biomedicine*, 22(1): 15-22.
 - 19.. Kavitha, A.V. and G. Jagadeesan, 2006. Recovery of phosphatase and transaminase activity of mercury intoxicated *Mus musculus* (Linn) liver tissue by *Tribulus terrestris* (Linn.) (Zygophyllaceae) extract. *Tropical Biomedicine* 23(1):45-51.
 20. Kissane, J.M., 1985. Liver. In: *Anderson's Pathology*. The C.V. Mosby company, St. Louis, 1099.
 21. Harper, H.A., 1961. The functions and tests of the liver. In: *Review of physiological Chemistry*. Large Medical publishers, Los Altos, California 1-44.
 22. Jagadeesan.G. and S. Sankar Samipillai, 2007. Hepatoprotective effects of taurine against mercury induced toxicity in rats. *J. Environ. Biol.* 28(4), 753-756.
 23. Rouiller, C.H., 1964. In: *Liver: Morphology, Biochemistry and physiology*. (Rouiller, C.H., Eds.), Academic Press, New York, London, Vol.2, 335-416.
 24. Dudley, R.E., D.J. Suoboda and C.D. Klassen, 1982. Acute exposure to cadmium causes severe liver injury in rats. *Toxicol. Appl. Pharmacol.*, 65: 302-313.
 25. Varley, H., 1988. Tests in liver and biliary tract disease. In: *Practical Clinical Bio- Chemistry*. 4th Eds., CBS Publishers and Distributors, Delhi, 349-359.
 26. Kavitha, A.V. and G. Jagadeesan, 2004. Influence of *Tribulus terrestris* (Linn.) against mercuric chloride induced hepatic damage in mice *Mus musculus* (Linn). *Tropical Biomedicine* 21: 1-7.
 27. Margarat A. and G. Jagadeesan, 2000. Effect of *Tribulus terrestris* extract on mercuric chloride poisoning in mice, *Mus musculus*-a biochemical study. *Ind. J. Environ. Toxicol.* 10: 14-15.
 28. 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.
 29. Rathore, H. S. and K. K. Nandi, 1992. Further studies on the protective effect of Liv- 52 against cadmium toxicity in mammalian system. *Indian Drugs*, 29(4): 149 -154.
- Originals not referred