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Recovery of phosphatase and transaminase activity of mercury intoxicated *Mus musculus* (Linn.) liver tissue by *Tribulus terrestris* (Linn.) (Zygophyllaceae) extract

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Abstract. The efficacy of the methanolic fraction (MF) of *Tribulus terrestris* fruit extract on mercury intoxicated mice, *Mus musculus* has been studied. At a median-lethal dose of mercuric chloride (12.9 mg/kg body wt.) administration an enhanced level of glutamate oxaloacete transaminase (GOT), glutamate pyruvate transminase (GPT) and simultaneously decreased level of acid phosphatase (ACP) and alkaline phosphatase (ALT) activities were noticed in the liver. Due to the mercury toxicity the liver cells are damaged to cause the alterations in their enzymes. During the recovery period, all the enzymological parameters are restored to reach near normal level. The result suggested that the oral administration of MF of *T. terrestris* fruit extract has (6 mg/kg body wt.) provided protection against the mercuric chloride induced hepatic damage in the mice, *M. musculus*.

INTRODUCTION

Pollution is one of the challenging problems to the environmental biologists, as varieties of heavy metals have potentially harmful effects on the biological organisms (Kavitha & Jagadeesan, 2003; Sankar Samipillai & Jagadeesan, 2005) including human beings. Heavy metal pollution seems to be the most persistent one (Harvey *et al.*, 1974). Heavy metals like Hg, Cu, Cd and Zn disorder mark physiological disorders in the organism and inhibit the enzyme activities (Ramalingam *et al.*, 2002). Phosphatases (acid and alkaline) are the hydrolytic enzymes occurring in the animal tissues like liver, kidney, brain, muscle and heart (Johal *et al.*, 2002). Acid phosphatase (ACP) is mainly a lysosomal enzyme, found in endoplasmic reticulam. Alkaline phosphatase (ALP) is a membrane bound enzyme, found in hepatocytes (Shakoori *et al.*, 1992). The liver is a major target organ for toxic compounds (Guillouzo *et al.*, 1995). The measurement of phosphatase activity is also useful as an indicator of liver function (Padmakumaran Nair *et al.*, 1998). Aminotransferases (GOT and GPT) are reliable marker enzymes of liver and they are the first enzymes to be used in diagnostic enzymology when liver damage has occurred (Whitby *et al.*, 1984; Kuchel & Ralston, 1988). The liver disorder is a serious health problem (Sethuraman *et al.*, 2003; Kavitha & Jagadeesan, 2004). As there is no reliable hepatoprotective drug in modern medicine, nowadays attention has been drawn towards medicinal recipes for liver ailments. Hence, it is of particular interest to explore the actions of *Tribulus terrestris* on mercury induced toxicity in mice, *M. musculus*.

MATERIALS AND METHODS

Plant procurement and Extraction

The fresh fruits of plant material were collected from October to December near

paddy fields located in and around Chidambaram area (5 km away from university campus) Tamilnadu, India and identified by a Taxonomist and preserved in the Department of Botany, Annamalai University, Annamalainagar, India.

Preparation of plant extract

Tribulus terrestris fresh fruits were collected and dried in shade at room temperature (25 + 2°C) and powdered in an electric blender. Then 250 g powder was kept in the soxhlet apparatus and soxhlation was done with the help of methanol solvent up to 24 hours for separating the contents which were present in it (Shiping Fang *et. al.*, 1999).

Experimental design

Thirty, forty-five days old laboratory bred white female mice, *M. musculus* (Linn.) of swiss strain were used in the experimental study. They were weighed upto 25 ± 0.50 g and were divided at random into 5 groups (each of six mice). The design of the experimental study is as shown in Table 1. Each group was housed separately in a suitable cage, fed with standard laboratory diet, and tap water *ad libitum*.

The diet was kept constant at 30 g pallets/ day/cage throughout the experiment. After the scheduled treatment, the animals were sacrificed by cervical dislocation and then the whole liver tissue was removed immediately in the cold room. The whole liver tissue was used for the following estimations.

Estimation of acid phosphatase

The activity of acid phosphatase was assayed with the method of Tennis Wood *et al.* (1976). The liver tissue was homogenized in glass homogeniser, using 10 ml distilled water and centrifuged at 3000 rpm for 10 minutes. 0.5 ml of supernatant was taken in a clean test tube and 0.5 ml of the substrate solution (*p*-nitrophenyl phosphate) and 0.5ml of 0.1N citrate buffer were added. The test tube with the above solution was kept in water bath maintained at 37ºC for 30 minutes. After completion of 30 minutes, the reaction was arrested in the extracts by adding 3.8 ml of 0.1N sodium hydroxide. The colour formed at the end was read at 415 nm in UV-visible spectrophotometer (Spectronic-20 Bausch and Lamb).

Values were expressed in µ moles of phenol liberated/min/100mg protein.

Estimation of alkaline phosphatase

The activity of alkaline phosphatase was assayed with the method of Tennis Wood *et al.* (1976). The liver tissue was homogenized in glass homogeniser, using 10ml of distilled water and centrifuged at 3000 rpm for 10 minutes. 0.5 ml of supernatant was taken in a clean test tube and 0.5ml of

Table 1. Experimental design of the study with groups treatment types and treatment procedure

Group I	Untreated control	Provided standard diet and clean water *ad libitum* and observed for 4 days
Group II	Mercuric chloride treatment	12.9 mg of mercuric chloride/kg body weight, oral administration daily up to 7 days
Group III	Post-treatment of MF of *T. terrestris* fruit extract	6 mg of *T. terrestris* fruit extract/kg body weight, oral administration daily up to 7 days on mercury intoxicated mice
Group IV	Pre-treatment of MF of *T. terrestris* fruit extract	6 mg of *T. terrestris* fruit extract/kg body weight, oral administration daily up to 7 days followed by $HgCl_2$ treatment for another 7 days
Group V	MF of *T. terrestris* fruit extract alone treatment	6 mg of *T. terrestris* fruit extract/kg body weight, oral administration daily up to 7 days

the substrate solution (*p*-nitrophenyl phosphate) and 0.5ml of glycine buffer were added. The test tube with above solution was kept in a water bath maintained at 37ºC for 30 minutes. After completion of 30 minutes the reaction was arrested in the extract by adding 10 ml of 0.2N sodium hydroxide. The colour formed at the end was read at 415 nm in UV-spectrophotometer (Spectronic-20, Bausch and Lamb).

Values were expressed in µ moles of phenol liberated/ min/100 mg protein.

Estimation of glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT)

The activities of GOT and GPT were determined using the method of King (1965). The liver tissue was homogenised with 5 ml of phosphate buffer and centrifuged at 3000 rpm for 10 minutes. 1 ml of each substrate [for GOT activities- 1.33g of L-aspartic acid and 15 mg of að-keto glutaric acid were dissolved in 20.5 ml of buffer and 1N sodium hydroxide to adjust the pH to 7.5 and made upto 50 ml with the phosphate buffer. GPT activities- 1.78 g of DL-alanine and 30mg of α-keto glutaric acid were dissolved in 20ml of buffer. The pH was adjusted to 7.5 with 1N sodium hydroxide and made upto 100 ml with buffer and then few drops of chloroform were added] was taken into clean test tubes and it was incubated for 5 minutes at 37ºC. Then 0.2 ml of tissue homogenate was added in the test tubes and incubated for 1 hour in the case of GOT and 30 minutes for GPT. The reaction was arrested by adding 1.0 ml of DNPH reagent and 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 the reagent blank in the UV-spectrophotometer (Spectronic-20, Bausch and Lamb). A set of pyruvic acid was also treated in a similar manner for the standard.

The activities of GOT and GPT values were expressed as IU/L.

RESULTS

Level of acid phosphatase (ACP)

In the normal untreated control, the level of ACP activity in the liver tissue showed 2.58 ± 0.13. At median-lethal dose of $HgCl_2$ treatment, the level of ACP activity in the liver tissue is significantly decreased up to 1.09 ± 0.01 µ moles of phenol liberated/ minute/100 mg protein (% COUTC: -57.47). During the recovery span, the post-treatment of fruit extract ($HgCl_2$ followed by MF of *T. terrestris*) enhanced the level of ACP activity in the mercury intoxicated liver to reach near normal level (% COHgT: +159.63). The pre-treatment of fruit extract (MF of *T. terrestris* followed by $HgCl_2$) also maintained the same increasing trend (%COHgT: +106.42). The fruit extract (MF of *T. terrestris*) alone treatment also showed slight increase in the level of ACP activity in the liver tissue (% COUTC: + 15.89).

Level of alkaline phosphatase (ALP)

In the normal untreated control, the level of ALP activity in the liver tissue showed 0.88 ± 0.02. At median-lethal dose of $HgCl_2$ treatment, the level of ALP activity in the liver tissue significantly decreased upto 0.63 ± 0.34 µ moles of phenol liberated/ min/100 mg protein (% COUTC: -27.74).

During the recovery span, the post-treatment of fruit extract ($HgCl_2$ followed by MF of *T. terrestris*) enhanced the level of ALP activity in mercury intoxicated liver tissue (% COHgT: + 31.19). The pre-treatment of fruit extract (MF of *T. terrestris* followed by $HgCl_2$) also enhanced the level of ALP activity in liver tissue (% COHgT: + 14.73). The fruit extract (MF of *T. terrestris*) alone treatment retained the near normal level of ALP activity in the liver tissue (% COUTC: -4.30).

Level of glutamate oxaloacetate transaminase (GOT)

In the normal untreated control, the level of GOT activity in the liver tissue showed 39.33 ± 0.83. At median-lethal dose of $HgCl_2$ treatment, the level of GOT activity

in the liver tissue significantly increased up to 62.65 ± 0.99 IU/L (% COUTC: + 59.28).

During the recovery span, the post-treatment of fruit extract ($HgCl_2$ followed by MF of *T. terrestris*) decreased the level of GOT activity (% COHgT: -41.33). The pre-treatment of fruit extract (MF of *T. terrestris* followed by $HgCl_2$) also maintained the same decreasing trend in the level of GOT activity in the liver tissue (% COHgT: -32.71). The fruit extract alone treatment (MF of *T. terrestris*) retained near the normal level of GOT activity in the liver tissue (% COUTC: + 2.01).

Level of glutamate pyruvate transaminase (GPT)

In the normal untreated control, the level of GPT activity in the liver tissue showed 43.69 ± 0.93. At median-lethal dose of $HgCl_2$ treatment, the level of GPT activity in the liver tissue significantly increased upto 58.06 ± 0.84 IU/L (% COUTC: + 32.90).

During the recovery span, the post-treatment of fruit extract ($HgCl_2$ followed by MF of *T. terrestris*) drastically decreased the level of GPT activity in mercury intoxicated liver tissue to reach near the normal level (% COHgT: -28.57). The pre-treatment of fruit extract (MF of *T. terrestris* followed by $HgCl_2$) also showed the decreased level of GPT activity in the liver tissue (% COHgT: -49.16). The fruit extract alone treatment (MF of *T. terrestris*) restored the near normal level of GPT activity in the liver tissue (% COUTC: + 1.76).

DISCUSSION

In the present study, the animals were treated with LD_{50} value of $HgCl_2$, the liver tissue showed decreased level of ACP and ALP activities. Loss of ALP activity in the liver tissue of mercury intoxicated mice is a consequence of changes in the permeability of plasma membrane in addition to changes in the balance between synthesis and degradation of enzyme protein (Hardonk & Koudstaal, 1976) thus lowering the enzyme activity. El-Dermerdash (2001), stated that $HgCl_2$ intoxication significantly decreases the ACP and ALP activities in rats. In the liver, it is closely connected with lipid membrane in the canalicular zone, so that any interference with the bile flow, whether extra-hepatic or intra-hepatic leads to decrease in ACP and ALP activities. Mercury is known to react with sulphydryl groups on the cell membrane and impair its function (Rothstein, 1959), which is possible cause for inhibition of both ACP and ALP activities. Shakoori *et al.* (1992) have suggested the decrease (or) inhibition of ACP and ALP activities are due to increased necrosis in the tissues like hepatocytes.

Aminotransferases (GOT and GPT) are the first enzymes to be used in diagnostic enzymology when liver damage has occurred (Whittby *et al.*, 1984; Kuchelb & Ralston, 1988). Because of their intra-cellular location in the cytosol, toxicity affecting the liver with subsequent breakdown in membrane architecture of the cells leads to their spillage into plasma, and their concentration rises in the latter. Mercury intoxication showed a significant increase in GOT and GPT activities due to hepatocellular necrosis, which caused increase in the permeability of cell membrane resulting in the release of transaminase in the blood stream (Vandenberghe, 1995). Mercury induced oxidative stress could be inhibited by antioxidants like reduced glutathione, SOD and CAT (Kavitha & Jagadeesan, 2004) and simultaneously increased the reliable marker enzymes of liver such as GOT and GPT (Rana *et al.*, 1996). Depletion of hepatic GSH inhibited damage to liver cell membrane, which indicate the increased activity of enzyme such as GOT and GPT by mice treated with CCl_4. Sharma *et al.* (2002) also reported the same result, this was due to the hepatocellular necrosis of liver. Flora *et al.* (1994) reported that mercury induced hepatic toxicity led to increased activities of transaminase such as GOT and GPT.

During the recovery span, the level of ACP, ALP, GOT and GPT activities reached to near the normal level, when the mercury intoxicated mice were again treated with MF of *T. terrestris* fruit extract. This result suggests that the plant, *T. terrestris* can been used for strengthening the body resistance, restoring normal function of the body to consolidate the constitution and promote blood circulation and protect the animals from the adverse effect of mercury toxicity. *T. terrestris* increases the level of GSH content in the liver tissue (Margarat, 2001). The increased GSH level increased the antioxidant protection (Kavitha & Jagadeesan, 2004) and decreased the level of GOT and GPT, when the mercury intoxicated mice was again fed with the MF *T. terrestris* fruit extract. Similar results were also observed by Khandelwal *et al.* (2002) in cadmium intoxicated rat with the crude extract of *Emblica officinalis* fruit. Sethuraman *et al.* (2003) also reported the decreased activities of GOT and GPT and increased level of ALP in CCl_4 intoxicated rats fed with a crude extract of *Sarcostemma brevistigma*. Sharma *et al.* (2002) also reported the same result in mercury fed mice treated with *Ocimum sanctum*. Thus these data clearly demonstrate antidotal effect of MF of *T. terrestris* fruit extract over mercury intoxicated mice. Finally, the time duration (7 days for fruit extract administration) was not enough for eliminating the heavy metal completely from the body, therefore further studies are required to evaluate the efficacy of *T. terrestris*.

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Table 1. Changes in the level of ACP, ALP, GOT and GPT in liver tissue of mice, *Mus musculus*, treated with 7 days of median-lethal dose of mercuric chloride followed by 7 days of *Tribulus terrestris* methanolic fruit extract treatment

Design of Treatment	ACP (µ moles of phenol liberated/min/ 100 mg protein)	ALP (µ moles of phenol liberated/min/ 100 mg protein)	GOT (IU/L)	GPT (IU/L)
Control	2.58 ± 0.13	0.88 ± 0.02	39.33 ± 0.83	43.69 ± 0.93
% COUTC				
% COHgT				
$HgCl_2$ treated	1.09 ± 0.01	0.63 ± 0.34	62.65 ± 0.99	58.06 ± 0.84
% COUTC	-57.47*	-27.74	+59.28*	+32.90*
% COHgT				
$HgCl_2$ + Extract [Po.Tr]	2.83 ± 0.02	0.83 ± 0.01	36.76 ± 0.88	41.47 ± 0.85
% COUTC	+ 9.68*	-5.20*	-6.55*	-5.07*
% COHgT	+159.63	+31.19*	-41.33	-28.57
Extract + $HgCl_2$ [Pr. Tr]	2.25 ± 0.02	0.73 ± 0.01	42.15 ± 0.92	29.51 ± 0.79
% COUTC	-12.79*	-17.10*	+7.17*	-32.44*
% COHgT	+106.42	+14.73	-32.71	-49.16
Extract alone	2.99 ± 0.03	0.84 ± 0.02	40.12 ± 0.06	44.46 ± 0.85
% COUTC	+15.89*	-4.30*	+2.01*	+1.76*
% COHgT				

Mean ± S.D. [Mean of six individual observation]
% COUTC – percentage change over untreated control
% COHgT – percentage change over mercury treated
• Significant at 5 percent level of 't' test.

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