



## Research Article

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### HEPATOPROTECTIVE ACTIVITY OF *IXORA COCCINEA* L. IN ANIMAL MODEL

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#### ABSTRACT

The present study aim was to assess the hepatoprotective effects of an ethanolic extract of *Ixora* flowers against carbon tetrachloride induced hepatocellular injury in rats. Healthy male Wistar rats (150-200 g body weight, 4-6 wk old) were used. They were randomly divided into eight groups. In the test group, ethanolic flower extract at a dose of 300 mg/kg was administered orally once daily. A single dose of carbon tetrachloride (CCl<sub>4</sub>, 0.5 ml/kg in olive oil) was administered i.p. to induce hepatotoxicity on the 7<sup>th</sup> day after half an hour of extract administration. Animals were euthanized 24 h after the administration of CCl<sub>4</sub>. Blood and liver tissue were collected for the assessment of serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), TP (Total protein), TB (Total bilirubin) and liver reduced glutathione level. The liver tissue was used for histopathological assessment of liver damage. Pre-treatment of rats with the flower extract of *Ixora coccinea* (300 mg/kg) orally for 7 days showed significant reduction in serum levels of ALT ( $P < 0.05$ ) and AST ( $P < 0.05$ ) enzymes by 5.5 % and 14 % respectively. TP (Total protein) increased by 96.4 % and TB (Total bilirubin) decreased by 42.8 % and similarly significant reduction was observed ( $P < 0.05$ ) in the liver reduced glutathione level by 42.32 % 24 h after the administration of carbon tetrachloride. Histopathological studies provided the evidence that the flower extract possess hepatoprotective activity. The study indicated that under the defined experimental conditions ethanolic extract of flowers of *Ixora coccinea* showed hepatoprotective abilities against carbon tetrachloride induced liver damage in rats.

**Keywords:** Hepatoprotective activity, Carbon tetrachloride, *Ixora coccinea* L.

#### INTRODUCTION

Indian medicinal plants have been used for a long time in many herbal formulations. *Ixora coccinea* L. of Rubiaceae Juss. family is an evergreen flowering shrub of Asian origin. This is a common shrub extensively used in Ayurvedic medicine. The flower extract has been found to contain triterpenoids, tannins and flavonoids which confer certain medicinal properties upon them. Extracts of different plant parts have been shown to be effective in treating diarrhea, dysentery, leucorrhoea, dysmenorrhoea, hemoptysis and catarrhal bronchitis<sup>1</sup>. Carbon tetrachloride is a widely used chemical to induce liver damage in experimental studies and its toxicity has been studied extensively. The resulting hepatic injury was characterized by leakage of cellular enzymes into the blood stream and by centrilobular necrosis<sup>2,3</sup>. Present study was undertaken to evaluate the use of ethanolic extract of *Ixora coccinea* flower as a hepatoprotective and anti-oxidative agent in carbon tetrachloride induced hepatotoxicity in rats.

#### MATERIALS AND METHODS

##### Preparation of Decoction

The flowers were collected from local garden. The plant was identified by Plant Taxonomist from Department of Botany, University of Calcutta, India. A voucher specimen has been submitted in the University of Calcutta Herbarium with Accession number: 20008-CUH. The flowers were air dried. Then ethanolic extract was prepared by crushing these flowers in 70 % ethanol and

keeping them in 4°C for fortnight. The extract was then filtered and the filtrate kept at -4°C for future use.

##### Chemicals

Chemicals used in the study were of analytical grade. All biochemical assay kits were purchased from Span Diagnostics Limited, India.

##### Screening and Maintenance of Animals

Healthy male Wistar rats (150-200 g body weight, 4-6 wk old) were used for the experimental study. They were preliminarily screened and rats with liver damage were excluded. Animals were provided a standard diet and filtered tap water was given *ad libitum*. Animals were maintained under standard 12 h dark-light cycle, 60 ± 10 % humidity and a temperature of 21.5 ± 1°C. Coprophagy was prevented by keeping the animals in cages with gratings on the floors. The distribution of animals in the groups was randomized. Freshly prepared solutions of drugs or chemicals were used throughout the study. After completion of the experiments, animals were euthanized by over-anesthetization with anesthetic ether. All experiments complied with Control and Supervision of Experiments on Animals (CPCSEA), India guidelines for animal experimentation. The protocols used in this study were approved and ethical clearance was obtained from R. G. Kar Medical College and Hospital, Kolkata, West Bengal, India. Institutional Animal Ethical Committee (RKC/IEAC/12/7).

### Study Design

The animals were randomly divided (to assure equal distribution of weights) into eight following groups containing six animals each. Group 1 (Normal Control): received distilled water orally (10 ml/kg); Groups 2 (Toxin Control): given carbon tetrachloride (0.5 ml/kg in olive oil) intra peritoneal (ip) and they were euthanized 24 h later; Group 3 (test drug control): received plant extract (300 mg/kg body weight) orally, once daily, for 7 days and euthanized on the following day. The dose of the plant extract was calculated according to an internationally accepted calculation where the normal therapeutic dosage of humans was extrapolated to rat<sup>4</sup>; Group 4 (standard drug control): received Liv52 (12.5 ml/kg of b.w) once daily for 7 days and euthanized on the following day; Group 5 (standard and test drug combination control): received both plant extract (300 mg/kg b.w.) and Liv52 (12.5 ml/kg of b.w) once daily orally for 7 days and euthanized on the following day; Group 6 were given the plant extract (300 mg/kg b.w.) once daily orally for 7 days. On the seventh day, carbon tetrachloride was administered intra-peritoneal (i.p.) half an hour after the administration of the last dose of plant extract. Rats were euthanized 24 h later. Rats in groups 7, 8 were given the Liv52 (12.5 ml/kg of b.w) once daily orally for 7 days and in addition for 8<sup>th</sup> group also plant extract (300 mg/kg b.w.). For both the groups on the seventh day, carbon tetrachloride was administered i.p. half an hour after the administration of the last dose of plant extract. Rats were euthanized 24 h later. Blood samples were collected from ether anaesthetized rats by cardiac puncture and liver tissue was excised for the determination of reduced glutathione and a part was fixed in buffered formalin for histopathological assessment of liver damage. Liver damage was assessed by the estimation of serum activities of AST (Aspartate transaminase), ALT (Alanine transaminase), TP (Total protein) and TB (Total bilirubin) using commercially available serum diagnostic kits supplied by Span Diagnostic Ltd. The activity of the antioxidant enzymes of reduced glutathione (GSH) in the homogenate were assayed following standard procedure<sup>5</sup>. Histopathological assessment of liver damage was done by studying haematoxylin and eosin stained slides of liver tissue.

### Statistical Analysis

All data obtained were expressed as Mean  $\pm$  SD. The results were analyzed for statistical significance by Student's t-test.  $P < 0.05$  was considered as statistically significant.

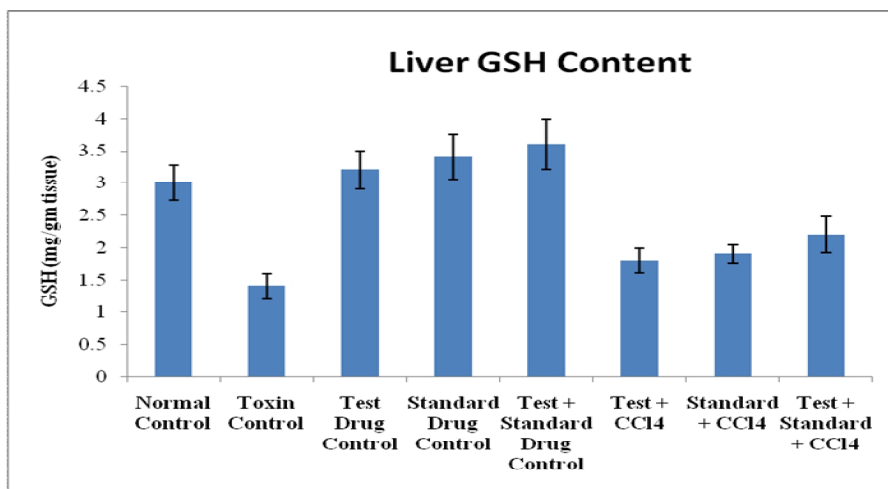
### RESULTS

Present study evaluated hepatoprotective activity of ethanolic extracts of *Ixora coccinea* flower, Table 1 showed the levels of serum enzymes namely AST, ALT, TP, TB and Graph 1 showed liver reduced glutathione level (GSH) in all the groups of rats. There was no

significant change in the activities of serum AST, ALT, TP, TB and liver reduced glutathione levels in Group 3, 4 and 5 rats as compared to Group 1 (Table 1). A significant increase ( $P < 0.05$ ) in the activities of serum enzymes and a significant decrease ( $P < 0.05$ ) in the liver reduced glutathione level occurred within 24 h of exposure to carbon tetrachloride in groups 6, 7 and 8 when compared with toxin control group 2. ALT levels were significantly ( $P < 0.05$ ) reduced in all treated groups in comparison to the toxin control group (group 2). It was most significantly reduced in  $\text{CCl}_4$  and *Ixora coccinea* flower ethanolic extract + Liv52-treated group of animals (group 8) i.e. 17.5 %, followed by group 6 and 7 to 5.5 % and 11.5 % respectively. Levels of AST were also reduced in all treated groups similarly like previous one. It was significantly ( $P < 0.05$ ) reduced to 25.2 % in group 8 followed by in group 6 and 7 by approximately 14 % and 25 % respectively. Similar results were obtained when Total protein (TP) and total bilirubin (TB) were estimated from serum. It is observed total bilirubin was significantly ( $P < 0.05$ ) reduced in the  $\text{CCl}_4$  and *Ixora coccinea* flower ethanolic extract and Liv52-treated group (Group 8). It was most significantly reduced by approximately 53 % and for group 6 and 7 it is 42.8 % and 46.2 %, while total protein (TP) levels were significantly ( $P < 0.05$ ) increased in all treated groups. It was most significantly increased by approximately 96.4 %, 116.5 % and 122.9 % for group 6, 7 and 8 respectively. In groups 6, 7 and 8 liver reduced glutathione level was found to be decreased significantly ( $P < 0.05$ ) in the 57.1 %, 35.7 % and 28.5 % 24 h after the administration of carbon tetrachloride with respect to  $\text{CCl}_4$  control group (group 2) (Graph 1). Results of histopathological studies provided supportive evidence for biochemical analysis. Histology of liver section of normal control animal (group 1) exhibited normal cellular architecture with distinct hepatic cells, each with well defined cytoplasm, prominent nucleus and nucleolus and well brought out central vein, sinusoidal spaces (Figure 1), whereas that of  $\text{CCl}_4$  intoxicated group (group 2) animal showed total loss of hepatic architecture with centrilobular hepatic necrosis, fatty changes, vacuolization and congestion of sinusoids, kupffer cell hyperplasia, crowding of central vein and apoptosis (Figure 2). The liver sections of the rats treated with plant extract and Liv 52 (Group 8) followed by  $\text{CCl}_4$  intoxication showed a sign of protection as it was evident by the absence of necrosis and vacuoles suggesting almost recovery (Figure 4).

### DISCUSSION

For screening the hepatoprotective drugs, liver damage is usually induced by  $\text{CCl}_4$ <sup>6</sup>. As a result there is a rise in different liver enzyme levels in serum i.e. AST, ALT and also for cholesterol which have been recognized as a sign to the damaged structural integrity of the liver, because they are cytoplasmic in location and released into circulation after cellular damages<sup>7</sup>.



Graph 1: Liver GSH content in different groups of rat

For each group (n = 6) the values are mean ± SD. P < 0.05 for all the groups with respect to their control

Table 1: Activity of serum biochemical parameters in different groups of rat

Group	Treatment	ALT (U/L)	AST (U/L)	Bilirubin (mg/dl)	Protein (g/dl)
Group 1	Normal	35.2 ± 0.85	51.1 ± 4.97	0.9 ± 0.03	5.64 ± 0.6
Group 2	CCl <sub>4</sub>	99.89 ± 4.14	124.72 ± 2.98	4.2 ± 0.3	3.09 ± 0.4
Group 3	I.C control	36.45 ± 0.41*	50.23 ± 0.49*	1.1 ± 0.1*	5.42 ± 0.5*
Group 4	Liv 52 control	35.63 ± 0.38*	51.02 ± 0.17*	1.1 ± 0.1*	5.5 ± 0.6*
Group 5	I.C + Liv 52	36.62 ± 0.5*	59.81 ± 0.43*	1.1 ± 0.2*	5.47 ± 0.7*
Group 6	I.C + CCl <sub>4</sub>	94.45 ± 1.73**	106.76 ± 3.37**	2.68 ± 0.2**	6.07 ± 0.8**
Group 7	Liv 52 + CCl <sub>4</sub>	88.44 ± 1.79**	99.92 ± 0.945**	2.41 ± 0.2**	6.69 ± 0.5**
Group 8	I.C + Liv 52 + CCl <sub>4</sub>	82.54 ± 1.6**	95.82 ± 0.56**	1.98 ± 0.2**	6.89 ± 0.7**

ALT: Alanine Amino Transferase; AST: Aspartate Amino Transferase

Data are expressed as Mean ± SD (n = 6); SD = Standard Deviation, \*P < 0.05 as compared with negative control i.e. group 1;

\*\*P < 0.05 as compared with positive control i.e. group 2

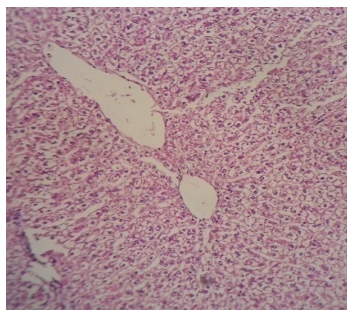


Figure 1: Micrograph of liver section from CCL4 induced rat

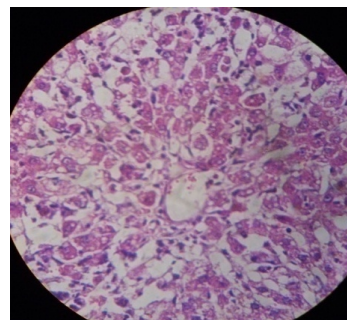


Figure 2: Micrograph of liver section from normal rat

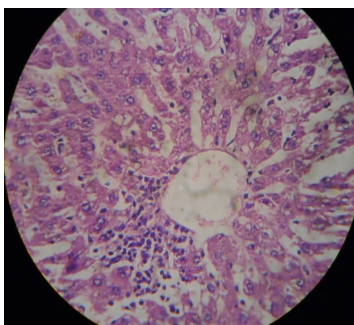


Figure 3: Micrograph of liver section from CCl<sub>4</sub> induced Ixora extract treated rat

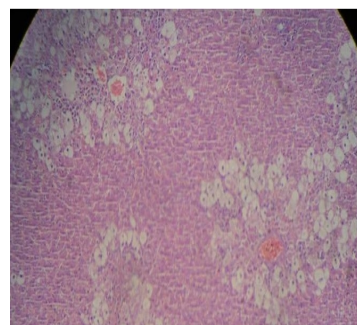


Figure 4: Micrograph of liver section from CCl<sub>4</sub> induced ethanolic extract and Liv52 treated rats

Carbon tetrachloride induces hepatotoxicity in rats by metabolic activation. Basically carbon tetrachloride is metabolically activated by a group of enzymes i.e. the cytochrome P-450 dependent mixed oxidase in the endoplasmic reticulum to form trichloromethyl free radical ( $^*CCl_3$ ) which is combined with cellular lipids and proteins in the presence of oxygen to induce lipid peroxidation<sup>8-10</sup> which induce change in the structures of the endoplasmic reticulum and other membrane, along with other metabolic changes i.e. loss of metabolic enzyme activation, reduction of protein synthesis and loss of glucose-6-phosphatase activation, apart from these up to even liver injury<sup>11-13</sup>. GSH is a critical determinant of tissue susceptibility to oxidative damage and with the depletion of hepatic GSH level is an indicator of hepatic toxicity to chemicals including  $CCl_4$ <sup>14</sup>. It is also observed that cell injury induced by xenobiotics due to the depletion of mitochondrial GSH<sup>15</sup>. In the present study, a significant decrease ( $P < 0.05$ ) in the liver GSH was observed after 24 h administration of  $CCl_4$  as compared to normal controls and drug control groups. Increase in hepatic GSH level in *Ixora* treated rat could either be due to an effect on the de novo synthesis of GSH, its regeneration or both. As a consequence, hepatic GSH level could be sufficiently maintained to counteract the increased formation of free radicals as in the case of carbon tetrachloride toxicity<sup>14</sup>. The observed protective effect of the plant extract against carbon tetrachloride may be attributed to the presence of flavonoids, ascorbic acid, carotenoids, tannin and lignins among the plant constituents<sup>16</sup>. Flavonoids are known to be antioxidants, free radical scavengers and anti lipoperoxidants leading to hepatoprotection. Histopathological observations suggested the possibility of the plant extract being able to condition the hepatic cells to a state of accelerated regeneration thus decreasing the leakage of AST, ALT, TP and TB into the circulation. Similar type of a significant increase ( $P < 0.05$ ) in the activities of serum enzymes and a significant decrease ( $P < 0.05$ ) in the liver reduced glutathione level (GSH) occurring within 24 h of exposure to carbon tetrachloride in *Epaltes* plant extract<sup>17</sup>. In conclusion, the results of the present study indicated that under the present experimental conditions, ethanolic extract of *Ixora coccinea* showed hepatoprotective effects against carbon tetrachloride induced liver damage in rats.

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#### REFERENCES

- Ghani A. Medicinal plants of Bangladesh with chemical constituents and uses. The Asiatic Society of Bangladesh; 2003. p. 500-508.
- Recknagel RO, Glende EA Jr, Dolak JA, Waller RL. Mechanisms of carbon tetrachloride toxicity. *Pharmacol. Ther* 1989; 43: 139-54. [http://dx.doi.org/10.1016/0163-7258\(89\)90050-8](http://dx.doi.org/10.1016/0163-7258(89)90050-8)
- Muriel P, Alba N, Perez Alvarez VM, Shibayama M, Tsutsumi VK. Kupffer cells inhibition prevents hepatic lipid peroxidation and damage induced by carbon tetrachloride. *Comp. Biochem. Physiol. C. Toxicol. Pharmacol* 2001; 130: 219-26. [http://dx.doi.org/10.1016/S1532-0456\(01\)00237-X](http://dx.doi.org/10.1016/S1532-0456(01)00237-X)
- Dhawan BN, Srimal RC. Acute toxicity and gross effects. In: Laboratory manual for pharmacological evaluation of natural products, Italy: United Nations Industrial Development Organization and International Centre for Science and High Technology; 1998. p. 17-19.
- Beutler E, Duron O, Kelly B. Reduced glutathione estimation. *J. Lab and. Clin. Med* 1963; 61: 882-885.
- Slater TF. Biochemical mechanism of liver injury. Academic Press, London; 1965. p. 1-44.
- Sallie R, Tredger JM, William R. Drugs and the liver. Part I. Testing liver function. *Biopharm. Drug. Dispos* 1991; 12: 251-259. <http://dx.doi.org/10.1002/bdd.2510120403>
- Recknagel RO, Glende RA Jr, Hruszkewycz AM. In: Pryor EA Jr (Ed.), Free Radicals in Biology, Vol. II. Academic Press, New York; 1976. p. 97-132.
- Recknagel RO. A new direction in the study of carbon tetrachloride hepatotoxicity. *Life Sci* 1983; 33: 401-408. [http://dx.doi.org/10.1016/0024-3205\(83\)90787-7](http://dx.doi.org/10.1016/0024-3205(83)90787-7)
- De Groot H, Noll T. The crucial role of low steady state oxygen partial pressures in haloalkane free-radical mediated lipid peroxidation. *Biochem. Pharmacol* 1986; 35: 15-19. [http://dx.doi.org/10.1016/0006-2952\(86\)90546-0](http://dx.doi.org/10.1016/0006-2952(86)90546-0)
- Recknagel RO, Glende EA Jr. Carbon tetrachloride hepatotoxicity: an example of lethal cleavage. *Critical Review Toxicology* 1973; 2: 263-297. <http://dx.doi.org/10.3109/10408447309082019>
- Gravel E, Albano E, Dianzani MU, Poli G, Slater TF. Effects of carbon tetrachloride on isolated rat hepatocytes: Inhibition of protein and lipoprotein secretion. *Biochem. J* 1979; 178: 509-512.
- Wolf CR, Harrelson WG Jr, Nastainczyk WM, Philpot RM, Kalyanaraman B, Mason RP. Metabolism of carbon tetrachloride in hepatic microsomes and reconstituted monooxygenase systems and its relationship to lipid peroxidation. *Mol. Pharmacol* 1980; 18: 553-558.
- Ko KM, Ip SP, Poon MKT, Wu SS, Che CT, Ng KH, et al. Effect of lignin enriched *Fructus schisandrae* extract on hepatic glutathione status in rats: protection against carbon tetrachloride toxicity. *Planta. Med* 1995; 61: 134-137. <http://dx.doi.org/10.1055/s-2006-958032>
- Ip SP, Poon MKT, Che CT, Ng KH, Kong YC, Ko KM. Schisandrin B protects against carbon tetrachloride toxicity by enhancing the mitochondrial glutathione redox status in mouse liver. *Free Radical Bio. Med* 1996; 21: 709-12. [http://dx.doi.org/10.1016/0891-5849\(96\)00179-7](http://dx.doi.org/10.1016/0891-5849(96)00179-7)
- Gilani AH, Janbaz KH. Preventive and curative effects of *Artemisia absinthium* on acetaminophen and  $CCl_4$ - induced hepatotoxicity. *Gen. Pharmacol* 1995; 47: 43-47.
- Hewawasam RP, Jayatilaka KAPW, Pathirana C, Mudduwa LKB. Hepatoprotective effect of *Epaltes divaricata* extract on carbon tetrachloride induced hepatotoxicity in mice. *Indian J. Med. Res* 2004; 120: 30-34.

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