Hepatoprotective Effects of *Thespesia lampas* Dalz & Gibs in CCl_4 Induced Liver Injury in Rats

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ABSTRACT: The extracts of the roots of *Thespesia lampas* (Malvaceae) were evaluated for hepatoprotective activity in rats by inducing chronic liver damage by subcutaneous injection of 50% v/v carbon tetrachloride in Tween 80 at a dose of 3ml/kg for a period of 4 weeks. The biochemical parameters like serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT), alkaline phosphatase (ALP), serum bilirubin and total proteins were estimated to assess the liver function. Hepatic steatosis, centrilobular necrosis, and often swelling of the hepatic cytoplasm were observed in carbontetra chloride treated group, while these were completely absent in the extracts of *T. lampas* (300 mg/kg b.wt) treated groups ($p<0.01$). The present investigation established pharmacological evidence to support the folkloric claim of hepatoprotective activity of *T. lampas*.

Key words: *Thespesia lampas*, Carbon tetrachloride, Hepatoprotective, Rats, Root extracts

INTRODUCTION

The liver is responsible for metabolism of foods and chemicals and for the regulation of internal chemical environment. Exogenous and endogenous chemicals are observed, concentrated and then processed by the liver into more usable or extractable form.[1] The major clinical manifestation of liver disorder is jaundice. Hepatocellular jaundice may occur due to the liver cell damage caused by hepatic viruses, bacterial toxins or toxic chemicals. There is no rational therapy available in western medicines as such for the cure of these diseases; usually supportive measures are practiced.[2] *T. lampas* Dalz & Gibs[3,4] belongs to the family Malvaceae and its roots and fruits are used for treating gonorrhea, jaundices, syphilis[5] and anti-microbial.[6]

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MATERIALS AND METHODS

Plant material. The roots were collected from the foot hills of Yercaud, Salem, in the month of September 2005. The plant was identified and authenticated by the experts in the department of Botany, Govt. Arts College, Salem, Tamil Nadu, India. A voucher specimen (TL-12) has been kept in our museum for future reference. The plant material after collection was dried in shade at room temperature for 10 days and coarsely powdered with the help of a hand-grinding mill and the powder was passed through sieve No. 60.

Preparation of the Extract. Powdered root weighing 1000g was packed in soxhlet apparatus and then subjected to methanol extraction for period of 45 hrs. The methanol extract was concentrated under vacuum condition at 50-60^{0}C (yielding 40.2g). Powdered root weighing 500g was subjected to aqueous extraction using 1.5 liters distilled water for 12 hrs. in a percolator. The aqueous extract was concentrated under vacuum at 40-45^{0}C (yielding 35

g), which was stored in desiccators and used for subsequent experiments.

Animals. Male Wister rats of approximately 9-12 weeks, weighing about 150-175 g were purchased from M/S Venkateshwara enterprises (P) Ltd, Bangalore and were used for the study. They were housed in polypropylene cages and fed with standard chow diet and water *ad libitum.* The animals were exposed to alternate cycle of 12 h of darkness and light each. The experimental protocol was approved by of the Institutional Animal Ethics Committee (IAEC NO: P.Cog-1/06).

Carbon tetrachloride- induced hepatotoxicity. Carbon tetrachloride (CCl_4) intoxication in rats is widely used to study necrosis and steatosis of the liver.[7] Liver cell damage was induced by subcutaneous injection of 50% CCl_4 in 1 % Tween 80 (3 ml/kg).[8] The experimental animals were randomized into 4 groups i. II to V of six rats in each. The group I served as a normal control that received 1 % Tween 80. The group II, III, IV and V animals were given CCl_4 (3 ml/kg/day) subcutaneous. Group III received standard drug silymarin (25 mg/kg/day), Groups IV and V received methanol and aqueous extracts of roots of *T. lampas* (300 mg/kg/day) respectively, by oral route. The treatment was carried out for 4 weeks. All the animals were sacrificed after 28 days.

Assay of serum bilirubin and serum alkaline phosphatase. Blood samples were collected by cardiac puncture and allowed to coagulate for 30 min at 37^0 C. Clear serum was separated and used for the estimation of SGPT, SGOT,[9] total protein,[10] serum alkaline phospatase[11] and serum bilirubin.[12]

Histopathology examination of hepatocytes. The livers were excised from the experimental animals of each group after collecting the blood samples and washed with normal saline. Weight of liver of each animal was noted (Table 1). After this, the livers were fixed in 10% neutral buffered formalin and with bovine solution they were processed for paraffin embedding following the standard microtecnique[13]. These sections of 4-5 µm were processed in alcohol xylene series and were stained with alum haematoxylin and eosin. The sections were examined microscopically for the evaluation of histopathological changes.

Statistical analysis. Statistical analysis was carried out using ANOVA. Results were expressed as mean ± SEM. The differences between the groups were evaluated by one-way analysis of variance (ANOVA) followed by the Dunnette multiple comparisons test. $P<0.01$ was considered as significant.

Table 1. Liver weight variation

Treatment	Dose (mg/kg)	Liver (mg/kg) (mg/kg)
Control	-	4.26 ± 0.14
CCl_4	1ml/kg	7.28 ± 0.08
Silymarin	25	5.20 ± 0.04**
Methanol extract	300	6.2 ± 0.02**
Aqueous extract	300	5.56 ± 0.06**

Values are expressed as mean ± SEM, n=6. **P<0.01 as compared with hepatotoxic control. Data were analyzed by using One way ANOVA followed by Dunnett's multiple comparison tests

RESULTS AND DISSCUSSION

Rats subjected to the CCl_4 regimen alone developed significant hepatocellular damage as evident from a significant elevation in serum levels of SGOT, SGPT, serum bilirubin, total protein and alkaline phosphatase. Oral administration of methanol and aqueous extracts of *T. lampas* at a dose of 300 mg/kg/day exhibited statistically significant reduction of the elevated enzymes levels: SGPT from 171.0 ± 2.6 to 142.2 ± 9.4 and 108.6 ± 9.4 (U/L), SGOT from 272.8 ± 2.9 to 231.0 ± 11.3 and 220.0 ± 6.9 (U/L), alkaline phosphatase from 374.9 ± 8.7 to 293.2 ± 13.6 and 284.5 ± 8.5 (U/L), serum bilirubin from 0.85 ± 0.02 to 0.70 ± 0.02 and 0.64 ± 0.02 (mg/dl) and total protein from 7.2 ± 0.11 to 6.80 ± 0.2 and 6.5 ± 0.16 (g/dl) respectively (Table 2). Treatment with silymarin at a dose of 25 mg/kg/day also exhibited significant reduction of enzymes like SGPT 89.9 ± 2.4 (U/L), SGOT 142.0 ± 2.4 (U/L), ALP 259.5 ± 2.6 (U/L), total bilirubin 0.6 ± 0.02 (mg/dl) and total protein 6.45 ± 0.16 (g/dl). The

activity of this plant extracts were compared with that of a standard hepatoprotective drug silymarin and was comparable in all the parameters tested.

Histopathological profiles of the liver from CCl_4 treated rats revealed intense centrilobular necrosis, steatosis and often swelling of the hepatic cytoplasm. The protective effect of *T. lampas* root extracts was confirmed by histopathological examination of the liver section. Administration of extracts to the experimental animals (300 mg/kg, p.o) showed a significant improvement of the hepaticcellular architecture over the CCl_4 treated control group, as evident from a considerable reduction in necrosis and fatty change. Thus, the present study confirms the liver protective action of the methanol and aqueous extracts of *T. lampas* against experimentally induced liver damage in rats, which was in comparison to that of a standard hepatoprotective drug and provides pharmacological evidence for its folkloric claim as hepatoprotective agent.

Table 2. Effects of *T. lampas* root extracts on different biochemical parameters in CCl4 induced liver injury in rats

Treatment mg/kg	SGPT U/L	SGOT U/ L	ALP U/L	Total bilirubin (mg/dl)	Total protein (g/dl)
Control	80.0 ± 5.6	109.2 ± 4.2	189.0 ± 3.2	0.6 ± 0.02	5.9 ± 0.2
CCl_4	171.0 ± 2.3	272.8 ± 2.9	374.9 ± 8.7	0.85 ± 0.02	7.2 ± 0.11
Silymarin 25	89.9 ± 2.4**	142.0 ± 2.4**	259.5 ± 2.6**	0.6 ± 0.02**	6.45 ± 0.16**
Methanol extract 300	142.2 ± 9.4**	231.0 ± 11.3**	293.2 ± 13.6**	0.70 ± 0.02**	6.80 ± 0.2**
Aqueous extract 300	108.6 ± 9.4**	220.0 ± 6.9**	284.5 ± 8.5**	0.64 ± 0.02	6.5 ± 0.16

Values are expressed as mean ± SEM, n=6. *P< 0.05, **P<0.01 as compared with hepatotoxic control. Data were analyzed by using one way ANOVA followed by Dunnett's Multiple Comparison test

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