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Hepatoprotective and antioxidant activity of *Thespesia lampas* (Cav.) Dalz & Gibs

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Abstract

Thespesia lampas (Cav.) Dalz & Gibs an important folk medicinal plant was evaluated for hepatoprotective and antioxidant activity against carbon tetrachloride (CCl_4) induced hepatic damage in rats. In the present study, the *T. lampas* stems extracts at dose of 200 mg/kg body wt. were administered orally once daily for nine days and on seventh day after one hour of drug administration CCl_4 (1ml/kg s.c.) given orally. After 24 h of ninth day, they were sacrifice and their livers were dissected for biochemical and histopathological studies. The extracts showed significant hepatoprotective and antioxidant effect by lowering the serum levels of transaminases (SGOT and SGPT), alkaline phosphatase (ALP), bilirubin, protein, cholesterol and triglyceride as compared to silymarin as a standard hepatoprotective agent. The extracts showing increased levels of superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH) and decreased level of lipid peroxidation (LPO). The biochemical observations were supplemented with histopathological examination of rat liver sections. The results of *T. lampas* stems extract showed significant protection to the liver against carbon tetrachloride induced damages. Our finding suggested that among comparative significance of various extracts, the methanolic extract of *T. lampas* stems having better efficacy and significant activity. The present study support the traditional believes of this plant and highlighted profound potential of *T. lampas* to be investigated for bioactve compounds responsible for hepatoprotective and antioxidant effect.

Keywords: *Thespesia lampas*, Malvaceae, carbon tetrachloride, hepatoprotective activity, antioxidant effect

Introduction

Thespesia lampas (Cav.) Dalz & Gibs (*T. lampas*) belong to the Malvaceae family, vernacularly known as "*Ranbhendi*" is found as a wild herb growing during monsoon on the hills in a throughout India and also in Eastern Tropical Africa (Nadkarni, 2007; Kirtikar et al., 2005). In the folk medicine, this plant has been considered to be hepatoprotective and

traditionally root paste used to cure jaundice in Korku tribe of Amravati district of Maharashtra and also in Nepal (Jagtap et al., 2006; Tamang et al., 2003). The roots of this plant are reported for anti-diabetic (Jayakar et al., 2008), anti-hyperlipidaemic (Sangameswaran et al., 2008), hepatoprotective (Sangameswaran et al., 2008), antioxidant (Kumaraswamy et al., 2008; Sangameswaran et al., 2009) and anthelmintic activity (Kosalge et al., 2009). Flowers contain quercetin and some protocatechuic acid (Perkin, 1909).

The stems of the plant are used as a folk remedy and it is traditionally used in the treatment of inflammation, acidity, bleeding nose, bronchitis, cough, dysentery, fever, sun stroke, urinary complaints, anthelmintic, carbuncle (Adhikari et al., 2007). *T. lampas* stems showed antimicrobial activity and reported for presence of gossypol (Valsaraj et al., 1997; Luckefahr et al., 1967). In the ethnobotanical claims, the stems are used for the treatment of jaundice and other hepatic diseases by the folk tribes of Trimbakeshwar Hills, Maharashtra state, India. However, no scientific information is available regarding the hepatoprotective effect of stems of *T. lampas*. Therefore, to justify the traditional claims we have assessed the hepatoprotective and antioxidant effect of *T. lampas* stems using carbon tetrachloride (CCl_4) intoxicated rats.

Materials and methods

Plant Material

The plant, *T. lampas* was collected in Trimbakeshwar Hills, Nashik District (Maharashtra) in May 2008. The plant was authenticated and herbarium deposited in Botanical Survey of India, Pune, Maharashtra under voucher specimen number CDSTL1. (Ref. No. BSI/WC/Tech/2008/79). The stems of the plant were dried, powdered and passed through 40 mesh sieve and stored in an airtight container for further use.

Extraction

The air-dried stems of *T. lampas* were made into a coarse powder. The powdered material was defatted with petroleum ether. The defatted material was extracted with methanol and distilled water using a Soxhlet extractor. Methanolic extract was further fractionated with ethyl acetate to get ethyl acetate soluble and ethyl acetate insoluble fractions. Then the extract was filtered through muslin and the filtrate was evaporated under reduced pressure and vacuum-dried (Mukherjee, 2002). All the extracts were administered to the animals as a suspension in gum acacia.

Animals

Adult Wistar rats (120–200 g) of either sex were obtained form the National Institute of Bioscience, Pune, Maharashtra, India. The rats were maintained under controlled temperature, 12 h light/12 h dark conditions for 1 week before the start of the experiments to acclimatize to laboratory conditions. They were allowed to feed standard rodent pellet diet and water ad libitum. The study protocol was approved by the IAEC (Institutional animal ethics committee of CPCSEA, Govt. of India).

Carbon tetrachloride induced hepatotoxicity

Adult wistar rats of either sex were divided into eight groups of six animals each. Group I received only gum acacia (5 mg/kg per day p.o.) for nine days and served as control. Group II animals received in a single dose of carbon tetrachloride (CCl_4) /olive oil (1:1,1ml/kg s.c.) on the seventh day as treated control group. Group III animals were treated with silymarin (25 mg/kg per day p.o.) for nine days and on the seventh day, a single dose of carbon tetrachloride/olive oil (1:1,1ml/kg s.c.) was given. Group IV - VIII animals were received petroleum ether, methanolic, ethyl acetate soluble fraction, ethyl acetate insoluble fraction and aqueous extract (200 mg/kg per day p. o.) respectively for nine days and on the seventh day, a single dose of carbon tetrachloride/olive oil (1:1,1ml/kg s.c.) was administered (Bhattacharya et al., 2003).

Assessment of liver functions

Blood samples of the rats were withdrawn from ratino bulber venous plexus with the help of a glass capillary under light anesthesia and were kept at room temperature for 2 h, so that the coagulation process gets completed. Blood was collected, allowed to clot and serum was separated at 2500 rpm for 15 min and biochemical investigations were carried out.

Biochemical determinations

The biochemical parameters like serum enzymes, serum glutamate oxaloactate transminase (SGOT), serum glutamate pyruvate transaminase (SGPT) (Reitman et al., 1957), serum alkaline phosphatase (SALP) (King, 1965), total bilirubin (Malloy et al., 1937), total proteins (Doumas et al., 1971), total cholesterol (Kaplan et al., 1983) and total triglyceride (Fossati et al., 1982) were assayed using assay kits (Varad Diagnostic, Ahmednagar).

Estimation of SOD, CAT, GSH and MDA levels

Grouping and dosing schedule in rats was followed similarly as mentioned in CCl_4 induced hepatotoxicity. The rats were sacrificed 36 h after CCl_4 injection animals were sacrificed by cervical dislocation. Liver samples were dissected out and washed immediately with ice cold saline to remove as much blood as possible. Liver homogenates (5% w/v) were prepared in cold 50mM potassium phosphate buffer (pH 7.4) using a Remi homogenizer. The unbroken cells and cell debris were removed by centrifugation at 1000 rpm using a Remi refrigerated centrifuge. The supernatant was used for the assay of marker enzymes namely superoxide dismutase (SOD) (Yasuhisa, 1972), Catalase (Luck, 1971) and GSH (Ellaman, 1959) levels. LPO was estimated by the standard method (Ohkawa et al., 1979). The total protein content was estimated by biuret method (Doumas et al., 1971).

Histopathological studies

The liver tissue was dissected out and fixed in 10% formalin, dehydrated in gradual ethanol (50–100%), cleared in xylene, and embedded in paraffin. Sections were prepared and then stained with hematoxylin and eosin (H–E) dye for photomicroscopic observation,

Table 1. Effect of extracts of *T. lampas* stems on serum levels of liver enzymes against CCl_4 induced liver damage in rats

Group	Treatment	SGOT (U/L)	SGPT (U/L)	SALP (U/L)
I	Control	31.31 ± 0.88	54.69 ± 0.88	116.85 ±15.92
II	CCl_4 treated	109.66± 1.60	249.52 ± 15.12	256.03 ± 5.64
III	Silymarin + CCl_4	39.69 ± 0.82**	58.36 ± 0.64**	120.99± 2.73**
IV	Petroleum ether extract + CCl_4	94.16 ± 5.42^{ns}	175.77 ± 1.08 **	133.51 ± 0.72^{ns}
V	Methanolic extract + CCl_4	49.91 ± 2.11**	69.48 ± 1.41**	142.60± 8.07**
VI	Ethyl acetate soluble fraction + CCl_4	68.31 ± 2.02**	89.48 ± 1.41**	284.50± 8.52*
VII	Ethyl acetate insoluble fraction + CCl_4	71.98 ± 19.01**	96.07 ± 1.37**	144.90± 6.52*
VIII	Aqueous extract + CCl_4	78.15 ± 7.28*	112.52± 2.62**	139.23± 51.41*

Values are mean ± SEM, n=6. Symbols represent statistical significance.
*P< 0.05, **P<0.01 as compared to CCl4 - intoxicated group; ns - not significant.

including cell necrosis, fatty change, hyaline regeneration, ballooning degeneration (Shanmusnaram et al., 2006).

Statistical analysis

The data are expressed as mean ± S.E.M. The difference among means has been analyzed by using one-way ANOVA followed by Dunnett's Multiple Comparison test. A value of $P < 0.05$ was considered as statistically significant.

Results

Hepatoprotective activity

The results of hepatoprotective effect of extracts on CCl_4-intoxicated rats are shown in Table 1 and Table 2. In the CCl_4-intoxicated group (II) serum SGPT, SGOT, ALP, TB, TP, TC and TG were increased to 109.66± 1.602 U/L, 249.52 ± 15.129U/L, 256.03 ± 5.646 IU/L, 1.975 ± 0.1990 mg/dL, 9.755 ± 0.06485 mg/dL, 105.31± 3.258 mg/dL and 219.97 ± 2.679 mg/dL, respectively, whereas these values were showed 31.315 ± 0.8861U/L, 54.697 ± 0.8803 U/L, 116.85± 15.923 IU/L, 0.6133 ± 0.03870 mg/dL, 5.832 ± 0.9279 mg/dL, 72.567 ±11.904 mg/dL, 162.43 ± 1.839 mg/dL in control group (I) respectively. The elevated levels of serum SGPT, SGOT, ALP, TB, TP, TC and TG were significantly reduced in the animals groups treated with various extracts. Treatment with methanolic extract showed highly significant activity ($P < 0.001$) with maximum inhibition. So, the methanol extract treated group was superior to the other extracts but not as effective as the silymarin (Table 1 and 2).

Antioxidant activity

The results of antioxidant activity of different extracts on CCl_4-intoxicated rats are shown in Table 3. Results of study clearly revealed increase in the levels of MDA in CCl_4-intoxicated rats compare to control group. Treatment with extracts significantly prevented

Table 2. Effect of extracts of *T. lampas* stems on biochemical parameters of liver against CCl_4 induced liver damage in rats.

Group	Treatment	TB (mg/dl)	TP (mg/dl)	TC (mg/dl)	TG (mg/dl)
I	Control	0.61 ± 0.03	5.83 ± 0.92	72.56 ± 11.90	162.43 ± 1.83
II	CCl_4 treated	1.97 ± 0.19	9.75 ± 0.06	105.31± 3.25	219.97 ± 2.67
III	Silymarin + CCl_4	0.65 ± 0.002**	6.21± 0.11**	79.10 ± 1.49**	174.43±11.08**
IV	Petroleum ether extract + CCl_4	1.41 ± 0.65^{ns}	9.48 ± 0.12^{ns}	97.61 ± 1.81^{ns}	189.82 ± 2.81**
V	Methanolic extract + CCl_4	0.72 ± 0.07**	6.43 ± 0.01**	81.90 ± 0.30**	177.10±11.75**
VI	Ethyl acetate soluble fraction + CCl_4	0.78 ± 0.05**	6.48 ± 0.60**	86.31 ± 2.39*	178.93 ± 1.01**
VII	Ethyl acetate insoluble fraction + CCl_4	0.82 ± 0.04*	6.57 ± 0.01**	89.11 ± 0.53^{ns}	181.77 ± 0.97**
VIII	Aqueous extract + CCl_4	0.98 ± 0.002*	7.58 ± 0.02**	2.51 ± 1.02*	187.60 ± 2.34**

Values are mean ± SEM, n=6. Symbols represent statistical significance.
*P< 0.05, **P<0.01 as compared to CCl_4 - intoxicated group; ns - not significant.

Table 3. Effect of extracts of *T. lampas* stems on liver enzymes against CCl_4 induced liver damage in rats.

Group	Treatment	MDA nMol/g	SOD U/mg	CAT U/mg	GSH nMol/mg
I	Control	0.82 ± 0.01	6.23 ± 0.39	72.46 ±15.92	45.26 ± 1.28
II	CCl_4 treated	1.38 ± 0.03	3.06± 0.06	43.33 ± 1.43	20.11 ± 1.73
III	Silymarin + CCl_4	0.92 ± 0.003**	5.66 ± 0.09**	71.33 ± 1.33**	42.11 ± 1.02**
IV	Petroleum ether extract + CCl_4	1.30 ± 0.03^{ns}	3.58 ±0.19^{ns}	47.76 ± 0.45*	25.11 ± 1.17*
V	Methanolic extract + CCl_4	1.20 ± 0.03**	5.31 ± 0.23**	67.66 ± 0.42**	38.48 ± 0.64**
VI	Ethyl acetate soluble fraction + CCl_4	1.80 ± 0.02**	4.76 ±0.08**	62.16 ± 1.22**	35.15 ± 1.08**
VII	Ethyl acetate insoluble fraction + CCl_4	2.80 ± 0.02**	4.15 ± 0.19**	60.66 ± 0.66**	32.48 ± 0.73**
VIII	Aqueous extract + CCl_4	1.28 ± 0.02^{ns}	3.01 ± 0.18^{ns}	57.50 ± 0.42**	28.65 ± 0.69**

MDA = nMol of MDA/mg of protein; SOD = U/mg of protein, CAT = nMol of H_2O_2 decomposed/min/mg/protein, GSH = nMol/mg of protein
Values are mean ± SEM of six rats. Symbols represent statistical significance.
*P< 0.05, **P<0.01 as compared to CCl_4 - intoxicated group; ns - not significant.

this raise in levels. SOD, CAT and GSH content have significantly increased in extract treated groups whereas CCl_4-intoxicated group has shown significant decrease in levels compare to control group. Methanolic extract has shown maximum protection as compare to the different extracts (Table 3).

Histopathological observations

Histology of the liver sections of control animals showed normal hepatic cells with well-preserved cytoplasm, prominent nucleus, nucleolus and visible central veins. The liver sections of CCl_4-intoxicated rats showed massive fatty changes, necrosis, ballooning degeneration and broad infiltration of the lymphocytes and the loss of cellular boundaries. The histological architecture of liver sections of the rats treated with different extracts showed more or less normal lobular pattern with a mild degree of fatty change, necrosis and lymphocyte infiltration almost comparable to the control and methanolic extract showed more normal lobular pattern but not as effective as the silymarin treated group (Figure1).

Discussion

Carbon tetrachloride (CCl_4) is believed to be metabolized by microsomal cytochrome P450 in the liver to a highly reactive trichloromethyl free radical ($\bullet CCl_3$) which can start a chain of reactive free radical formation resulting in peroxidation of lipids and damage to proteins and components of the cell which can result in cell lyses (De Groot et al., 1986; Tomasi et al., 1987; Clawson, 1989). Toxicity begins with the change in endoplasmic reticulum, which results in the loss of metabolic enzymes located in the intracellular structures (Recnagal, 1983).

In this view, the reduction in levels of SGPT and SGOT by the *T. lampas* stems extracts is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damage caused by $CC1_4$. This effect is agreement with the commonly accepted view that serum levels of transaminases return to normal with the healing of hepatic parenchyma and regeneration of hepatocytes (Thabrew et al., 1987). The *T. lampas* stems extract induced suppression of the increased SALP activity with the concurrent depletion of raised bilirubin suggest the possibility of the extracts to have ability to stabilize biliary dysfunction in rat liver during hepatic injury with CCl_4. The *T. lampas* stems extract also suppress the increased level of cholesterol and triglyceride.

A major defense mechanism involves the antioxidant enzymes, including SOD, CAT and GSH which convert active oxygen molecules into non-toxic compounds. The lipid peroxidation is accelerated when free radicals are formed as the results of losing a hydrogen atom from the double bond in the structure of unsaturated fatty acids. Scavenging of free radicals is one of the major antioxidation mechanisms to inhibit the chain reaction of lipid peroxidation (Constantin et al., 1990). Reduced lipid peroxidation was revealed by significant decrease in MDA level in extracts treated groups. Simultaneously significant increase in GSH, SOD and CAT content of liver suggested antioxidant activity of *T. lampas* stems extracts and silymarin.

The hepatoprotective effect of *T. lampas* stems extract was confirmed by histological examination of the liver tissue of control and treated animals. The histological architecture of

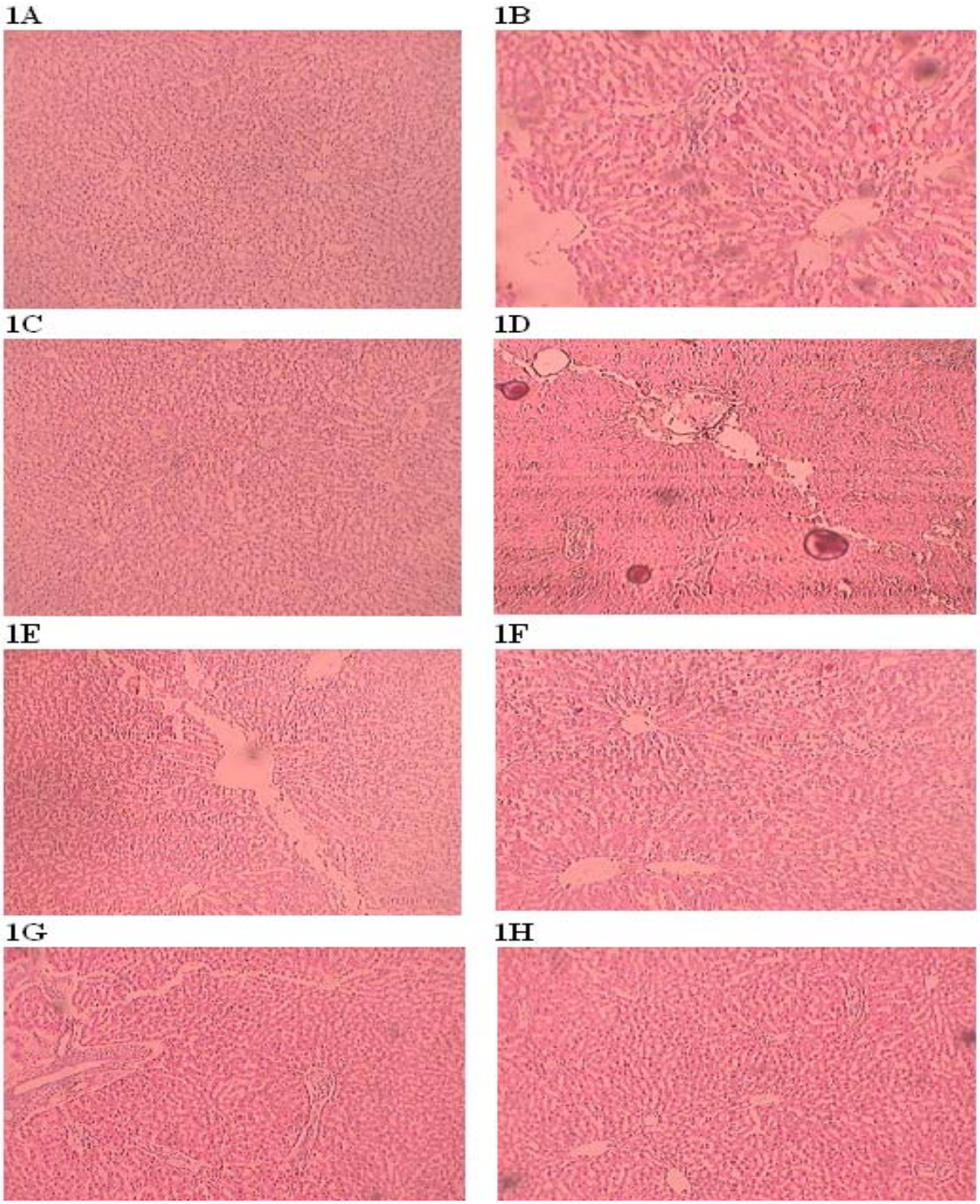

Figure 1. Effect of *T. lampas* extracts on histopathological changes against CCl_4 induced liver damage in rats. (1A) Normal control; (1B) CCl4 treated; (1C) CCl_4 + silymarin treated; (1D) CCl_4 + petroleum ether extract treated; (1E) CCl_4 + methanolic extract treated; (1F) CCl_4 + ethyl acetate soluble fraction treated; (1G) CCl_4 + ethyl acetate insoluble fraction treated; (1H) CCl_4 + aqueous extract treated

carbon tetrachloride treated liver section showed fatty degeneration of hepatocytes. However administration of *T. lampas* stems extract (200 mg/kg) almost normalized these defects in the histological architecture of the liver, almost to the level of the Silymarin treated groups,

showing its potent hepatoprotective effects. The administration of methanolic extract of *T. lampas* stems revealed significant protection in hepatocyte regeneration against the toxic effect of carbon tetrachloride. Hence, the histological examination of *T. lampas* stems extract treated group showing hepatoprotective effects and it supported to biochemical studies.

Thus, it can be concluded that, present study gives some scientific evidences on effect of extraction solvents was made to find out the therapeutically better efficacious extract. Among comparative significance of various extracts, the methanolic extract of *T. lampas* stems having better efficacy and significant hepatoprotective and antioxidant activity. Therefore, the present study support the traditional believes of this plant and highlighted profound potential of *T. lampas* to be investigated for bioactive compounds responsible for hepatoprotective and antioxidant effect.

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