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Hepatoprotective Activity of *Thespesia populnea* Bark Extracts against Carbon Tetrachloride-Induced Liver Toxicity in Rats

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Abstract – In the present study, *Thespesia populnea* (Malvaceae) bark was extracted with methanol and water. The extracts were vacuum dried to yield the respective methanol (MET) and aqueous extract (AET). The extracts were evaluated for hepatoprotective activity against carbon tetrachloride (CCl_4) induced liver damage at 2 dose levels (250 and 500 mg/kg). The biochemical parameters observed in serum were total bilirubin, alkaline phosphatase (ALP), serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT) levels and total protein. Aspartate transaminase (AST), alanine transaminase (ALT) and total protein levels in liver were also evaluated. Histopathological study on the liver tissue was also performed. The extracts exhibited dose dependent reduction in total bilirubin, ALP, SGOT, SGPT, AST, ALT and increase in total protein (serum and liver) levels. The extracts also exhibited only mild hepatocytic damage compared to the CCl_4 treated group. MET was found to exhibit higher hepatoprotection than AET.
Key words – *Thespesia populnea*, carbon tetrachloride, hepatoprotective

Introduction

Thespesia populnea Soland ex Correa (Malvaceae) is a large avenue tree found in the tropical regions and coastal forests in India. Various parts of *T. populnea* are claimed (Anonymous, 1995) to used as gastrointestinal antibacterial, anti-inflammatory, antimalarial and purgative. It is also claimed to be used in the treatment of cutaneous infections, herpetic diseases, cholera, dysentry, hemmorrhoids, urethritis and gonnorhea. Hepatoprotective activity of the flowers of *T. populnea* (Shirwaikar *et al.*, 1992; Shirwaikar *et al.*, 1995; Shirwaikar *et al.*, 1996; Rajesh *et al.*, 2000) and antibacterial activity (Hewage *et al.*, 1998) of the stem were reported. To our knowledge, there are no scientific reports on the pharmacological properties of *T. populnea* bark that promoted us to pursue a systematic hepatoprotective activity evaluation of *T. populnea* bark extract. In the present study, *T. populnea* bark was extracted with methanol and water by soxhlet extraction. The extracts were vacuum dried to yield the respective methanol (MET) and aqueous extract (AET). The extracts were evaluated for hepatoprotective activity against carbon tetrachloride (CCl_4) induced liver damage at 2 dose levels (250 and 500 mg/kg).

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Materials and Methods

Plant material and extraction – The leaves of *Thespesia populnea* Soland ex Correa (Fam: Malvaceae) were collected from Chennai, India during August 2002 and identified by Dr. E. Sasikala, Botanist, Central Research Institute for Sidda, Chennai. The barks were dried in shade (7 days), powdered and extracted successively with methanol (MET) and water (AET) by soxhlet extraction (24 h) to yield the respective extracts. The extracts were vacuum dried in a rotary vacuum film evaporator and the extractive yields of MET and AET were 14.7 and 24.7% (w/dry w of bark), respectively.

Animals – Inbred Wistar albino rats (120-200 g) of either sex were used for the evaluation of pharmacological activities. They were kept in colony cages at 25±2°C, relative humidity 45-55% under 12 h light and dark cycles. All the animals were acclimatized for a week before use. They were fed with standard animal feed (Hindustan Lever Ltd.) and water *ad libitum*. The test compounds and the standard drugs were administered in the form of a suspension using 1% carboxymethylcellulose as vehicle. Each group consisted of six animals. Acute oral toxicity was performed for the extracts. All the pharmacological experimental protocols

were performed according to the recommendation of the institutional animals ethics committee.

Acute oral toxicity – Acute oral toxicity (Ecobichon, 1997) was performed as per OECD-423 guidelines (acute toxic class method). Wistar albino mice (n=3) of either sex selected by random sampling technique was used for the study. The animals were kept fasting for 3-4 hr providing only water, after which the extracts (suspended in olive oil) were administered orally at the dose level of 5 mg/kg by intragastric tube and observed for 3 days. If mortality was observed in 2-3 animals, then the dose administered was assigned as toxic dose. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher doses such as 50, 300 and 2000 mg/kg.

Hepatoprotective activity – Wistar albino rats (Mandal *et al.*, 1992) of either sex selected by random sampling technique was used. MET and AET (250 and 500 mg/kg/day) was administrated orally for seven days and on the seventh day, a single dose of equal mixture of CCl_4 and olive oil (50% v/v-5 ml/kg/ i.p) was administrated. Silymarin (25 mg/kg/day/p.o) was used as standard for comparison.

All the animals were sacrificed by cervical decapitation under light ether anesthesia on the eighth day. Blood was collected from jugular veins and centrifuged (3000 rpm for 10 min) to obtain serum. The serum was used for the assay of total bilirubin (Jendrassik and Grof, 1938), alkaline phosphatase (ALP) (Kind and Kings, 1954), serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT) (Reitman and Frankel, 1957), and total protein (Doumas *et al.*, 1971). The liver was dissected out immediately after sacrifice, washed in ice-cold saline, and a homogenate was prepared in 0.1M Tris-HCl buffer (pH 7.4). The homogenate was centrifuged (3000 rpm for 10 min) and the supernatant was used for the assay of aspartate transaminase (AST), alanine transaminase (ALT) and total protein. Small pieces of liver tissue were collected and preserved in 10% formalin solution for histopathological studies.

Statistical analysis – The statistical analysis (Spiegel and Meddis, 1980) was carried out using one way analysis of variance (ANOVA) followed by Dunnet's *t*-test. *P*-values <0.05 were considered as significant.

Results and Discussion

The extracts did not cause mortality upto 2000 mg/kg and were considered as safe (x-unclassified). ALP, SGOT, SGPT, AST, ALT, and total protein were significantly increased and total protein (serum and liver) was significantly decreased in CCl_4 treated group. The extracts exhibited dose dependent reduction in total bilirubin, ALP, SGOT, SGPT, AST, ALT, and increase in total protein (serum and liver) levels (Table 1).

Histology of liver from normal control group (Fig. 1a) showed the central vein surrounded by cords of hepatocytes. Microscopical examination of CCl_4 treated liver (Fig. 1b) showed diffuse hepatocytic damage with areas of necrosis. AET (250 mg/kg) treated animals (Fig. 1c) showed individual focal hepatocyte damage and necrosis. AET (500 mg/kg) treated animals (Fig. 1.d) showed the mild focal hepatocytic damage and necrosis. MET (250 mg/kg) treated animals (Fig. 1e) showed the mild focal groups of heptocytic damage and MET (500 mg/kg) treated animals (Fig. 1f) showed reduced hepatocytic damage. The silymarin treated group (Fig. 1g) showed central vein with cords of hepatocytes with occasional focal hepatocytic damage.

The present study has demonstrated that both AET and MET exhibited significant dose dependent hepatoprotective

Table 1. Hepatoprotective parameters of AET and MET against CCl_4 induced liver damage

Biochemical parameters	Control	CCl_4 treated group	*Thespesa populnea* bark extracts + CCl_4				Silymarin (25 mg/kg) + CCl_4
			AET (mg/kg)		MET (mg/kg)		
			250	500	250	500	
Total bilirubin (mg/dl)	0.48 ± 0.03	25 ± 0 16$^{\dagger a}$	2.13 ± 0.20$^{\dagger\dagger d}$	0.8 ± 0.05$^{\dagger\dagger a}$	1.93 ± 0.08$^{\dagger\dagger a}$	0.65 ± 0.1$^{\dagger\dagger a}$	0.55 ± 0.03$^{\dagger\dagger a}$
ALP (KA units)	115.83 ± 7.16	256.67 ± 5.02$^{\dagger a}$	236.67 ± 4.92$^{\dagger\dagger d}$	178.83 ± 6$^{\dagger\dagger a}$	219.5 ± 3.93$^{\dagger\dagger a}$	158.83 ± 8.01$^{\dagger\dagger a}$	145.67 ± 5.83$^{\dagger\dagger a}$
SGOT (U/ml)	29.17 ± 1.7	82.83 ± 4.69$^{\dagger a}$	74.82 ± 1.72$^{\dagger\dagger d}$	35.5 ± 2.11$^{\dagger\dagger a}$	71.5 ± 2.06$^{\dagger\dagger b}$	34.00 ± 2.06$^{\dagger\dagger a}$	32.83 ± 1.29$^{\dagger\dagger a}$
SGPT (U/ml)	24.67 ± 3	61.50 ± 4.07$^{\dagger a}$	52.17 ± 1.82$^{\dagger\dagger d}$	28.5 ± 0.76$^{\dagger\dagger a}$	49.33 ± 2.93$^{\dagger\dagger b}$	27.17 ± 4.08$^{\dagger\dagger a}$	26.5 ± 0.76$^{\dagger\dagger a}$
Total protein (mg/dL)	5.47 ± 0.11	3.77 ± 0.15$^{\dagger a}$	4.47 ± 0.23$^{\dagger\dagger c}$	4.7 ± 0.16$^{\dagger\dagger b}$	4.6 ± 0.17 $^{\dagger\dagger b}$	5.13 ± 0.19$^{\dagger\dagger a}$	5.17 ± 0.16$^{\dagger\dagger a}$
AST (µmol of pyruvate liberated/mg protein/ min)	39.33 ± 0.88	75.83 ± 2.09$^{\dagger a}$	68.83 ± 1.6$^{\dagger\dagger d}$	45 ± 1.31$^{\dagger\dagger a}$	67.5 ± 2.93$^{\dagger\dagger b}$	41.83 ± 1.83$^{\dagger\dagger a}$	41.33 ± 2.67$^{\dagger\dagger a}$
ALT (µmol of pyruvate liberated/mg protein/ min)	36.83 ± 2.27	85.17 ± 3.88$^{\dagger a}$	76.83 ± 2.88$^{\dagger\dagger d}$	43.83 ± 1.68$^{\dagger\dagger a}$	72 ± 2.44$^{\dagger\dagger a}$	41.83 ± 1.22$^{\dagger\dagger a}$	38.67 ± 1.98$^{\dagger\dagger a}$
Total protein (mg/g of tissue)	0.52 ± 0.01	0.33 ± 0.01$^{\dagger a}$	0.34 ± 0.01$^{\dagger\dagger a}$	0.43 ± 0.02$^{\dagger\dagger a}$	0.37 ± 0.01$^{\dagger\dagger a}$	0.48 ± 0.01$^{\dagger\dagger a}$	0.49 ± 0.01$^{\dagger\dagger a}$

†Control compared with CCl_4 treated animals, $^{\dagger\dagger}CCl_4$ treated animals compared with AET and MET. $^{a}P<0.001$, $^{b}P<0.01$, $^{c}P<0.02$, $^{d}P<0.05$. Data are expressed as mean ± SE (n=6).

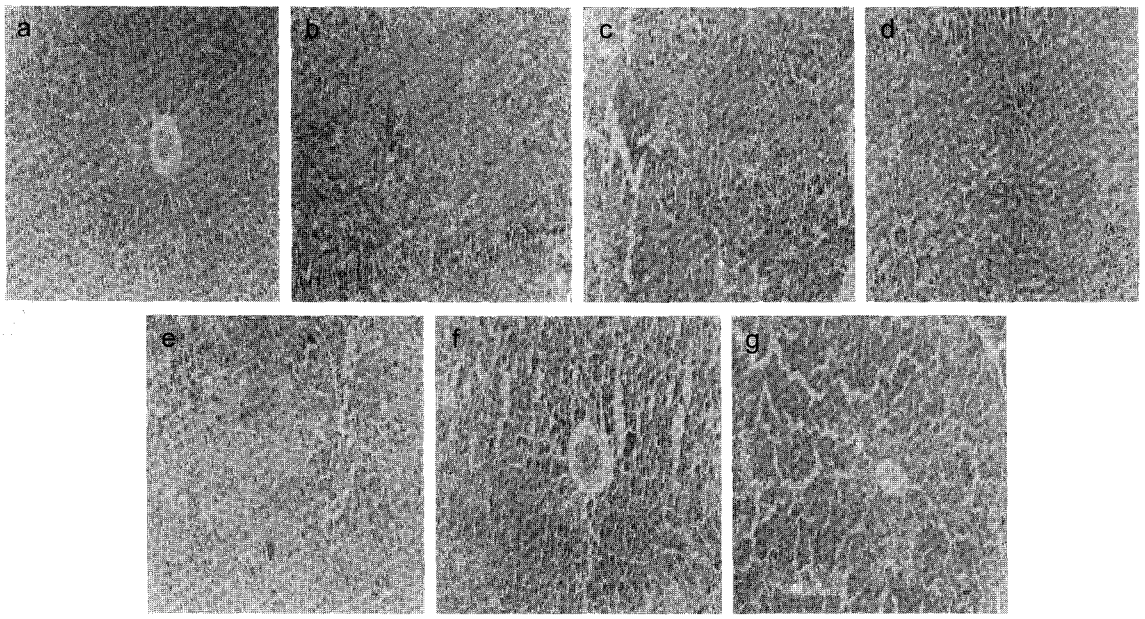

Fig. 1. a) The central vein surrounded by cords of hepatocytes (control group). b) The diffused hepatocytic damage with areas of necrosis (CCl_4 treated group). c) Individual focal hepatocytic damage and necrosis (AET-250 mg/kg). d) Mild focal hepatocytic damage and necrosis (AET-500 mg/kg). e) Mild focal groups of hepatocytic damage (MET-250 mg/kg). f) Reduced hepatocytic damage (MET-500 mg/kg). g) The central vein with cords of hepatocytes with occasional focal hepatocytic damage (Silymarin group).

activity against liver injury induced by CCl_4. CCl_4 induces hepatotoxicity by metabolic activation, therefore it selectively causes toxicity in liver cells maintaining semi-normal metabolic function. CCl_4 is metabolically activated by the cytochrome P450 in the endoplasmic reticulum to form a trichloromethyl free radical ($CCl_3^{\bullet}$) which combine with cellular lipids and proteins in presence of oxygen to induce lipid peroxidation. These results in changes of structures of endoplasmic reticulum and other membrane, loss of metabolic enzyme activation, reduction of protein synthesis and elevation of serum transaminases leading to liver damage (Lim *et al.*, 2000). Aminotransferases contribute a group of enzymes that catalyze the interconversion of aminoacids and α-ketoacids by transfer of amino groups. These are liver specific enzymes and are considered to be very sensitive and reliable indices for necessary hepatotoxic as well as hepatoprotective or curative effect of various compounds (Hayes *et al.*, 1986).

Both AST and ALT levels increase due to toxic compounds affecting liver cells integrity (Subramaniam and Pushpangadan, 1999). ALP is a membrane bound glycoprotein enzyme, with high concentration in sinusoids and endothelium. ALP reaches the liver mainly from bone. It is excreted into the bile so its elevation in serum occurs in hepatobiliary diseases (Burtes and Ashwood, 1986). Results of the present study indicates that both AET and MET probably stabilize the hepatic plasma membrane from CCl_4 induced damage.

The liver is known to play a significant role in the serum protein synthesis, being the source of plasma albumin and fibrinogen and also the other important components like α and β-globulin. The liver is also concerned with the synthesis of γ-globulin. The serum albumin level is low in hepatic diseases. The result reveals that animals pretreated with hepatoprotective agents prior to the challenge with CCl_4, the liver biosynthesis of protein continues to be unaffected.

The metabolic transformation of amino acid in liver by synthesis, transamination, etc. may be impaired due to the escape of both non-proteins and protein nitrogenous substances from injured liver cells as mediated by raise in the serum enzyme levels of ALP, AST and ALT. The protective activity of the extracts may be attributed to the membrane stabilizing agents present in the AET and MET which may avert enzyme leakage in tissue in response to CCl_4 poisoning leading to enhanced metabolic transformation of aminoacids in liver through synthesis and transformation (Mandal *et al.*, 1992).

The results of biochemical observations are supplemented by histopathological examination of rat liver section. The histological changes induced by CCl_4 treatment are reversed by administration of AET and MET. It can be concluded that *T. populnea* bark possess potential hepatoprotective activity. MET was found to exhibit higher hepatoprotection than AET.

References

Anonymous., *The Wealth of India*, Publication and Information Directorate (CSIR), New Delhi, 1995, pp. 223-275.

Burtes, C. A. and Ashwood, E. R., *Textbook of Clinical Chemistry*, WB Sunders Company, Philadelphia, 1986, p. 56.

Doumas, B. T., Watson, W. A. and Biggs, A. G., Estimation of Total Protein. *Clin. Chem. Acta*. **31,** 87-96 (1971).

Ecobichon, D.J., *The basis of toxicology testing*, CRC press, New York, 1997, pp. 43-86.

Hayes, J. R., Londie, L. W. and Leeca, B. J. F., Acute 14 days repeated dosage and 90 days subchronic toxicity studies of CCl_4 in CDI mice. *Fund. Appl. Toxicol*. **7**, 454, 1986.

Hewage, C. M., Bandara, B. M. R., Karunaratne, V., Wannigama, G. P., Pinto, M. R. M. and Sundara, D. S. A. W., Antibacterial activity of some medicinal plants of Srilanka., *J. Nat. Sci. Coun. Srilanka*. **26**, 27-34 (1998).

Jendrassik, L. and Grof, P., Simplified photometric method for determination of blood bilirubin. *Biochem. J.* **297**, 81, (1938).

Kind, P. R. N. and Kings, E. J., Estimation of plasma phosphatase by determination of hydrolysed phenol with aminopyrines. *J. Clin. Path*. **7**, 322-326, (1954).

Lim, H. K., Kim, H. S., Choi, H. S., Oh, S. and Choi, J., Hepatoprotective effects of bergenin, a major constituent of *Mallotus japonicus* on CCl_4 intoxicated rats. *J. Ethnopharmacol*. **72**, 469-474 (2000).

Mandal, P. K., Bishayee, A. and Chatterjee, M., Studies of hepatic protein synthesis in response to *Mikania cordate* root extract in CCl_4 induced hepatotoxicity in mice. *Ital. J. Biochem*. **431**, 345-350 (1992).

Rajesh, M. G., Paul B. and Latha, M. S., Efficacy of kamilari in alcoholic liver cirrhosis. *Antiseptic*. **97**, 320-321 (2000).

Reitman, S. and Frankel, S., A colorimetric method for the determination of serum glutamate oxaloacetate transaminases. *Am. J. Clin. Path*. **28**, 56-63 (1957).

Shirwaikar, A., Sreenivasan, K. K., Kumar, A. A. and Krishnanand, B. R., Antihepatotoxic activity of "kamalari" a marketed ayurvedic preparation. *Indian Drugs*. **29**, 219-224 (1992).

Shirwaikar, A., Kumar, A. A., Krishnanand, B. R. and Sreenivasan, K. K., Chemical investigation and antihepatotoxic activity of Thespesia populnea. *International J. Pharmacog*. **33**, 305-310 (1995).

Shirwaikar, A., Sreenivasan, K. K. and Nagaraj K., Clinical evaluation of kamalari-An ayurvedic drug in the management of viral hepatitis and jaundice. *Antiseptic*. **93**, 412-413 (1996).

Spiegel, M. R. and Meddis, R., *Probability and Statistics*, McGraw-Hill Book Company, New York, 1980, pp. 108-151

Subramoniam, A. and Pushpangadan, P., Development of phytomedicines for liver diseases. *Indian J. Pharmacol*. **31**, 166-175 (1999).

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