Hepatoprotective activity of *Pterocarpus santalinus* L.f., an endangered medicinal plant

B.K. Manjunatha

Department of Botany, S.R.N.M.N. College of Applied Sciences, Shimoga, Karnataka, India.

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Correspondence to:
B.K. Manjunatha
E-Mail:
doctor_bkm@yahoo.com

ABSTRACT

Objective: To evaluate the hepatoprotective activity of crude aqueous and ethanol stem bark extracts of *Pterocarpus santalinus* (Fabaceae) using CCl_4 induced hepatic damage in male Wistar albino rats.

Materials and Methods: The aqueous (45 mg/ml) and ethanol (30 mg/ml) extracts of stem bark in 1% gum tragacanth was administered orally for 14 days and the hepatoprotective activity studied in CCl_4 induced hepatic damage model. The hepatoprotective activity was assessed using various biochemical parameters like serum bilirubin, protein, alanine transaminase, aspartate transaminase and alkaline phosphatase along with histopathological studies of liver tissue.

Results: There was a significant increase in serum levels of bilirubin, alanine transaminase, aspartate transaminase and alkaline phosphatase with a decrease in total protein level, in the CCl_4 treated animals, reflecting liver injury. In the aqueous and ethanol extracts treated animals there was a decrease in serum levels of the markers and significant increase in total protein, indicating the recovery of hepatic cells. Histological study of aqueous extract treated group exhibited moderate accumulation of fatty lobules and cellular necrosis where as ethanol extract treated animals revealed normal hepatic cords without any cellular necrosis and fatty infiltration.

Conclusion: The ethanol and aqueous stem bark extract of *P. santalinus* afforded significant protection against CCl_4 induced hepatocellular injury.

KEY WORDS*:* CCl_4, red sander, stem bark extract.

Introduction

Pterocarpus santalinus L.f. (Fabaceae) is commonly called as Red sander (English), Kempu honne (Kannada) and Raktachandan (Sanskrit). It is an endangered plant species, endemic to the state of Andrapradesh in India.[1] The plant is renowned for its characteristic timber of exquisite color, beauty and superlative technical qualities and ranks among the finest luxury in Japan.[2] Wood is used as astringent, tonic, as external application for wounds, cuts and inflammations, in treating headache, skin diseases, fever, boils, scorpion sting and to improve sight.[3] The red wood yields a natural dye santalin which is used as a coloring agent in pharmaceutical preparations, food stuffs; fruit extract is used as astringent, diaphoretic, in inflammations, headache, skin diseases, bilious infections and chronic dysentry.[4] Heart wood is known to possess isoflavone glucosides,[5]-[7] savinin, calocedrin[8] and triterpene.[9] The lignan isolated from the heartwood is known to inhibit tumor necrosis factor–alpha production and T-cell proliferation.[8] Ethanolic stem bark extract is known to possess antihyperglycemic activity.[10]

The tribal groups of Western Ghats, Shimoga region use stem bark extract of *Pterocarpus santalinus* in treating diabetes, fever, snake bite and jaundice (About 100 g of powdered stem bark is boiled in 500 ml of water for 3-4 h till the volume is reduced to half, cooled and 10 g of jaggery added to the extract and made into pills. Two to three pills a day for 10 days is administered for acute jaundice).

Review of the literature revealed that this rare medicinal plant remained unexplored for many of its claimed pharmacological activities. In the present study, an effort has been made to evaluate the hepatoprotective activity of *Pterocarpus santalinus* L.f.

Materials and Methods

Plant material

Stem bark of *Pterocarpus santalinus* was collected during the month of January 2003 from the Ayurvedic medicinal garden at Gajanur, Shimoga district (Forest Department of Shimoga). The voucher specimens (BKM-430, BKM-431) were deposited in the department Herbaria, SRNMN College of Applied Sciences, Shimoga for future reference.

Extraction

Stem bark was shade dried for a week and powdered mechanically (Sieve No. 10/44). About 250 g of the powder was extracted with 70% ethanol for 48 h using Soxhlet apparatus. The solvent was distilled off at low temperature under reduced pressure using rotary flash evaporator (Buchi, Flawil, Switzerland). The yield was 21% w/w. Another 250 g of the powdered material was boiled in distilled water for 30 min, kept for 3 days with intermittent shaking, filtered and concentrated using rotary flash evaporator to obtain the aqueous extract. Both the extracts were dried in desiccator. The yield was 16.2% w/w. Both the extracts were subjected to preliminary phytochemical tests.[11]

Drug formulations

Oral suspensions containing 45 mg/ml and 30 mg/ml of the aqueous and ethanol stem bark extracts, respectively, were prepared in 1% w/v gum tragacanth.

Animals

Male Wistar albino rats weighing 150-200 g were procured from the National College of Pharmacy, Shimoga and maintained under standard housing conditions. The animals were fed with commercial diet (Hindustan Lever Ltd., Bangalore) and water *ad libitum*. The study was permitted by the Institutional Animal Ethical Committee with Reg. No. 144/1999/CPCSEA/SMG.

Acute toxicity studies

Acute toxicity study was conducted for both the extracts by stair case method.[12] One tenth of the LD_{50} doses were selected for the evaluation of hepatoprotective activity.[13]

The animals were divided into five groups of six rats each. Group I served as control and received the vehicle (1 ml/kg/day of 1% w/v gum tragacanth p.o. for 14 days). Group II to V received 0.1 ml/kg/day of CCl_4 i. p. (E-Merck, Mumbai, India) for 14 days. Group III animals received the standard drug silymarin (Ranbaxy Lab, Dewas) in the dose of 100 mg/kg/day, p.o. for 14 days, while the aqueous and ethanol stem bark extracts of *P. santalinus* were administered to groups IV and V in the dose of 45 mg and 30 mg/kg/day, p.o. respectively for 14 days. The CCl_4 and silymarin or the extracts were administered concomitantly to the respective groups.

All the animals were sacrificed on 14th day under light ether anesthesia. The blood sample from each animal was collected separately in sterilized dry centrifuge tubes by carotid bleeding and allowed to coagulate for 30 min at 37°C. The clear serum was separated at 2500 rpm for 10 min and subjected to biochemical investigations viz., total bilirubin.[14] total protein.[15] serum alanine transaminase, aspartate transaminase[16] and alkaline phosphatase.[17]

Results of biochemical estimations are reported as mean±SEM of six animal in each group. The data were subjected to one-way ANOVA followed by Tukey's multiple comparision test. $P<0.001$ was considered statistically significant.

Histopathology

The liver was excised from the animals and washed with the normal saline. The materials were fixed in 10% buffered neutral formalin for 48 h and then with bovine solution for 6 h and processed for paraffin embedding. Sections of 5m thickness were taken using a microtome, processed in alcohol-xylene series and were stained with alum-haematoxylin and eosin[18] and subjected to histopathological examination.

Results

The LD_{50} of aqueous and ethanol stem bark extracts were found to be 450 mg/kg, b.w. and 300 mg/kg, b.w, respectively. One tenth of these doses (45 mg/kg, b.w. and 30 mg/kg, b.w.) were selected for the evaluation of hepatoprotective activity. Effect of aqueous and ethanol stem bark extracts of *Pterocarpus santalinus* on CCl_4 induced liver damage in rats with reference to biochemical changes in serum is shown in Table 1. The CCl_4 treated control group showed a significant increase in serum total bilirubin (2.45±0.01), alanine transaminase (1413.00±1.99), aspartate transaminase (2213.50±32.79) and alkaline phosphatase (444.33±1.56) and a decrease in total protein (5.93 ± 0.01) indicating the

Table 1

Effect of aqueous and ethanol stem bark extract of *Pterocarpus santalinus* on CCl_4 induced hepatotoxicity in rats

Group (N)		*Total bilirubin (mg/dl)*	*Total protein (gm%)*	*AST (IU/L)*	*ALT (IU/L)*	*ALP (IU/L)*
Control (1% w/v gum tragacanth p.o.)		0.44±0.01	9.44±0.02	153.52±1.43	54.08±1.16	174.99±1.80
CCl_4 (0.1ml/kg/day i.p.)		2.45±0.01*	5.93±0.01*	2213.50±32.79*	1413.00±1.99*	444.33±1.56*
CCl_4 +silymarin (0.1ml/kg/day i.p. +100mg/kg/day p.o.)		0.54±0.01†	8.82±0.01†	208.50±2.17†	75.18±1.17†	184.40±1.16†
CCl_4 + aqueous extract (0.1ml/kg/day i.p + 45mg/kg/day p.o.)		0.93±0.02$^{\dagger@}$	7.40±0.02$^{\dagger@}$	242.17±2.02$^{\dagger@}$	193.36±1.49$^{\dagger@}$	244.30±1.91$^{\dagger@}$
CCl_4 + ethanol extract (0.1ml/kg/day i.p. +30mg/kg/day p.o.)		0.62±0.01$^{\dagger@}$	8.41±0.02$^{\dagger@}$	221.67±2.59$^{\dagger@}$	125.06±1.27$^{\dagger@}$	204.43±1.64$^{\dagger@}$
One-way ANOVA	F	3282.0	8643.0	3691.0	1.63	4659.0
	df	4,25	4,25	4,25	4,25	4,25
	P	0.01	0.01	0.01	0.01	0.01

Values are expressed as mean±SEM. n = 6 in each group. *P≤0.01 when compared to control. †P≤0.01 when compared to CCl_4. $^{@}$P≤0.01 when compared to silymarin.

Figure 1. Section of the liver tissue of control animal showing normal histology and a portal triad showing portal vein (V), hepatic artery (arrow) and bile duct (arrow head). (H & E, 100X)

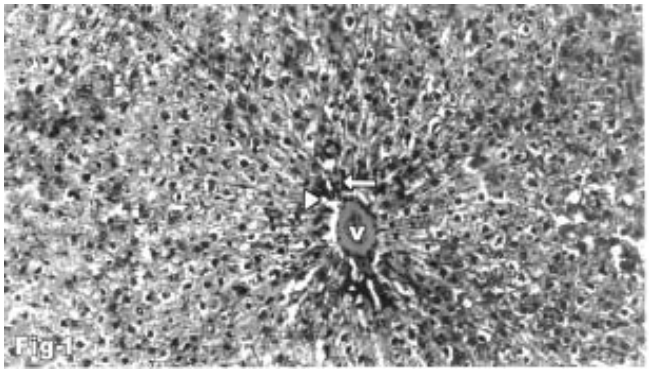


Figure 2. Section of the liver tissue of animal treated with CCl_4 showing a central hepatic vein (V), necrosis (N) and fatty change (F). (H & E, 100X)

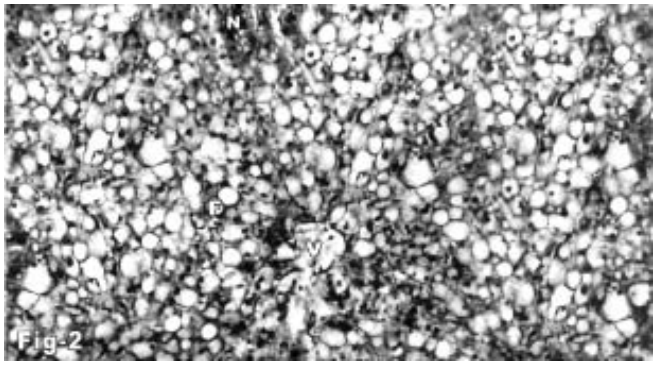


Figure 3. Section of the liver tissue of silymarin treated animals showing normal hepatocytes with central hepatic vein (V). (H & E, 100X)

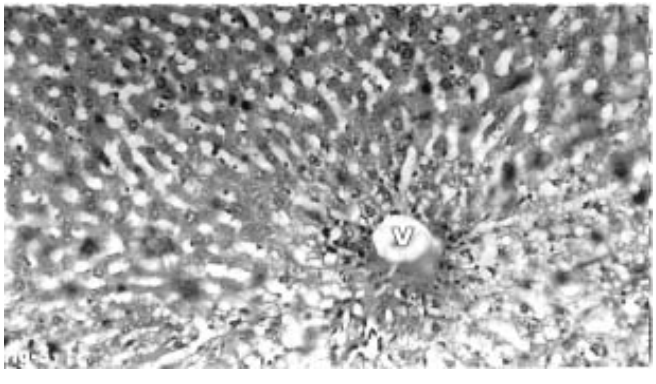


Figure 4. Section of the liver tissue of aqueous stem bark extract treated animals showing normal arrangement of hepatocytes around the portal vein (V), hepatic artery (arrow), bile duct (arrow head), absence of necrosis and few fatty vacuoles**.** (H & E, 100X)

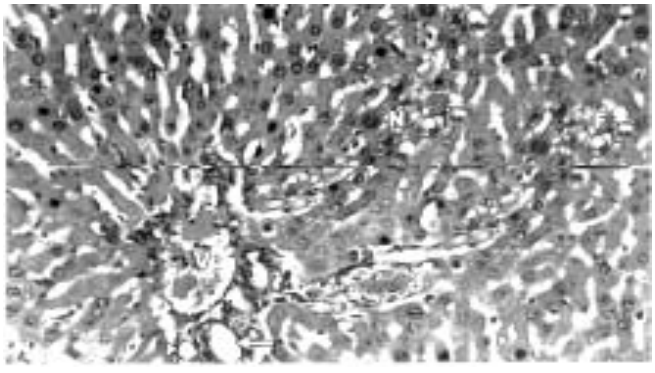
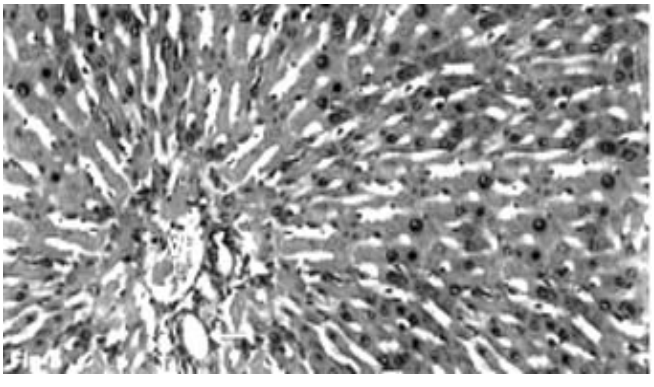


Figure 5. Section of the liver tissue of ethanol stem bark extract treated animals showing normal arrangement of hepatocytes around the portal vein (V), hepatic artery (arrow), bile duct (arrow head), absence of necrosis and fatty vacuoles**.** (H & E, 100X)



liver injury caused by CCl_4. Whereas animals treated with aqueous and ethanol stem bark extracts exhibited a decrease in total bilirubin (0.93 ± 0.02; 0.62 ± 0.01), alanine transaminase (193.36 ± 1.49; 125.06 ± 1.27), aspartate transaminase (242.17 ± 2.02; 221.67 ± 2.59) and alkaline phosphatase (244.30 ± 1.91; 204.43 ± 1.64) along with a significant increase in total protein (7.40 ± 0.02; 8.41 ± 0.02).

Histologically, control animals showed normal hepatic architecture (Figure 1), -the group II animals exhibited intense centrilobular necrosis (N), vacuolization and macrovesicular fatty changes (F) (Figure 2). Silymarin treated animals showed a normal hepatic architecture (Figure 3). Moderate accumulation of fatty lobules and cellular necrosis (Figure 4) were observed in the animals treated with aqueous extract. However, the ethanol extract treated animals exhibited significant liver protection against CCl_4 induced liver damage, as evident by the presence of normal hepatic cords, absence of necrosis and fatty infiltration (Figure 5).

Discussion

The present investigation indicated that both the extracts of *Pterocarpus santalinus* provide significant protection against CCl_4 induced hepatotoxicity in rats. CCl_4 is widely used as hepatotoxin in the experimental studies. The CCl_4 is biotransformed by the cytochrome P450 system to produce the trichloromethyl free radicals, which in turn covalently binds to cell membranes and organelles to elicit lipid peroxidation.[19] Several plants viz., *Cassia aungustifolia,*[20] *Wrightia tinctoria,*[21] *Foeniculum vulgare*[22] and *Panax notoginseng*[23] have been tested for their efficacy in controlling the CCl_4 induced liver damage. Further it has been evident that several phytoconstituents have the ability to induce microsomal enzymes either by accelerating the excretion of CCl_4 or by inhibition of lipid peroxidation induced by CCl_4.[24] Phytoconstituents like flavonoids,[25] triterpenoids,[26] saponins[27] and alkaloids[28] are known to possess hepatoprotective activity. Phytochemical investigations of aqueous and ethanol extract of stem bark revealed the presence of alkaloids, phenols, saponins, glycosides, flavonoids, triterpenoids, sterols and tannins. The present study revealed that among the two extract tested, ethanol extract of stem bark of *P. santalinus* found to possess significant protective effect against hepatotoxicity induced by carbon tetrachloride which may be attributed to the individual or combined action of phytoconstituents present in it. The component(s) of the extract responsible for this effect however was not investigated. Further investigations are needed for identification of the active compounds responsible for hepatoprotective activity. The present finding provides scientific evidence to the ethnomedicinal use of this rare plant genetic resource by the tribal group of Western Ghats in treating jaundice.

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