Hepatoprotective activity of active fractions of *Thespesia lampas* Dalz and Gibs (Malvaceae)

Sir,

In the absence of satisfactory treatment using conventional medicine, treatment of liver disorders remains as one of the major medical problems.[1] Numerous medicinal plants are used in local health traditions to treat liver disorders.[1] In the recent past, studies have shown varying levels of hepatoprotective properties in traditional plants found in Kerala, India, such as *Phyllanthus maderaspatensis*, *Phyllanthus rheedii*, *Thespesia populena, Momordica subangulata*, *Naregamia alata*, *Lygodium flexuosum*, *Cheilanthes farinosa, Physalis peruviana,* and *Trichopus zeylanicus*.[2-6] However, many medicinal plants used in remote villages and tribal pockets of Kerala remain to be studied. *Thespesia lampas* Dalz. and Gibs (family Malvaceae) is one such plant. This plant root is used in folklore medicine to treat liver diseases in Attapadi Hills of Western Ghats, Kerala. In traditional medicine, its roots and fruits are used for treating gonorrhea, jaundice, and syphilis. Studies have shown that the plant possesses anti-microbial, antioxidant, anti-lipoxygenase, anti-hyperlipidaemic, and anthelmintic activities. The traditional use of this plant as a hepato-protective agent has not been verified. This study was carried out to get insights into the utility of *T. lampas* to develop a satisfactory hepatoprotective medicine.

Male albino rats of Wistar strain weighting 190 ± 10 g were used for the study. They were reared under standard conditions. The experimental protocol was approved by Institute Animal Ethics Committee controlled by Committee for the Purpose of Control and Supervision of Experiments on Animals.

The plant roots were collected from Attapadi Hills and authenticated by Dr. Mathew Dan of TBGRI. A voucher specimen (No. SAS 1450) was deposited in TBGRI.

The plant root powder was sequentially extracted with petroleum ether, ethanol, and water. The yield of ethanol extract (the most active extract) was 4.4% of the root powder. The ethanol extract (40 g) was fractionated sequentially into *n*-butanol, ethyl acetate, and double-distilled water (each 50 × 3 ml) fractions. The fractions were dried in a water bath at 50 °C. The yield of *n*-butanol fraction was 32% of the ethanol extract. The ethanol extract and *n*-butanol fraction were subjected to HPLC analysis.

To isolate major compounds from the ethanol extract, the extract was subjected to column chromatography (500 × 30 mm) using Merck silica gel (60–120 mesh) and increasing polarity of solvents (petroleum ether, chloroform, ethyl acetate, and methanol in different ratios) for elution. Identification of isolated compounds was done by comparison with standards in HPLC and with spectral data (IR, NMR, and mass spectra).

Hepatoprotective property of *T. lampas* extracts and different fractions on paracetamol-induced hepatic damage was done essentially as described.[6] Briefly, the rats were divided into indicated number of groups. The extracts were given orally for 5 days. Paracetamol was administered (single dose in 1% CME, 2 g/kg, p.o.) to all the groups except the normal control group on the third day of the experiment. The rats were sacrificed on the fifth day after blood collection under ether anesthesia by ocular puncture of retro-orbital plexus.[7] Serum was separated under cold conditions, and biochemical parameters were measured using assay kits (SPAN Diagnostics Ltd., Surat, India). Hepatoprotective activity of the butanol fraction against CCl_4-toxicity in rats was performed as described.[4]

As shown in Table 1, *T. lampas* root extracts showed varying levels of hepato-protection against paracetamol-induced liver damage. Ethanol extract showed better activity compared to water and petroleum ether extracts. All the three fractions of the ethanol extract showed varying levels of hepatoprotection (data not shown). However, *n*-butanol fraction (200 mg/kg) exhibited maximum hepatoprotective activity which was concentration dependent and comparable to that of 50 mg/kg silymarin [Table 1]. Similarly, the fraction showed hepatoprotective action against CCl_4-induced hepatic damage also (data not shown).

Table 1: Effect of various extracts and active fraction of the alcohol extract of *T. lampas* roots on paracetamol-induced liver injury in rats

Groups	SGOT (IU/L)	SGPT (IU/L)	SALP (IU/L)	Bilirubin (μ mol/L)
Saline control (5 ml/kg)	91.4 ± 2.4	57.3 ± 1.8	74.6 ± 3.1	14.3 ± 0.3
Paracetamol (2 g/kg)	427.2 ± 3.2	299.6 ± 2.3	178.8 ± 5.7	25.5 ± 0.2
Paracetamol + Silymarin (50 mg/kg)	138.7 ± 1.9 (86)*	88.2 ± 1.8 (87)*	76.1 ± 1.3 (98)*	15.2 ± 0.2 (92)*
Paracetamol + PE (200 mg/kg)	331.6 ± 2.9 (29)*	189.4 ± 2.0 (46)*	140.9 ± 0.5 (36)*	20.2 ± 0.3 (48)*
Paracetamol + EE (200 mg/kg)	209.3 ± 1.7 (65)*	121.3 ± 2.3 (74)*	109.6 ± 0.5 (66)*	17.1 ± 0.2 (75)*
Paracetamol + WE (200 mg/kg)	222.1 ± 3.3 (61)*	140.9 ± 1.0 (65)*	120.2 ± 0.9 (56)*	17.4 ± 0.3 (72)*
Paracetamol + BF (50 mg/kg)	176.2 ± 1.3 (74)*	126.3 ± 2.5 (71)*	98.0 ± 0.5 (81)*	17.9 ± 0.3 (77)*
Paracetamol + BF (100 mg/kg)	160.1 ± 1.1 (79)*	110.6 ± 1.2 (78)*	88.6± 0.7 (89)*	16.6 ± 0.3 (81)*
Paracetamol + BF (200 mg/kg)	132.4 ± 3.6 (88)*	89.9 ± 2.9 (86)*	82.9 ± 0.7 (95)*	15.4 ± 0.3 (92)*

Values are mean ± SEM of six animals; values marked with ostrich are statistically significant (compared to paracetamol control), *$P < 0.001$; one-way ANOVA followed by Dunnet's *t*-test. PE, Petroleum ether extract; EE, ethanol extract; WE, water extract; BF, butanol fraction of ethanol extract; SGPT, serum glutamate pyruvate transaminase; SGOT, serum glutamate oxaloacetate transaminase; SALP, serum alkaline phosphatase. Values in parentheses are % protection.

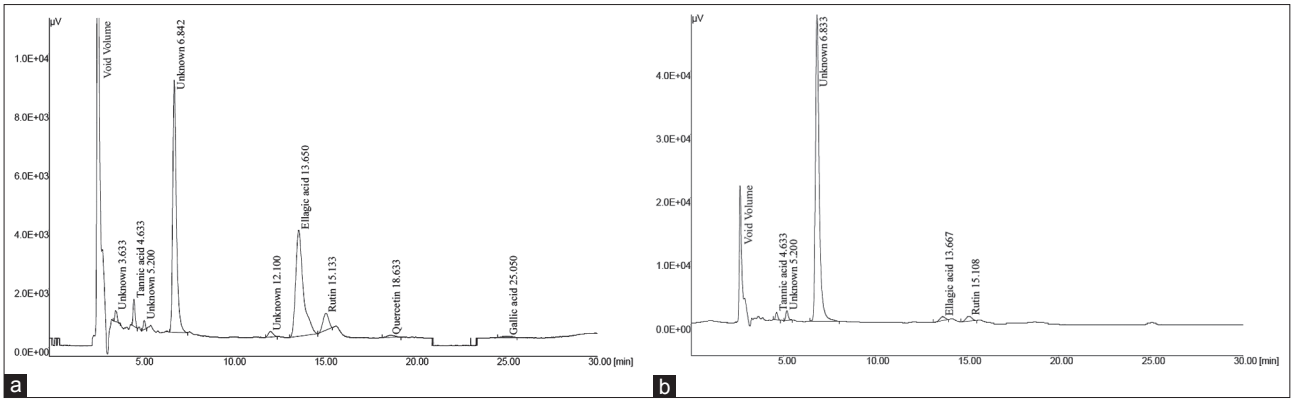

Figure 1: HPLC chromatogram of the ethanol extract and butanol fraction of the ethanol extract of *T. lampas.* (a) Ethanol extract. (b) Butanol fraction. Mobile phase: acetonitrile–methanol–25 mM KH_2PO_4 (10:5:85 v/v) pH 2.5. Column: Grace C18 (250 × 4.6) mm; particle size: 5 μm; wavelength: 261 nm; flow: 1.0 mL/min; injection volume: 20 μl from 500 μg/mL sample

HPLC chromatogram of the ethanol extract showed the presence of nine peaks and two very minor peaks [Figure 1a]. Four compounds were isolated from the ethanol extract by column chromatography. One of the two major compounds was identified as ellagic acid and the other (retention time: 6.81 min) was an unidentified phenolic compound. Other compounds identified were rutin and tannic acid. As judged from retention time-matching with standards in HPLC, quercetin and gallic acid were detected as very minor compounds [Figure 1a].

In the HPLC profile of the *n*-butanol fraction of the ethanol extract, one major peak and 4 minor peaks were seen [Figure 1b]. The major peak (retention time: 6.81 min) was the unidentified phenolic compound purified from the ethanol extract. Other peaks were rutin, ellagic acid, tannic acid, and an unidentified compound [Figure 1b].

During the course of our study, a preliminary report appeared on the hepatoprotective property of *T. lampas* root against CCl_4-toxicity in rats In that study, the water extract of the plant root was reported to have more hepatoprotective activity than the methanol extract.[8] In this study, the ethanol extract was found to be better than the water extract.

We show for the first time that *T. lampas* contains three known hepatoprotective compounds (tannic acid, ellagic acid, and rutin) and a major unidentified hepatoprotective phenolic compound. Tannic acid is the active principle in the plant *Polygonum bistorta* which shows protection against paracetamol-induced liver damage in rats.[9] Ellagic acid is known to have hepatoprotective, anti-oxidant, and anticancer activities.[10] Rutin (20 mg/kg) prevents paracetamol-induced liver damage.[11]

The ethanol extract contained more amount of the known hepatoprotective compounds, ellagic acid in particular, whereas relative concentrations of these compounds were low in the *n*-butanol fraction. However, the hepato-protective activity of the *n*-butanol fraction was higher compared to the ethanol extract. The *n*-butanol fraction contained the major unidentified phenolic compound which could be the major active principle. We have tentatively identified this compound as geraniin. Studies are in progress to confirm the same.

T. lampas root is used in traditional medicine without any recorded or known toxicity. Further, in our preliminary investigation the root powder was well tolerated in rats up to 5 g/kg tested.

Since the active extract and fraction contain several bio-active compounds, they may perhaps act against the toxic chemicals by additive or synergistic actions. Low doses of several phytochemicals with hepato-protective and other beneficial effects could probably serve as a better and safer hepato-protective medicine. *T. lampas* is very promising for the development of safe and effective hepatoprotective phytomedicine and/or conventional medicine.

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