

Full Length Research Paper

Hepatoprotective Activity of Geraniin Isolated from *Thespesia lampas* Dalz. and Gibson

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Accepted 02 April, 2012

***Thespesia lampas* Dalz. and Gibson (root) is used in traditional medicine for liver ailments in Kerala, India. Preliminary studies showed the hepatoprotective activity of the butanol fraction of alcohol extract of *T. lampas* root against paracetamol toxicity in rats. In this follow up studies, the major active principle was isolated by column chromatography and identified as geraniin using spectral data. Geraniin showed promising hepatoprotective activity against both CCl₄ and paracetamol-induced liver damages in rats. The promising anti-hepatotoxic activity of geraniin was not reported previously. Further, this study revealed the presence of geraniin in *T. lampas* root.**

Keywords: *Thespesia lampas* Dalz. and Gibson; geraniin; hepatoprotection; hepatotoxicity.

INTRODUCTION

Liver disorders remain as one of the major medical problems; the conventional modern medicine is devoid of satisfactory treatment to severe liver diseases and the herbal drugs are used, to a large extent, to manage these diseases (Mumoli et al., 2006; Subramoniam and Pushpangadan, 1999). The major causes of liver damages are viral infection and exposure to toxic chemicals including drugs and alcohol. Drug induced liver injury is responsible for 5% of all hospital admissions and 50% of all acute liver failures (Mumoli et al., 2006). Paracetamol, commonly used anti-pyretic drug, is usually well tolerated in prescribed doses but overdose causes liver toxicity (Wallace, 2004). To determine anti-hepatotoxicity of drugs, paracetamol as well as CCl₄ (carbon tetrachloride) are commonly used to induce liver damage in rodents.

A number of medicinal plants are used in traditional systems of medicine and local health traditions for the management of liver disorders (Subramoniam and Pushpangadan, 1999). Examples of hepato-protective traditional medicinal plants of Kerala, India, which are scientifically verified in the recent past for their liver protective action include *Phyllanthus maderaspatensis*

(Asha et al., 2004; Asha et al., 2007), *Phyllanthus rheedii* (Suresh and Asha, 2008), *Thespesia populena* (Yuvaraj and Subramoniam, 2009), *Momordica subangulata* (Asha, 2001), *Naregamia alata* (Asha, 2001), *Lygodium flexuosum* (Wills and Asha, 2006; Wills and Asha, 2007), *Chilanthus farinose* (Krishna et al., 2010), *Physalis peruviana* (Arun and Asha, 2007) and *Trichopus zeylanicus* (Subramoniam et al., 1998). However, many medicinal plants used for liver protection in remote villages and tribal pockets of Kerala remain to be studied. *Thespesia lampas* Dalz and Gibson (family Malvaceae) is one such plant used in the treatment of liver disorders in folklore medicine.

T. lampas is an ever green tree commonly known as Kattupoovarssu in Malayalam. The plant occurs in many parts of India, Africa, Philippines, etc. In traditional medicine, its roots and fruits are used for treating gonorrhoea, jaundice and syphilis (Stephan Ambrose et al., 2012). Studies have shown that the plant possesses antioxidant, anti-lipoxygenase (Kumaraswamy and Satish, 2008), anti-hyperlipidaemic (Sangameswaran et al., 2008) and anthelmintic (Satish et al., 2009) activities. Our preliminary studies have shown strong hepatoprotective activity in the butanol fraction of alcohol extract of the plant root against paracetamol induced toxicity (Stephan Ambrose et al., 2012). The objective of the present study was to isolate the major hepato-protective principle from the butanol

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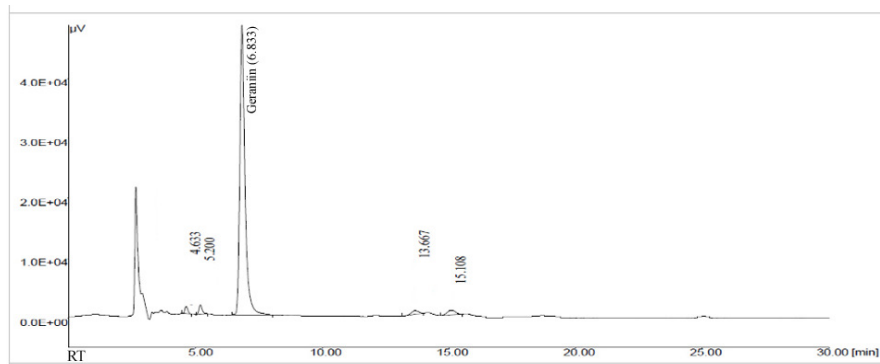


Figure 1. HPLC chromatogram of butanol fraction of ethanol extract of *T. lampas*
 Mobile Phase: ACN-Methanol-25 mM KH_2PO_4 . (10:5:85 v/v) pH 2.5;
 Column: Grace C18 (250X4.6) mm 5 μ ; Wavelength: 261 nm; Flow: 1.0 mL/min;
 Injection volume: 20 μ L from 500 μ g/mL sample
 Collection of plant materials separation of butanol fraction from the ethanol extract of the plant root, etc are described recently (Stephan Ambrose et al., 2012).

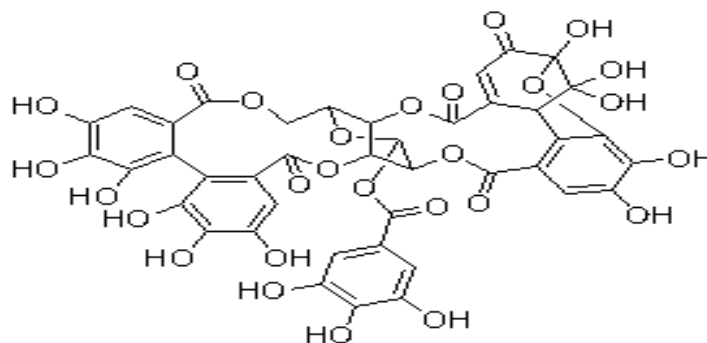


Figure 2. Structure of geraniin; mw 954; MF: $\text{C}_{41}\text{H}_{28}\text{O}_2$

Activity guided isolation of the major active principle from butanol fraction: Geraniin, the major compound in the active fraction (AF) was isolated by column chromatography. The AF (500 mg) was mixed with 1.4 g of Silica gel (60-120 mesh) to obtain an admixture. The column (diameter: 1.6 cm, bed height: 11cm, sample height: 3.7 cm) was packed with 10.3 g of silica gel (60-120 mesh) and 2.1 g of admixture with chloroform. The column was eluted with increasing solvent polarity from chloroform to methanol such as 1-3 fractions from chloroform 100%, 3-6 fractions from chloroform: methanol 5: 95, 7-9 fractions from 20: 80, 10-12 fractions from 50 : 50, 13-15 fractions from 80: 20 and 16-18 fractions from 100% methanol. One to fifteen fractions did not yield any significant amount of the major compound, but fractions sixteen to eighteen yielded 0.035 g of dark brown powder (the major peak in HPLC). The compound obtained was identified using spectral data (UV-Vis, IR, $^1\text{H-NMR}$, $^{13}\text{C-NMR}$, DEPT 135 and HREI MS) as geraniin.

fraction and evaluate its anti-hepatotoxic activity.

RESULTS AND DISCUSSION

In HPLC chromatogram of the butanol fraction (active fraction), there were 5 peaks---single major component (Rt: 6.892) and 4 minor components (Figure 1). The major compound with Rt: 6.892 was more than 60 % of

the butanol fraction; it was isolated in pure form by column chromatography. It was identified as geraniin from the $^1\text{H-NMR}$ and Mass data with a molecular weight of 954 and molecular formula of $\text{C}_{41}\text{H}_{28}\text{O}_{27}$. The structure of geraniin is shown (Figure 2).

As shown in Table 1, geraniin showed a concentration dependent hepato-protective activity against paracetamol-induced liver damage as judged from the activities of serum marker enzymes for liver

Table 1. Effect of geraniin isolated from *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 \pm 2.4	57.3 \pm 1.8	74.6 \pm 3.1	12.6 \pm 1.1
Paracetamol (2 g/kg)	427.2 \pm 3.2	299.6 \pm 2.3	178.8 \pm 5.7	22.5 \pm 1.0
Silymarin (50 mg/kg) + Paracetamol	138.7 \pm 1.9 (86)*	88.2 \pm 1.8 (87)*	76.1 \pm 1.3 (98)*	14.0 \pm 1.4 (86)*
BF (50 mg/kg) + Paracetamol	209.3 \pm 1.7 (65)*	121.3 \pm 2.3 (74)*	109.6 \pm 2.5 (66)*	15.9 \pm 1.2 (67)*
Geraniin (50 mg/kg) + Paracetamol	132.4 \pm 3.6 (88)*	89.9 \pm 2.9 (87)*	82.9 \pm 2.7 (92)*	14.6 \pm 1.2 (80)*
Geraniin (100 mg/kg) + Paracetamol	111.2 \pm 1.1 (94)*	74.6 \pm 1.2 (93)*	79.9 \pm 1.7 (95)*	13.6 \pm 0.9 (90)*
Geraniin (200 mg/kg) + Paracetamol	93.4 \pm 3.8 (99)*	57.6 \pm 2.8 (100)*	75.9 \pm 2.7 (99)*	13.1 \pm 1.3 (95)*

Values are mean \pm 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; 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. The hepatoprotective activity was determined essentially as described (Subramoniam et al., 1998). The BF or geraniin was given orally (5 ml/kg) daily for 5 days. Paracetamol was administered (single dose in 1% CME, 2 gm/kg, 5 ml/kg, p.o) to all the groups except normal control group on the 3rd day of the experiment. The rats were sacrificed under ether anesthesia on the 5th day after blood collection. Biochemical parameters were measured using assay kits (SPAN Diagnostics Ltd, Surat, India). Male albino rats of Wistar strain weighting 190 \pm 10 g (about 9 week-old rats) were used for the study. The experimental protocol was approved by Institutional Animal Ethics Committee constituted as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals.

Table 2. Effect of different doses of geraniin from *T. lampas* root on CCl₄-induced liver injury in rats

Groups	SGOT (IU /L)	SGPT (IU /L)	SALP (IU /L)	Bilirubin [indirect](μ mol/l)	Liver weight (g)
Saline control (5 ml/kg)	98.4 \pm 1.8	62.5 \pm 2.0	71.2 \pm 1.2	11.9 \pm 1.0	3.13 \pm 0.01
CCl ₄ (0.7 ml/kg)	442.2 \pm 3.2	302.0 \pm 2.7	186.7 \pm 2.5	26.5 \pm 2.5	5.06 \pm 0.05
Silymarin (50 mg/kg) + CCl ₄	121.7 \pm 2.6 (93)*	75.3 \pm 1.3 (95)*	74.5 \pm 1.1 (97)*	14.6 \pm 1.8 (82)*	3.35 \pm 0.08 (89)*
Geraniin (50 mg/kg) + CCl ₄	122.9 \pm 4.9 (93)*	73.0 \pm 1.9 (96)*	79.3 \pm 1.8 (93)*	14.9 \pm 1.9 (80)*	3.39 \pm 0.09 (87)*
Geraniin (100 mg/kg) + CCl ₄	108.2 \pm 4.1 (97)*	66.2 \pm 3.0 (98)*	74.1 \pm 2.1 (97)*	14.4 \pm 1.1 (83)*	3.31 \pm 0.07 (91)*
Geraniin (200 mg/kg) + CCl ₄	98.6 \pm 4.4 (99)*	63.0 \pm 2.9 (99)*	70.4 \pm 2.7 (100)*	14.0 \pm 1.7 (86)*	3.20 \pm 0.09 (96)*

The hepatoprotective activity against CCl₄ toxicity was performed as described (Youvaraj and Subramoniam, 2009). Values are mean \pm SEM of six animals; Values marked with ostrich are statistically significant compared to CCl₄ control values, * $P < 0.001$; Values in parentheses are % protection. CCl₄ was administered intraperitoneously. Other details as given for table 1.

damage (glutamate pyruvate transaminase, glutamate oxaloacetate transaminase, alkaline phosphatase) and bilirubin levels. The elevated levels of these parameters found in the serum of paracetamol control rats were almost normalized by the drug treatment in a concentration dependent manner. Geraniin (50 mg/kg) isolated from the butanol fraction, exhibited more activity than the butanol fraction (50 mg/kg). The hepatoprotective activity of geraniin was almost comparable to that of silymarin, a standard herbal drug. As shown in Table 2, in the case of CCl₄-induced liver damage also, geraniin showed protective activity. In this case also, the efficacy of geraniin was almost comparable to that of silymarin.

As far as our literature survey could ascertain, no studies on the anti-hepatotoxic activity of geraniin, hydrolysable tannins, have been carried out previously. In the present study, purified geraniin exhibited hepatoprotective activity against hepatotoxic chemicals-induced liver damage.

Geraniin is known to occur in plants such as *Phyllanthus urinaria*, *P. amarus*, *P. embilica*, *Nephelium lappaceum* and *Geranium sibiricum*. It has many biological properties. Geraniin from *P. amarus* is able to effectively inhibit HIV-1 replication (Notka et al., 2003). Further, geraniin from *Phyllanthus urinaria* has been reported to be active against HSV (herpes simplex virus) (Young et al., 2007). However, anti-hepatitis viral activity of this compound remains to be studied.

Geraniin, isolated from *Phyllanthus urinaria*, possesses anti-oxidant, anti-semi carbazide-sensitive amine oxidase and anti-hypertensive activities (Lin et al., 2008). Geraniin is one of the major anti-oxidant components of *P. embilica* fruits (Liu et al., 2008). Further, geraniin from *Geranium sibiricum* (Zhexiong and Renshuang, 2011) and *Nephelium lappaceum* fruits (Thitilertdecha et al., 2010) has *in vivo* and *in vitro* anti-oxidant activities. Synthetic anti-oxidants such as butylated hydroxytoluene (BHT) are suspected of being responsible for liver damage (Barlow, 1990). In the present study, the naturally occurring anti-oxidant, geraniin exhibited very promising hepatoprotective activity against both paracetamol and carbon tetrachloride induced toxicity. The mechanism action of geraniin is not clear. It could have more than one mechanisms of action and one of them may be due to its anti-oxidant activity.

Phyllanthus species such as *P. amarus* and *P. urinaria* are known for their hepatoprotective properties and geraniin is present in these plants. In light of the present studies, it is clear that the hepato-protective properties of these plants could be partly due to geraniin.

The dried *T. lampas* root powder contains about 1 % of geraniin. Further, this compound is easily available from other sources also. *Phyllanthus amarus*, which can be easily cultivated, also contains this compound. The compound can also be obtained from waste materials such as rind waste of fruit of *Nephelium*

lappaceum (Thitilertdecha et al., 2010). Thus the raw materials for the isolation of geraniin for drug development could be made available easily and likely to be cheaper than silymarin, the commonly used hepato-protective agent.

Since this compound is known to have activities such as anti-hypertensive and anti-oxidant properties, it could prove to be an ideal drug to patients with hypertension and oxidative stress in addition to liver damage. There is an urgent need for clinical trial of this compound.

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