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## Recommended Citation

Yaesh, S., Jamal, Q., Shah, A., Gilani, A. (2010). Antihepatotoxic activity of *Saussurea lappa* extract on D-galactosamine and lipopolysaccharide-induced hepatitis in mice.. *Phytotherapy Research*, 24, S229-S232.

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## SHORT COMMUNICATION

# Antihepatotoxic Activity of *Saussurea lappa* Extract on D-galactosamine and Lipopolysaccharide-Induced Hepatitis in Mice

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The effects of aqueous-methanol extract of *Saussurea lappa* Clarke root (Sl.Cr) was investigated against D-galactosamine (D-GalN) and lipopolysaccharide (LPS)-induced hepatitis in mice. Co-administration of D-GalN (700 mg/kg) and LPS (1 µg/kg) significantly raised the plasma transaminase levels (ALT/AST) as compared to the control group ( $p < 0.05$ ). Pretreatment of mice with different doses of Sl.Cr (150, 300 and 600 mg/kg) significantly prevented the D-GalN and LPS-induced rise in plasma levels of ALT and AST in a dose-dependent manner ( $p < 0.05$ ). Post-treatment with Sl.Cr (600 mg/kg) significantly restricted the progression of hepatic damage induced by D-GalN and LPS ( $p < 0.05$ ). The improvement in plasma enzyme levels was further verified by histopathology of the liver, which showed improved architecture, absence of parenchyma congestion, decreased cellular swelling and apoptotic cells in treatment groups as compared to the toxin group of animals. These data indicate that the Sl.Cr exhibits hepatoprotective effect in mice and this study rationalize the traditional use of this plant in liver disorders. Copyright © 2009 John Wiley & Sons, Ltd.

**Keywords:** *Saussurea lappa*; antihepatotoxic; D-galactosamine; lipopolysaccharide, hepatoprotective.

## INTRODUCTION

In traditional herbal medicine, dried roots of *Saussurea lappa* Clarke (Asteraceae) are considered as antiseptic, astringent, diuretic, aphrodisiac, antispasmodic, anti-helminthic, and sedative and are used for the treatment of asthma, dyspepsia, rheumatism, cough, throat infections, tuberculosis, leprosy, malaria, convulsions, fever, helminth infestation and many other diseases (Nadkarni, 1976). They are being used either alone or, more commonly, as a part of compound preparation for the treatment of various liver disorders (Said, 1982). The *in vitro* antiviral activity of *Saussurea lappa* root against hepatitis B virus had been reported (Chen *et al.*, 1995). There is, however, no reported *in vivo* activity related to liver damage. This study was conducted to evaluate the hepatoprotective activity of *Saussurea lappa* root extract.

## MATERIALS AND METHODS

**Plant material and preparation of crude extract.** Dried roots of *Saussurea lappa* were purchased from a local herbalist and authenticated by a taxonomist, Mr Afzal Rizvi, at Hamdard University. The voucher specimen

(AV-PL-03-02-44) was submitted to the herbarium at the Department of Biological and Biomedical Sciences, Aga Khan University, Karachi. Plant material was cleaned of adulterants, crushed and soaked in 70% aqueous-methanol solution in a large container for 10 days with occasional shaking. It was then filtered through filter paper and concentrated into thick semi-solid paste under reduced pressure on a rotary evaporator, with an approximate yield of 5.5%. The extract (Sl. Cr) was soluble in normal saline and distilled water.

**Animals and chemicals.** Balb-C mice of either sex (20–25 g) were obtained from the animal house of Aga Khan University. Experiments performed complied with the rulings of the Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council (NRC, 1996), approved by the Ethical Committee of the Aga Khan University. D-Galactosamine (D-GaIN) and Lipopolysaccharide (LPS) (E.Coli 055:B5) were purchased from Sigma Chemicals Co. (St Louis, MO, USA). Alanine transaminase (ALT) and Aspartate transaminase (AST) estimation kits were purchased from Randox Laboratories Ltd (Antrim, UK).

**Induction of hepatic injury.** Hepatic injury was induced by intraperitoneal co-administration of D-GalN and LPS at doses of 700 mg/kg and 1 µg/kg, respectively (Tiegs *et al.*, 1989). Control animals received 0.01 mL/kg normal saline. Mice were sacrificed by cervical dislocation 8 h after the administration of toxins. Blood samples, collected in sterilized and heparinized syringes by direct cardiac puncture, were centrifuged and plasma

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obtained was used for ALT and AST estimation spectrophotometrically (Reitman and Frankle, 1957). Liver excised out was placed in 10% buffered formal saline that was changed after 12 h to remove any residual blood. Tissue was then dehydrated in increasing concentrations of alcohol, cleared with xylene, impregnated and embedded in paraffin to form block. Approximately 5 µm sections were cut and fixed on glass slides, stained with hematoxylin and eosin for microscopic examination. The following parameters were observed and quantified for liver histopathology score (LH score); (hpf: high power field)

1. Lobular architecture:
    - Intact ----- 0
    - Intact with cell swelling ----- 1
    - Mild disruption ----- 2
    - Marked disruption ----- 3
  2. Apoptosis:
    - None ----- 0
    - Occasional ----- 1 (if <5 cells per hpf in 10 or less hpf)
    - Mild ----- 2 (if <10 cells per hpf in 10 hpf)
    - Marked ----- 3 (if >10 cells per hpf in 10 hpf)
  3. Congestion:
    - None ----- 0
    - Occasional ----- 1 (if 1 or 2 foci in 10 hpf)
    - Mild ----- 2 (if >2 foci in 10 hpf)
    - Marked -- 3 (if 1 or 2 foci per hpf in 10 or > hpf)
- On the basis of above mentioned criteria, hepatic damage was considered as:
- None; if the score is 0–1.
  - Mild; if the score is 2–3.
  - Moderate; if the score is 4–6, and
  - Marked; if the score is 7–9.

**Hepatoprotective study.** Animals were divided into six groups of seven mice each. Group 1 received normal saline (0.01 mL/kg) twice. Group 2 received normal saline followed by, after 1 h, D-GalN 700 mg/kg and LPS 1 µg/kg. Groups 3, 4 and 5 received Sl.Cr at doses of 150 mg/kg, 300 mg/kg and 600 mg/kg, respectively, 1 h before the co-administration of toxins. Group 6

received Sl.Cr (600 mg/kg) 1 h after the administration of toxins. Extract, toxins and vehicle were administered intraperitoneally.

**Acute toxicity study.** Sl.Cr administered to a group of 10 mice at a dose of 5 g/kg intraperitoneally. The animals were kept under constant observation for 6 h to note any behavioral change and mortality was recorded after 24 h.

**Statistical analysis.** Plasma ALT and AST were presented as mean ± standard deviation and the mean of two groups were compared by Student's *t*-test. Analysis of variance (ANOVA) was used for comparison between different pretreatment groups. In all statistical analysis, *p*-value of <0.05 was considered as significant. Liver histopathology score (LH score) was presented as mode and range. Percent reduction in serum enzyme levels was calculated as follows;

$$100 - \left[ \frac{\text{Treatment group mean} - \text{control mean}}{\text{toxin group mean} - \text{control mean}} \times 100 \right]$$

## RESULTS AND DISCUSSION

D-galactosamine (D-GalN) and LPS-induced hepatitis in mice is a commonly used test model (Hishinuma *et al.*, 1990). The major cytokines responsible for hepatocellular damage and apoptosis in this animal model are tumor necrotic-alpha (TNF-α) (Joseph *et al.*, 2000) and nitric oxide (NO) (Moncada and Higgs, 1993). In our study, co-administration of D-GalN (700 mg/kg) and LPS (1 µg/kg) to Group 2 (toxin group) significantly raised the plasma levels of ALT and AST to 2808 ± 204 and 2862 ± 387 IU/L, as compared to the control group (Table 1). Liver histopathology of toxin group showed marked hepatic damage with LH score of 9 (Table 1, Fig. 1). The plasma ALT and AST levels in groups of mice pre- and post-treated with different doses of Sl.Cr were significantly lower than the toxin

**Table 1.** Effect of *Saussurea lappa* extract on D-galactosamine/Lipopolysaccharide-induced increase in hepatic enzyme levels

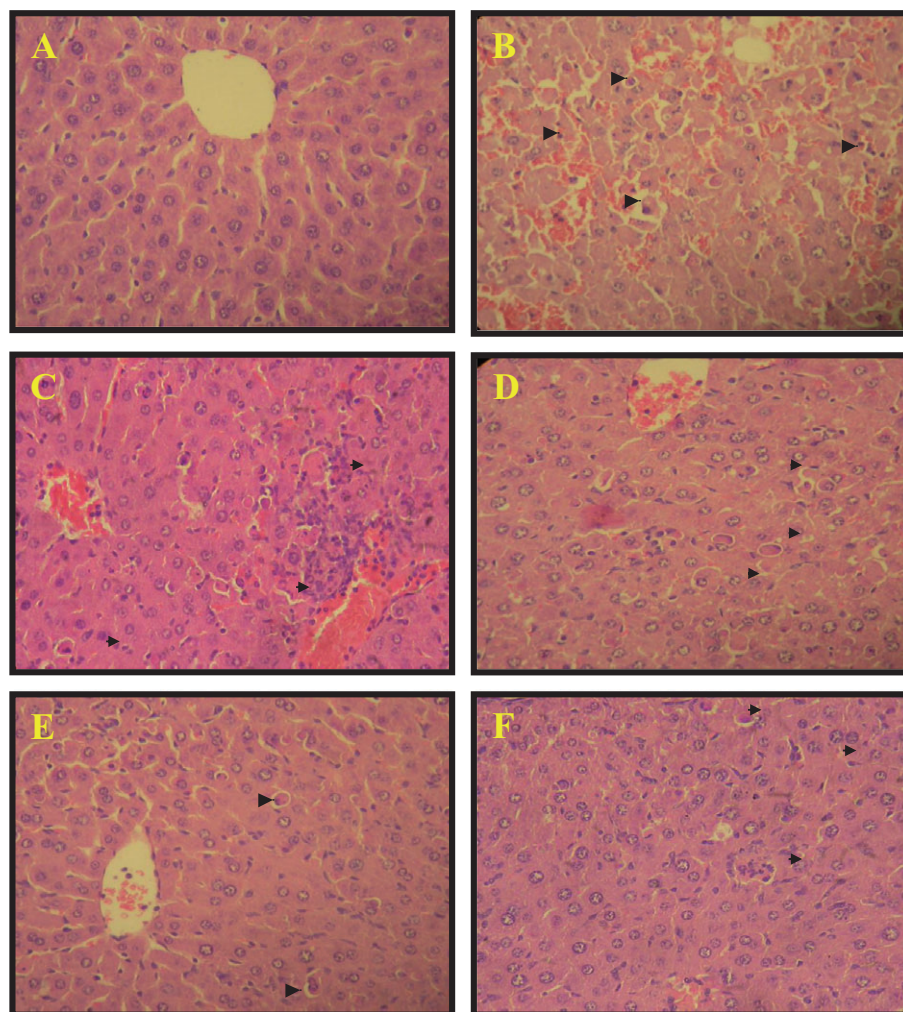
Group	Treatment	ALT (IU/L)	AST (IU/L)	LH score
1	Normal saline (0.01 mL/kg)	253 + 52	377 + 80	0 (0–1)
2	Normal saline (0.01 mL/kg)	2808 + 204*	2862 + 387*	9 (8–9)
3	<i>Saussurea lappa</i> (150 mg/kg)	1677 + 129# (44.25%)	1830 + 295# (41.56%)	4 (3–6)
4	<i>Saussurea lappa</i> (300 mg/kg)	1429 + 175# (53.99%)	1662 + 199# (48.28%)	3 (3–4)
5	<i>Saussurea lappa</i> (600 mg/kg)	1225 + 180# (61.96%)	1477 + 342# (55.75%)	1 (1–2)
6	D-galactosamine (700 mg/kg) + Lipopolysaccharide (1 µg/kg)	1911 + 107# (35.11%)	1992 + 311# (35%)	4 (4–5)

\*: *p* < 0.05; group 1 vs 2.

#: *p* < 0.05; group 2 vs 3, 4, 5 and 6.

ANOVA used for comparing pretreatment groups (3, 4, and 5) that showed *p* < 0.05 for ALT and *p* > 0.05 for AST.

Values represent mean + standard deviation of seven determinations with % reduction given in parenthesis beneath each value. Liver histopathology score (LH score) is presented as mode and range (given in parenthesis) beneath each value. Extract, toxins and normal saline were given intraperitoneally.



**Figure 1.** Representative microphotographs (40x magnification, hematoxylin and eosin staining). Control group (A) showed normal parenchyma architecture and sinusoidal spaces with no apoptosis and congestion. Toxin group (B) showed disturbed architecture with marked cellular swelling, loss of cell adhesion and many apoptotic cells (arrow heads). Treatment groups (C, D, E and F) showed improved architecture, less marked cellular swelling and apoptosis with increasing dose of treatment.

group and showed dose-dependent improvement (Table 1). The LH scores of treatment groups were much lower than that of the toxin group (Table 1).

The observed protective effects of SI.Cr might be due to the reported facts of its identified phytochemicals. The compounds from *Saussurea lappa* root like costunolide and dehydrocostus lactone (Lee *et al.*, 1999), reynosin and santamarine (Cho *et al.*, 1998) and saussureamine (Matsuda *et al.*, 2003) have shown inhibitory

effect on expression of TNF- $\alpha$  and NO, respectively, from activated macrophages. Calcium channel blockers have shown hepatoprotection (Farghali *et al.*, 2000) and we have recently identified calcium channel blocking (CCB) constituents in *Saussurea lappa* (Gilani *et al.*, 2006).

Furthermore, SI.Cr is relatively safe as it did not produce any behavior change and mortality when given at a dose as high as 5 g/kg.

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