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Antiulcerogenic activity of *Saussurea lappa* root

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Abstract

The study was designed to investigate the antiulcer activity of ethyl acetate extract of the *Saussurea lappa* root using different models of gastric and duodenal ulceration in rats. Gastric ulcers were induced by oral administration of ethanol, aspirin and by pyloric ligation and duodenal ulcers were induced by oral administration of cysteamine HCl. The extract was administered at a dose of 200 and 400 mg/kg orally 30 min prior to ulcer induction. Ranitidine (50 mg/kg) was used as a reference standard. The antiulcer activity was accessed by determining and comparing the ulcer index in the test group with that of the standard drug treated group. Gastric volume, total acid and free acid were estimated in the pylorus-ligated rats. *Saussurea lappa* root (400mg/kg) showed maximum inhibition of gastric acid, free acid and total acid to 53.54%, 52.55% and 30.30%, respectively. The ulcer index in the *Saussurea lappa* root treated animals was found to be significantly less in all the models compared to standard drug treated cases. The antiulcer activity of *Saussurea lappa* root was, however, less than that of ranitidine. The results suggest that *Saussurea lappa* root possesses significant antiulcer property which could be due to cytoprotective action of the drug or strengthening of gastric and duodenal mucosa with the enhancement of mucosal defense.

Key-Words: *Saussurea lappa* root, antiulcer**Introduction**

Man has used plants as medicines for thousands of years¹. Traditionally peptic ulcers have been described as an imbalance between the luminal acid peptic attacks versus the mucosal defense². The treatment of peptic ulcers with plant products used in folk medicine and the protection of induced gastric ulcer in laboratory animals using medicinal plants was reported³. Generally plant flavonoids have been found to be effective against ulcer in experimental animals⁴ and exhibit several biological effects⁵. Herbal medicine deals with plants and plant extracts in treating diseases. These medicines are considered safer because of the natural ingredients with no side effects⁶.

Costus is the root of *Saussurea lappa*, (Decne.) C.B. Clarke., Asteraceae, an aromatic perennial plant growing in the open slopes of India and Kashmir. The Commonly it is called as *costus*, *kuth*, *kushta*, *kust*, *muxiang*, *patchak*, *quang mu xiang*. The modern researches suggest that it is a blood purifier, antiseptic and increases the cetaceous circulation. It is a good insect repellent.

Hence, might be used to keep off the insects from the clothes. Internally it is a good expectorant, anti-spasmodic and neurotoxin, hence, might be used for cough, bronchitis, bronchial asthma, paralysis, facial palsy and neurasthenia⁷⁻¹⁰. Recently, it has been reported that betulinic acid, betulinic acid methyl ester and guaiane sesquiterpenoids: mokko lactone and dehydrocostuslactone from the roots of *Saussurea lappa* C.B. Clarke have significant PTP1B inhibitory activity¹¹⁻¹².

Saussurea lappa roots have been widely recommended in inflammation-related diseases characterized by rheumatoid arthritis, chronic gastritis, asthma and bronchitis in traditional medicine. The scientific evidences of their significance are inadequate. Akhtar and Farah reported chemical contents exerting anthelmintic effects in animals. Recently, a small number of *In Vitro* studies have been published describing effects of the methanolic root extracts of *Saussurea lappa* on cell mediated immunity in rats. However, the toxicological effects of these preparations on individual's general health remain yet to be ascertained¹³⁻¹⁴. The *Saussurea lappa* root has been claimed to have antiulcer activity¹⁵, but no detailed scientific investigations have been carried out to define the antiulcer activities of *Saussurea lappa*. Thus the present investigation sets out to study the

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antiulcer activity of *Saussurea lappa* root extract. The effect produced by *Saussurea lappa* was compared with that of ranitidine, a standard drug.

Material and Methods

Collection of plant materials

The Fresh Plant of *Saussurea lappa* were collected in the month of August from the local Nursery bed in Etawah, Uttar Pradesh state, India, and authenticated by Dr. Harish Kr. Sharma, Ayurvedic Medical College, Davangere, Karnataka, India.. A voucher specimen was submitted at Institute's herbarium department for future reference. The fresh root were collected and fixed immediately using FAA (formalin: Acetic acid: ethyl alcohol) as fixative agent for anatomical studies.

Preparation of extract

The samples were washed with running tap water and separated before being chopped into pieces. They were oven-dried at 45°C for 2 days and ground to powder. The ground powder was extracted with methanol in a water bath at room temperature for 24 h. The solvent was then removed by filtration and fresh solvent was then added to the plant material. The extraction process was twice repeated. The combined filtrates were then evaporated under reduced pressure to give a dark green viscous mass. This methanol crude extract was further extracted with ethyl acetate and water, and then separated using separating funnels. These ethyl acetate-soluble fractions were later evaporated and afforded the ethyl acetate extract¹⁶. The extract was stored at 0-4 °C. This extract was used for animal administration.

Acute toxicity studies

Albino mice weighing 22-25 g selected by random sampling technique were used in the study. Acute oral toxicity was performed as per OECD- 423 guidelines¹⁷. The animals were fasted overnight, provided only water after which extract was administered to the groups orally at the dose level of 5 mg/kg body weight by gastric intubation and the groups were observed for 48 hrs. If mortality was observed in 2 or 3 animals among 6 animals then the dose administered was assigned as a 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 2,000 mg/ kg body weight. The animals were observed for toxic symptoms such as behavioral changes, motor reflexes and mortality for 48 hours.

Experimental animals

Inbred colony strains of Wistar rats of either sex weighing 150-250 g procured from the animal house of Sir Madanlal Institute of Pharmacy were used for the study.

The animals were maintained in polypropylene cages of standard dimensions at a temperature of $28 \pm 1^\circ\text{C}$ and standard 12 hour: 12 hour day/night rhythm. The animals were fed with standard rodent pellet diet (Hindustan Lever Ltd.) and water *ad libitum*. Prior to the experiment, the animals were acclimatized to the laboratory conditions. The experimental protocol was approved by Institutional Animal Ethical Committee (IAEC) constituted under CPCSEA. IAEC ref no: IAEC/XII/06/CLBMCP/2007-2008 dated 20-04-2007.

Preliminary phytochemical analysis

The ethyl acetate extract of the root of *Saussurea lappa* was subjected to preliminary phytochemical screening¹⁸.

Aspirin-induced gastric lesions

Aspirin (0.2 g/kg x 3 days) were administered once per day to groups of animals for the number of day's specified¹⁹. Animals of control group received 1% carboxy methyl cellulose (CMC) suspension and test groups received *Saussurea lappa* suspension orally at two dose levels (200 and 400 mg/kg) for 10 days. From day 8 the animals received CMC/ *Saussurea lappa* two hours prior to the administration of aspirin. Overnight fasted animals 348 *Antiulcer Activity* were sacrificed by cervical dislocation one hour after the last dose of ulcerogen. The stomach was incised along the greater curvature and examined for ulcers.

Alcohol-induced gastric lesions

Groups of rats fasted for 24 h received either *Saussurea lappa* (200 and 400mg/kg) or control vehicle. After 30 min, ulceration was induced by oral administration of 50 %ethanol (5 ml/kg). The animals were sacrificed after 1 h following administration of ethanol²⁰. The stomach was removed, opened along the greater curvature and sum of length of lesions (mm) was calculated and expressed as lesion index.

Pylorus ligated rats

Saussurea lappa (200 and 400 mg/kg) was administered for a period of 7 days. On day 7, after the last dose of *Saussurea lappa*, the rats were kept for 24 hours fasting and care was taken to avoid coprophagy. Under light ether anesthesia, the abdomen was opened and pylorus was ligated without causing any damage to its blood vessels. The stomach was replaced carefully and the abdominal wall was closed with interrupted sutures. The animals were deprived of water during the postoperative period²¹ four hours after ligation, stomachs were dissected out and contents were collected into clean tubes. Volume, pH, free acid and total acid content of gastric juice were determined. The contents were centrifuged, filtered and subjected to titration for estimation of free and total acidity. 1ml of centrifuged and filtered gastric secretion was titrated against 0.1N Sodium hydroxide using Topfers reagent

as indicator for determination of free acidity and 1% phenolphthalein as indicator for combined acidity. The sum of the two titrations was total acidity²². The stomach was opened along the greater curvature and examined for ulcers. The ulcer index was evaluated using the method described earlier²³.

Cysteamine HCl induced duodenal ulcers

Rats were treated with *Saussurea lappa* (200 & 400 mg/kg) orally for a period of 7 days. On day 8, the overnight fasted animals were given a single subcutaneous injection of cysteamine hydrochloride (30 mg/kg) and the animals were killed by cervical dislocation after 18 hours²⁴ duodenum was examined for the presence or absence of ulcers.

Statistical analysis

Statistical analysis was carried out by using ANOVA followed by Dunnet's multiple comparison tests using Graph pad PRISM software version 4.03 (2005). 'P' values <0.05 were considered significant.

Results and Conclusion

The preliminary phytochemical screening carried out on ethyl acetate extract of *Saussurea lappa* revealed the presence of phytoconstituents such as alkaloids, flavanoids, carbohydrates, glycosides, phenols, gums and mucilage. (Table 1)

Table 1: Preliminary phytochemical test of *Saussurea lappa* root ethyl acetate extract

Phytochemical Tests	Results
Test for Alkaloids	+
Test for Carbohydrates	+
Test for Proteins	+
Test for Steroids	-
Test for Sterols	-
Test for Phenols	+
Test for Flavanoids	+
Test for Gums and mucilage	+
Test for Glycosides	+
Test for Saponins	-
Test for Terpenes.	-

+ Indicates the presence of compounds.

- Indicates the absence of compounds.

The extract did not produce any toxic symptoms of mortality up to the dose level of 2000 mg/kg body weight in rats, and hence the drugs were considered safe for further pharmacological screening, the 1/10 and 1/5 of the LD₅₀ were taken as dose for the evaluation of antiulcer activity. According to the OECD-423 guidelines for acute oral toxicity, the LD₅₀ dose of 2000 mg/kg and above is categorized as unclassified. The values are shown in Table 2.

Table 2: Acute toxicity studies of extract for 48 h

Extract toxicity (48 hr)		
	Motor reflex	Behaviour pattern
Dose (mg)	Ethyl acetate	Ethyl acetate
Test-200	Normal	Normal
Test-400	Normal	Normal
Control	----	----

During the course of the study, the incidence and severity of aspirin and alcohol induced ulcerations were significantly reduced by *Saussurea lappa*. Induction of duodenal ulcers in rats with cysteamine hydrochloride showed the presence of ulcers in all the animals in the control group, which was significantly reduced in the *Saussurea lappa* treated group. The values are shown in Table 3 & 4

Table 3: Anti ulcer activity of the *Saussurea lappa* root ethyl acetate extract

Ulcerogen and Dose	Ulcer Index			
	Control	<i>Saussurea lappa</i> (200mg/kg)	<i>Saussurea lappa</i> (400mg/kg)	Ranitidine (50mg/kg)
Aspirin (200 mg/kg)	25.00 ± 0.37	18.38 ± 0.22*	13.44 ± 0.37*	8.48 ± 0.11*
Alcohol (80%)	36.76 ± 0.33	23.24 ± 0.36*	22.06 ± 0.31*	16.25 ± 0.17*
Cysteamine HCl (30mg/kg)	32.30 ± 0.46	19.66 ± 0.29*	15.66 ± 0.28*	13.54 ± 0.32*

Values are mean ± SE of 8 animals in each group (n = 8); *P<0.05 compared with respective control group.

Effect of *Saussurea lappa* on gastric volume, free acid, total acid and ulcer in pylorus ligated rats were studied. *Saussurea lappa* (200 and 400 mg/kg) inhibited the volume of gastric juice secreted by the control rats by 21.559% and 53.54%, respectively. The free acid and the total acid were reduced by the extract to 39.03, 17.77% and 52.55, 30.30%, respectively for the 200 and 400mg/kg. *Saussurea lappa* administered in doses 200 and 400 mg/kg orally cause a dose dependent decrease in ulcer index in pylorus ligated rats. The dose of 400 mg/kg showed maximum ulcer protection of 55.6%. The values are shown in Table 4.

Table 4: Effect of *Saussurea lappa* root ethyl acetate extract on gastric volume, free acid, total acid and ulcer index in pylorus ligated rats

Parameters	Groups				
	Control	<i>Saussurea lappa</i> (200mg/kg)	Percentage inhibition	<i>Saussurea lappa</i> (400mg/kg)	% inhibition
Gastric volume (ml/100g)	7.64 ± 0.33	5.99±0.37*	21.59	3.55 ± 0.13	53.54
Free acid (μEq/100g/4h)	392.0 ± 4.5	239.11 ± 2.06*	39.03	186±1.88*	52.55
Total acid (μEq/100g/4h)	489.29 ± 3.35	402.33 ± 2.46*	17.77	341.11± 2.60*	30.30
Ulcer Index	27.28 ± 0.69	22.88± 0.42*	16.13	17.99 ± 0.80*	34.05

Values are mean ± SEM of 8 animals in each group;
*P<0.05 compared with respective control group.

In most of the cases the etiology of the ulcer is unknown. It is generally accepted that it results from an imbalance between aggressive factors and the maintenance of the mucosal integrity through the endogenous defense mechanism²⁵. To regain the balance, different therapeutic agents including plant extracts are used to inhibit the gastric acid secretion or to encourage the mucosal defense mechanisms by increasing mucus production, stabilizing the surface epithelial cells, or interfering with the prostaglandin synthesis.

Even though many products in the market for the treatment of gastric ulcers, including antacids, proton pump inhibitors, anticholinergics and histamine H₂-antagonists, are used, most of these drugs produce several adverse reactions, such as gynecomastia, hematopoietic changes, acute interstitial nephritis²⁶, thrombocytopenia²⁷, anaphylaxis reactions²⁸, nephrotoxicity and hepatotoxicity²⁹. Medicinal plants are amongst the most attractive sources of new drugs, and have been shown to give promising results in treatment of gastric and duodenal ulcers.

The anti ulcerogenic activity of *Saussurea lappa* was evaluated by employing aspirin and alcohol induced

ulcerations in rats. Non-steroidal anti-inflammatory drugs (NSAIDs) like aspirin are known to induce gastric ulceration. The reason being attributed principally to inhibition inhibited the production of pro-inflammatory cytokines such as interleukine – 8 (IL-8) and tumor necrosis factor (TNF-α) in murine like macrophages, resulting in overproduction of nitric oxide in lipopolysaccharide (LPS) sesquiterpene, a dehydrocostus lactone³⁰. Hence, the protective action of *Saussurea lappa* against aspirin- induced gastric lesions could possibly be due to production of pro-inflammatory cytokines effect.

Ethanol-induced gastric lesion formation may be due to stasis in gastric blood flow, which contributes to the development of the haemorrhage and necrotic aspects of tissue injury³¹. It has also been reported that effect of *Saussurea lappa* roots extract in ethanol on leukocyte phagocytic activity lymphocyte proliferation and interferon-Gamma. It suppressed the secretion of IFN-γ by mitogen-activated (PHA; 2.5μg/ml) of peripheral mononuclear cells in dose-dependent manner³².

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