

Original Article

GASTROPROTECTIVE POTENTIAL OF *LASIA SPINOSA* IN ALBINO RATS

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ABSTRACT

Objective: The intent of the present study was to assess the gastroprotective impact of *Lasia spinosa* leaf ethanolic concentrate (LSEE) by method of Indomethacin and Cold Restrain stress induced Ulcers in Albino rats. *Lasia spinosa* is a plant found in Assam on rough slope top of dry deciduous timberlands.

Methods: Indomethacin 5mg/kg body weight p. o; for five days and Cold restraint stress models, were utilized for actuating gastric ulcers in rats. Biochemical parameters such as Glutathione, Malondialdehyde, acidity, Gastric volume, gastric pH were determined in order to assess the gastro protective activity of LSEE in both the models.

Results: Treatment of rats with Indomethacin and subjecting them to Cold restraint stress (CRS) elevated the levels of Gastric volume, acidity, Glutathione, Malondialdehyde and gastric pH in negative control group in comparison with normal group. The elevated levels were significantly reversed when treated with standard drug Ranitidine 50 mg/kg body weight p. o; and *Lasia spinosa* leaf ethanolic extracts (LSEE).

Conclusion: All in all it can be expressed that *Lasia spinosa* leaf ethanolic concentrates demonstrated a critical inversion of ulcerative parameters. It could be imagined that it applies its action because of the vicinity of flavonoids which have been accounted for to secure the mucosa by development of a defensive layer.

Keywords: Gastro protective, *Lasia spinosa*, Ranitidine, Stomach.

INTRODUCTION

Home grown drugs have as of late pulled in much consideration as option pharmaceutical helpful for treatment and aversion of way of life related disorder [1]. Nonetheless, moderately almost no information is accessible about their mode of activity and safety. The earliest recorded utilization of natural cures originates from Hippocrates, who bolstered utilization of straightforward plants, for example, garlic [2].

Analysts reported that peptic ulcers were created by an irregularity between the forceful components (increment in gastric emission) and various known barrier mechanisms (bodily fluid generation) [3]. Peptic ulcer illness (PUD) happens when the stomach lining or the proximal duodenum is eroded which is created by *Helicobacter pylori* (*H. pylori*) disease, long haul and high dosages utilization of medications, for example, nonsteroidal anti inflammatory drugs (Nsoids), ailments like Zollinger- Ellison syndrome and huge numbers of the psychosocial elements including enthusiastic anxiety, overabundance liquor utilization and smoking is thought to be an upgrading element for ulcers [4]. Gastrointestinal surgery for over a century has been considered as front line treatment for peptic ulcer disease. Peptic ulcers are deep gastrointestinal disorders that involve erosion of the entire mucosal thickness, penetrating the muscular mucosa. For decade it was believed that gastrointestinal ulcerations were believed to be caused by an increased secretion of gastric acid, but the secretion rates were found to be normal in the majority of the patients suffering with the said type of ulcers.

Pharmacological treatment for ulcers such as proton pump inhibitors (PPI), H₂-receptor antagonist, antacids and antibiotics for *H. pylori* are available commercially to ease the patient's discomfort⁴. Owing to the fact that these medications have many untoward effects their use by the patient is declining. As a result many people are turning to traditional system of medicine which comparatively has fewer side effects in accordance with its counterpart. *Lasia spinosa* has been reported to contain Polyphenols, Ascorbic acid, dietary fibers and Hydrocyanic acid [5]. Traditionally, the leaves of this herb are commonly employed in the treatment of

gastrointestinal diseases, respiratory diseases and in skin infections [5, 6]. *Lasia spinosa* is rich in flavonoids and saponins which are reported to have antioxidant property and lowering the levels of reactive oxygen species released due to the oxidation process. The present study incorporates *Lasia spinosa* leaves ethanolic extract, due to the antioxidant activity [7] to evaluate its gastro protective effect by using Indomethacin and Cold restraint stress model in albino rats.

MATERIALS AND METHODS

Collection and authentication of the plant material

Lasia spinosa is commonly known as Phak Naam, Lasia (English). The plant was checked for data in www. plantlist. org with the following statement (This name is accepted name of a species in the genus *Ficus* (family Araceae). The record derives from WCSP (in review) which reports it as an accepted name with original publication details: *Ann. Mus. Bot. Lugduno-Batavi* 3:285 1867. The plant parts like fruit is used in heart diseases while liver and bark are used in liver and skin ailments. Leaves of *Lasia spinosa* plant were obtained from the forest patches of Karbi, Anglong district in Assam, India. The authentication of plant material was done by Dr. K. Madhava Chetti, Assistant Professor, Department of Botany, Sri Venkatesh wara University during the month of March 2013.

Experimental animals

Healthy Wistar Albino Rats weighing about (120-160 gm) of either sex were obtained from animal house. The animals were maintain under standard condition i. e., housed in polypropylene cages and maintained at a temperature 27 ± 2°C, relative humidity 65 ± 10% under 12 hour light and dark cycle. The animals were acclimatized for 10 days under laboratory condition before carrying out the experiments. The animal's house approved by the Committee for the Purpose of Control and Supervision on Experimental Animals (CPCSEA)-Registration number – 1330/AC/10/CPCSEA [8].

Chemicals

All the chemicals were Analytical grade.

Ulcer inducing agent: Indomethacin (PubChem CID: 3715), 5mg/kg body weight, p. o.

Standard Drug: Ranitidine at a dose (PubChem CID: 3001055) of 50 mg/kg body weight administered by oral route.

Method of preparation of extract

The collected leaves were washed thoroughly under running water, cut into smaller pieces and air dried for eight days. Then the dried leaves were coarsely powdered using grinder and were continuously extracted in a Soxhlet apparatus at 30°C with 2500 ml ethanol. The extract was filtered through a fine muslin cloth and evaporated under reduced pressure by the rotary evaporator. The obtained extracts were stored in amber colored glass bottle for further processing [9].

Preliminary Phytochemical screening

The solution of the methanolic extract was prepared using distilled water and subjected to preliminary phytochemical screening. Test for common phytochemicals were carried out by standard methods described in practical pharmacognosy by Kokate, Khandelwal and Trease and Evans [10-12].

Determination of acute toxicity (OECD guideline 423)

The acute toxicity for ethanolic extract of leaves of *Lasia spinosa* (FDEE) was determined in albino rats following OECD guideline 423, maintained under standard conditions [8].

Evaluation of gastro protective activity

As following:

Table 1: Indomethacin-induced gastric ulcers [13]

Group no.	Treatment and label	Dose (b.w. p.o.)
Group 1	Normal control.	Distill water 5 ml/kg orally.
Group 2	Negative control (untreated group)	Indomethacin (5 mg/kg, p. o) for 5 days
Group 3	Positive control	Indomethacin (5 mg/kg, p. o) for 5 days + Ranitidine 50 mg/kg body weight, p. o.
Group 4	Test dose 1	Indomethacin (5 mg/kg, p. o) for 5 days + LSEE 100 mg/kg b. w; p. o.
Group 5	Test dose 2	Indomethacin (5 mg/kg, p. o) for 5 days + LSEE 200 mg/kg b. w; p. o.
Group 6	Test dose 3	Indomethacin (5 mg/kg, p. o) for 5 days + LSEE 400 mg/kg b. w; p. o.

After the completion of the test period the stomach was removed by humanely sacrificing of the rats, ulcer index was then measured. Acidity, volume of gastric juice, pH of gastric acid, endogenous antioxidant like glutathione (GSH) and Malondialdehyde was measured.

Table 2: Cold restraint stress - induced ulcers [14]

Group no.	Treatment and label	Dose (b.w. p.o.)
Group 1	Normal control.	Distill water 5 ml/kg orally.
Group 2	Negative control (untreated group)	Cold restraint at 4°C for 1 hour daily for 7 days.
Group 3	Positive control	Cold restraint at 4°C for 1 hour daily for 7 days + Ranitidine 50 mg/kg b. w; p. o.
Group 4	Test dose 1	Cold restraint at 4°C for 1 hour daily for 7 days + LSEE 100 mg/kg b. w; p. o.
Group 5	Test dose 2	Cold restraint at 4°C for 1 hour daily for 7 days + LSEE 200 mg/kg b. w; p. o.
Group 6	Test dose 3	Cold restraint at 4°C for 1 hour daily for 7 days + LSEE 400 mg/kg b. w; p. o.

Animals were humanely sacrificed on 7th day using ether and the stomachs were excised. Stomachs that were excised from control and treated groups were placed in chilled ice cold saline solution after the evaluation of ulcer index. A 10% stomach homogenate in 1.15% KCl was prepared for estimation of GSH, Malondialdehyde, Acidity, Volume and pH of Gastric acid.

Statistical analysis

Results were expressed as Mean ± SEM. Statistical analysis were performed with Graph pad prism software using one way Analysis of Variance followed by Dunnett's *t*-test.

P values were considered significant when *P<0.05, **P<0.01, ***P<0.001 when the test and standard were compared with the untreated groups [8].

RESULTS

Preliminary phytochemical analysis

The phytochemical screening of ethanolic extract of *Lasia spinosa* leaves showed the presence of Carbohydrates, glycosides, alkaloids, tannins, flavonoids and proteins.

Acute toxicity studies

The acute toxicity studies of *Lasia spinosa* ethanolic leaves extract was carried out as per OECD guideline no. 423. There was no gross evidence of any abnormality observed up to a period of 4-6 hrs or mortality up to a period of 24 hrs at the maximum tolerated dose level of 2000 mg/kg body weight p. o. Further pharmacological screening were carried out with three dose ranges i. e. 100 mg/kg b. w. p. o., 200 mg/kg b. w. p. o. and 400 mg/kg b. w. p. o.

Effect of *Lasia spinosa* leaves ethanolic extract on indomethacin and Cold Restraint Stress (CRS) induced gastric ulcers

Administration of Indomethacin (5 mg/kg) and subjecting of rats to CRS produced superficial and deep erosions which lead to the

formation of ulcers. However, treatment with LSEE reduced the severity of gastric ulcer. Marked elevated levels of Acidity, volume, pH, GSH and Malondialdehyde was observed when treated with Indomethacin and also when subjected to CRS in comparison with the normal group. Ranitidine (50 mg/kg) showed a marked reversal of the elevated parameters. LSEE 400 mg/kg showed the maximum level of reversal (p< 0.001) of the elevated parameters in comparison to normal group.

A dose dependent inhibition of ulcer area and ulcer index was seen with LSEE extracts. LSEE 400 mg/kg produced a significant (p<0.001) low ulcer area and ulcer index when compared with negative control group. Ranitidine treated group exhibited a maximum inhibition of ulcer area and ulcer index.

DISCUSSION

Lasia spinosa leaves ethanolic concentrate did not demonstrate any untoward impact when managed orally up to dosage of 2000 mg/kg body weight every oral (p. o). As there was no mortality, 1/5th, 1/10th and 1/20th of the greatest endured measurement was taken i. e. 400 mg/kg, 200 mg/kg and 100 mg/kg.

The preparatory phytochemical screening of ethanolic concentrate of *Lasia spinosa* leaves uncovered the vicinity of Carbohydrates, glycosides, alkaloids, tannins, flavonoids and proteins. Tests were negative for Anthraquinones, and Reducing Sugars. As flavonoids and saponins have cell reinforcement property they may have assumed a huge part in Gastro defensive mechanism. The intent of the present study is to assess the ulcer hindrance/protection of *Lasia spinosa* leaves ethanolic concentrate in albino rats wherein the ulcers were affected by toxicant Indomethacin and Cold anxiety.

Table 3: Effect of LSEE on Biochemical parameter in Indomethacin-induced Gastric Ulcers

Groups	Free acidity	Total acidity	Volume of gastric juice	P ^H of gastric acid	GSH (min/mg protein)	MDA (nmol/mg tissue)
Group-I Control(Distilled water)	83.00±0.37	113±0.62	6.25±0.025	4.0±0.034	4.99 ± 0.06	53.35±0.47
Group-II –ve control (Indomethacin 5mg/kg)	111.00±0.66 ^a	145.25±0.56 ^a	10.32± 0.045 ^a	6.23±0.046 ^a	8.67± 0.065 ^a	103.29± 0.49 ^a
Group-III Standard (Ranitidine 50mg/kg)	87.23±0.30***	109.25±0.69***	6.28±0.054***	4.14±0.036***	5.23 ± 0.25***	60.28±0.23***
Group-IV test 100mg/kg LSEE	105.35 ± 0.55*	140.50± 0.35*	9.57± 0.26**	6.0± 0.45	8.21± 0.52*	97.97± 0.26
Group-V test 200mg/kg LSEE	97.78± 0.85**	131.25±0.56**	7.10 ± 0.35**	5.17±0.39*	6.86 ±0.54**	77.17 ±0.54*
Group-VI test 400mg/kg LSEE	90.59 ± 0.69**	126.58±0.36**	6.55±0.58**	4.59±0.48**	5.97±0.24**	65.34±0.634**

Values are mean ± SEM; N = 6 in each group, P values: a < 0.001 Negative Control group VS Normal Control group, * < 0.05, ** < 0.01, *** < 0.001 Test Groups VS Negative Control Group

Table 4: Effect of LSEE on Biochemical Parameter in Cold Restraint Stress-Induced Ulcers

Groups	Free acidity	Total acidity	Volume of gastric juice	P ^H of gastric acid	GSH (min/mg protein)	MDA(nmol/mg tissue)
Group-I Control(Distilled water)	102.00 ±0.065	113 ± 0.24	5.11 ± 0.028	4.17± 0.037	5.32± 0.025	61.33 ±0.49
Group-II –ve control cold restraint stress	135 ±0.025 ^a	149 ±0.26 ^a	8.26 ±0.41 ^a	8.35 ±0.78 ^a	8.42± 0.65 ^a	95.35 ±0.69 ^a
Group-III Standard (Ranitidine 50mg/kg)	93.37±0.32***	109±0.36***	5.27± 0.25***	4.08±0.34***	5.36 ± 0.35***	61.89±0.23***
Group-IV test 100mg/kg LSEE	128.55± 0.025	140±0.32*	7.1±0.36	7.97± 0.71	8.17± 0.5*	89.11±0.62
Group-V test 200mg/kg LSEE	119.17±0.45*	128.3± 0.36**	6.66±0.3*	6.66± 0.65**	6.95± 0.56**	75.33± 0.35*
Group-VI test 400mg/kg LSEE	109.00± 0.58**	118.00 ± 0.59*	5.89±0.4*	4.83 ± 0.5**	5.94± 0.25**	68.68± 0.33**

Values are mean ± SEM; N = 6 in each group, P values: a < 0.001 when Negative Control group VS Normal Control group, * < 0.05, ** < 0.01, *** < 0.001 Test Groups VS Negative Control Group

Non-steroidal anti-inflammatory drug (NSAID) like Indomethacin is normally utilized as a commonly prescribed prescription for fever, agony and swellings. Indomethacin works by restraining prostaglandins creation by nonselective hindrance of cyclooxygenase (COX) 1 and 2. Prostaglandins are present all through the body and perform different capacities, for example, bringing about pain, fever and inflammation [15].

Since Indomethacin inhibits both COX-1 and COX-2, it thereby inhibits the production of prostaglandins in various organs such as stomach and intestine, in stomach prostaglandins play a vital role in stimulating the secretion of bicarbonate and mucus, which maintains the mucosal blood flow, mucosal turnover and repair and mucosal lining of the gastrointestinal tract. Thereby, excess administration of Indomethacin results in ulcers. These ulcers can result in serious bleeding and perforation [16-18].

In the present study, administration of Indomethacin to rats resulted in severe ulcers. However, administration of LSEE 200 mg/kg and LSEE 400 mg/kg produced a significant gastric protection which is evident in parameters like mean score and ulcer index. Reduction in damage to the mucosal lining which was induced by free radicals may seem to be related to the gastro protective activity of LSEE extracts and this may be attributed to the plants antioxidant property. There was an increase in the levels of MDA and reduction in the levels of GSH, which were reversed when treated with Ranitidine 50 mg/kg and LSEE 400 mg/kg.

Stress induced ulcers might probably be triggered by the release of histamine. Histamine results in an increased gastric secretion and also causes disturbances in gastric mucosal microcirculation resulting in abnormal motility and decreases the mucus production in the stomach. Acetylcholine released by the increased stimulation of vagus nerve, interacts with the muscarinic receptors resulting in excess acid secretion in stomach. As these receptors are located on the cell surface of parietal cells and histamine secretory cells, the increased acid secretion is a consequence of acetylcholine action on parietal and histamine cell activity [19].

Subjecting of rats to cold exposure and immobilization individually and collectively is responsible for generation of reactive oxygen species (ROS). The generation of ROS results in lipid peroxidation in membranes and results in tissue injury. Increased levels of end products produced in lipid peroxidation were observed in rats subjected to cold restraint stress. Increased MDA and reduced GSH levels indicate increased peroxidation finally leading to tissue damage. Treatment with LSEE 400 mg/kg and Ranitidine 50 mg/kg significantly reversed the elevated levels. Hence, it may be interpreted that the likely mechanism of action of *Lasia spinosa* leaves ethanolic extracts is due to its antioxidant potential.

CONCLUSION

In conclusion, the present study indicates that the *Lasia spinosa* leaves ethanolic extracts possess a significant ulcer protective effect. This effect may be attributed to the free radical scavenging activity of the phytochemical constituents found in the plant and its ability to inhibit the process of lipid peroxidation. Based on the results obtained a conclusion can be made that, *Lasia spinosa* leaves extracts may have a significant potential as an alternate to commercially available drugs for the treatment of ulcer or in reducing the severity of the ulcers.

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CONFLICT OF INTERESTS

We declare that we have no conflict of interest.

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