

Anti Ulcer Activity of *Leea Indica* in Wistar Albino Rats

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Abstract

Objective: *Leea indica* is a well known plant with numerous pharmacological activities owing to the presence of the active constituents. In spite of numerous therapeutic uses, efficacy of the plant in treating ulcers is not yet evaluated scientifically. Hence an attempt has been made to evaluate the Antiulcer activity of *Leea indica*.

Methods: The effect of *Leea indica* methanolic extract (LIME) on gastric ulcer in pylorus ligation-induced and aspirin induced models was studied by employing LIME at 200 mg/kg, 400 mg/kg. In both the models Ranitidine (40mg/kg) was employed as the standard. Depending on the model, parameters evaluated were total acidity, free acidity, volume and pH of gastric fluid, ulcer score and percent inhibition of ulcer index.

Results: Data were analyzed following graph pad instat version 3.0 followed by Dunnett's multiple comparison test. LIME (400mg/kg) significantly ($P < 0.01$) reduced gastric ulcer index in pylorus ligation-induced and Aspirin induced ulcer models comparable to that of Standard drug. Histopathological studies confirmed that LIME (400mg/kg) possess anti ulcer activity in both models. The elicited activity might be owing to the existence of secondary metabolites such as flavonoids, tannins, and saponins.

Conclusion: Obtained results elucidate the Antiulcer activity of *Leea indica* methanolic extract. Further investigations on isolation of specific phyto chemicals and elucidating mechanisms of action are needed.

Keywords: Antiulcer, Pylorus Ligation Model, Aspirin Induced Model, Histopathology, *Leea Indica*; Peptic Ulceration; Vitaceae; Anti Hyper Lipidaemic; Antitumor; Hydrocarbons

Mini Review

Peptic ulceration is one of the common modern age epidemics affecting nearly 10% of world population [1]. Even though numerous potent anti-ulcer drugs are marketed, most of them were associated with toxicities, thus emphasizing the essentiality in search for new alternatives. Approximately 80% of the world's population trust on herbal-derived medicines reinforcing herbs was advantageous sources for new drugs. *Leea indica* a traditional Chinese medicine found to be distributed in India, Srilanka, Bangladesh, Burma, Nepal, Thailand, Laos, Cambodia, China, Vietnam, New Guinea, Malasia, Solomon islands, North Australia, Santa Cruz island, New Hebrides and Fiji [2-4]. *Leea indica*, an evergreen large shrub of family vitaceae [5,6] grows up to 8mt. Leaflets are ovate-lanceolate with crenate to serrate margins. Leaves are 1-3 pinnate bearing 7 leaflets, with petioles 7-20cm long. Fruits are purplish black,

bearing six seeds with 1 cm in diameter. Flowers are greenish white with 5mm across. Stems are glabrous to pubescent.

Leea indica leaves were scientifically evidenced for wide pharmacological activities like antitumor [7], antiviral [8], analgesic [9], sedative and anxiolytic [10], Nitric oxide inhibitory [11], phosphodiesterase inhibitory [12], antidiabetic [13]. Anti hyper lipidaemic [14]. Leaf decoction is employed in pregnancy and delivery for birth control and body pain [4,15]. Dried leaves ingested were effective against cancer [16]. Roasted leaves relieve vertigo. Leaves (31.4%) constitute to be the most widely used parts in medicine preparation then fruits and roots (16.05%) [17]. Therefore the current investigation was designed to screen the leaves for the antiulcer activity. Twenty three chemical constituents were detected in *Leea indica* leaves by GC-MS analysis, Spectroscopic

techniques, and Co-TLC. Compounds identified were eleven hydrocarbons, palmitic acid, phthalic acid, 1-eicosanol, farnesol, soanesol, gallic acid, three phthalic acid esters, lupeol, β -sitosterol and finally ursolic acid [18]. Though the plant was reported with numerous chemical constituents antiulcer activity was not yet screened. Hence the present study was taken up to investigate antiulcer activity of *Leea indica*.

Materials and Methods

Chemicals

Ranitidine (Lee Pharmaceuticals, Hyderabad.), Aspirin (Shalga pharmaceuticals, Goregaon, Mumbai.)

Plant

(Figure 1) *Leea indica* leaves were procured from Karthikavanam forest, Dhulapally, Hyderabad. These were authenticated by Dr. Madhavachetty, Professor, Sri Venkateshwara University, Tirupathi. The plant was placed in the college herbarium with a voucher specimen no. 438.

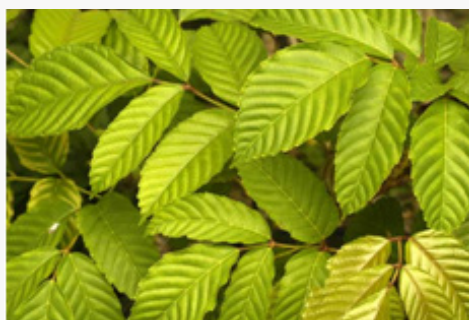


Figure 1: *Leea indica* leaves.

Animals

Male Wistar albino rats (200–250g) were purchased at Albino Research Center, Bachupally, Hyderabad. They were accommodated in polypropylene cages with standard diet and water *ad libitum*. Experiment was designed as per ethical norms of CPCSEA and IAEC Malla Reddy Institute of Pharmaceutical Sciences (1662/PO/a/12/CPCSEA).

Extraction

Fresh leaves were shade dried, coarsely powdered and sieved with sieve no.40. Powdered material (500gm) was packed in soxhlet apparatus and subjected to extraction with methanol and water for about 48h. Solvents were evaporated under reduced pressure by rotary vacuum evaporator. Obtained methanolic extract (MELI) and aqueous extracts of *Leea indica* (AQLI) were subjected to phytochemical screening and acute toxicity study [19].

Acute Toxicity Study

This study was performed as per OECD guideline 425 using limit test dose 2000mg/kg [20]. Rats were fasted overnight and a limit dose of 2000mg/kg was administered and observed for autonomic profiles (defecation and urination), behavioural profile (alertness,

restlessness, irritability and fearfulness), neurologic profile (spontaneous activity, reactivity, touch response, pain response and gait), physical states such as lacrimation, loss of appetite, tremors, hair erection, salivation, diarrhoea and for morbidity or mortality continuously for 2 h, periodically during the first 24 h and daily thereafter, for a total of 14d.

Antiulcer Activity

Pylorus Ligation Method: Male Wistar albino rats were segregated into four groups comprising five each [21]. Group I negative control, received distilled water, Group II positive control, received ranitidine 50mg/kg; Group III LIME (200mg/kg, p.o); Group IV LIME (400mg/kg, p.o). Plant extracts were administered for 10 d. At the end of experimental period rats were fasted overnight with water *ad libitum*. Standard, Ranitidine (40mg/kg) and LIME (200, 400mg/kg) were administered. Under light ether anaesthesia, the abdomen was cut opened by incision below the xiphoid process. The Pylorus end of stomach was slightly lifted up and ligated avoiding damage to blood vessels. After completing ligation stomach was replaced and the abdominal wall is sutured and closed. Rats were deprived of water during post operative period for 3 to 4h [22]. Four hours later, stomach was dissected out. Stomach contents were drained into tubes and were subjected to centrifugation at 2000 r/m for 10 min. Supernatant obtained was analysed for gastric volume, pH, free acidity and total acidity. The stomach was incised along the greater curvature, and observed for ulcers and the ulcer index (UI) was calculated. Ulcers were scored and severity was assessed microscopically (10 X) employing hand lens (10 X). The scores were given as mentioned.

0 = normal stomach.

0.5 = red coloration.

1.0 = spot ulcers.

1.5 = hemorrhagic streaks.

2.0 = ulcer > 3 but < 5.

3.0 = ulcer > 5

The ulcerated area and the total mucosal area were calculated. Ulcer index (UI) was determined as

Ulcer Index = $10 \times X$; where X = total mucosal area/total ulcerated area.

% Ulcer protection = $\{(UI \text{ of control} - UI \text{ of test}) \times 100\} / UI \text{ of control}$

Determination of Free Acidity And Total Acidity

One ml of gastric juice was pipetted into 100ml conical flask. To these 2 to 3 drops of topfer's reagent was added and titrated with 0.01N NaOH until red colour disappears and the colour turns to yellowish orange. The volume of the alkali added was noted. Obtained volume corresponds to free acidity. Now 2 to 3 drops of

phenolphthalein solution was added and titration was continued until red tinge reappear. Total volume of alkali added was noted. This volume corresponds to total acidity. Acidity = Volume of NaOH x Normality / 100_{meq / 1.0}

Aspirin induced model

Wistar albino rats were segregated into four groups comprising five each. Group I negative control, received Aspirin (200mg/kg, p.o); Group II Standard, Ranitidine (40mg/kg, p.o); Group III LIME (200mg/kg p.o); Group IV LIME (400mg/kg p.o). Standard, Ranitidine (40mg/kg) and LIME (200,400mg/kg) were administered for 10d. Rats were fasted overnight Aspirin (200mg/kg), Ranitidine (40mg/kg) and LIME (200, 400mg/kg) were administered. 4 h later rats were sacrificed. Stomach was then excised along the greater curvature and examined for ulcers and the ulcer index (UI) was calculated [23-25].

- 0 = normal stomach.
- 0.5 = red coloration.
- 1.0 = spot ulcers.
- 1.5 = hemorrhagic streaks.
- 2.0 = ulcer > 3 but < 5.

f) 3.0 = ulcer > 5

The ulcerated area and the total mucosal area were calculated. Ulcer index (UI) was determined as

Ulcer Index= 10xX; where X = total mucosal area/total ulcerated area. % Ulcer protection = {(UI of control- UI of test) x 100}/ UI of control.

Histopathological Evaluation

The stomachs were immersed in 10% formalin solution for histopathological examination. These tissues were processed, embedded in paraffin wax. The central part of damaged or ulcerated tissue (if present) was cut on half along the greater curvature. If the damage is not seen then sections of 5µm thickness were cut by a rotary microtome and stained with haematoxylin and eosin. Stained sections were examined under microscope for histopathological changes such as congestion, haemorrhage, necrosis, inflammation, infiltration, erosion and ulcer.

Statistical Analysis

Results were expressed as mean ± SEM. Statistical analysis was done by using Graph pad instate version 3 followed by Dunnet's t test. Values of P less than <0.05 were considered as statistically significant (Tables 1-4) and (Figures 1-3).

Table 1: Phytochemical screening of *Leea indica*.

Phyto Constituents	Methanolic Extract	Aqueous Extract
Carbohydrates	+	+
Proteins	-	-
Flavonoids	+	+
Steroids	+	-
Tannins	+	-
Phenolics	+	+
Saponins	+	-
Cardiac glycosides	-	-
Starch	-	-
Alkaloids	+	+
Glycosides	-	-

+ve - Present; -ve - Absent

Table 2: Effect of LIME on gastric secretion in pylorus ligation induced ulceration in rats.

S. No	Treatment	Vol of Gastric Juice (ml)	Free Acidity	Total Acidity	Ph
I	Control + Pylorus ligation	6.86± 0.13	84.10±3.12	115.21±2.42	1.42±0.51
II	Ranitidine (40mg/kg)	2.33 ±1.12*	35.25±2.44**	42.62±1.16**	6.7±0.18**
III	MELI (200mg/kg)	4.02 ±0.65 ^{ns}	77.56±3.2ns	106.72± 2.53ns	2.81±0.30*
IV	MELI (400mg/kg)	2.86 ±0.81*	35.8 ±1.98**	47.34±1.98**	6.60±0.25**

mean ±S.E.M. *P 0.05, **P < 0.01 compared with Control.

Table 3: Effect of LIME on ulcer index and % protection in pylorus ligation induced ulceration in rats.

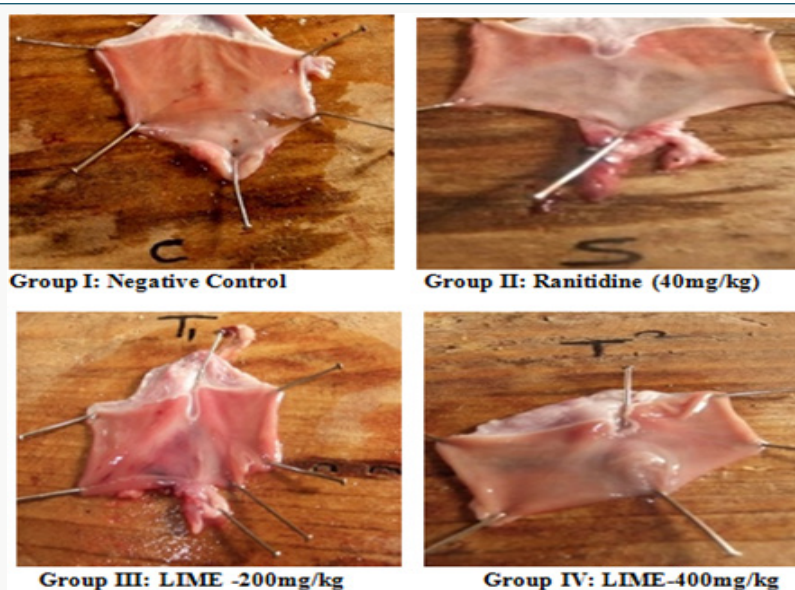
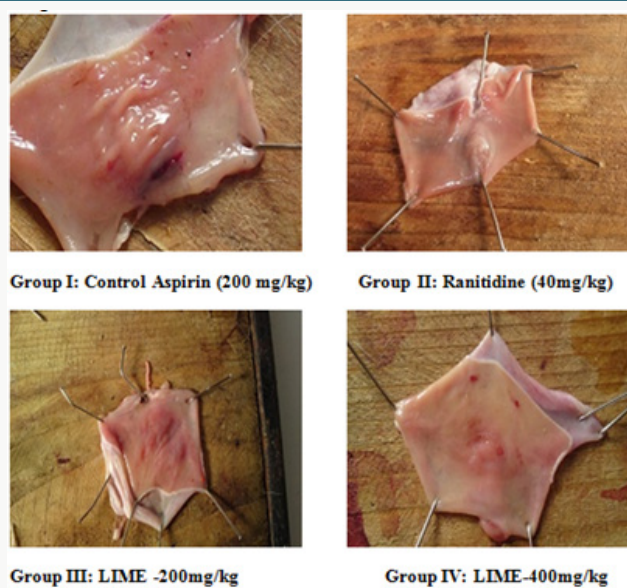
S.No	Treatment	Ulcer index	% protection
I	Control + Pylorus ligation	6.44±0.71	-
II	Ranitidine (40mg/kg)	1.8±0.69**	72.04
III	MELI (200mg/kg)	6.1±0.55 ^{ns}	7.20
IV	MELI (400mg/kg)	1.73± 0.42**	73.13

Mean ±S.E.M. *P <0.05 & **P < 0.01 when compared with Control.

Table 4: Effect of LIME on ulcer index and % protection in Aspirin induced ulceration in rats.

S.No	Treatment	Ulcer index	% Protection
I	Control + Aspirin	7.45±0.54	-
II	Ranitidine (40mg/kg)	1.95±0.34**	74.49
III	MELI (200mg/kg)	5.25±0.84*	29.53
IV	MELI (400mg/kg)	1.65±0.12**	77.85

mean ± S.E.M. *P 0.05, **P < 0.01 when compared with Control.

**Figure 2:** Pylorus ligation method**Figure 3:** Aspirin induced method

Results

Phytochemical screening of methanolic extract of *Leea indica* revealed the existence of phyto constituents like carbohydrates, flavonoids, steroids, tannins, phenols, saponins and alkaloids. Aqueous extract of *Leea indica* is constituted with phyto constituents like carbohydrates, flavonoids, phenols and alkaloids. In acute toxicity studies MELI was found to be nontoxic upto 2000mg/kg b.wt. Hence 1/10th, 1/5th of dose was selected for further pharmacological screening. In pylorus ligated ulcer model Ranitidine (40mg/kg) and MELI (400 mg/kg) produced a significant decrease in volume of gastric acid (P< 0.05), free acidity (P< 0.05), total acidity (P< 0.01) and increase in pH (P< 0.01). MELI (200mg/kg) did not produce significant decrease in volume of gastric acid, free acidity, total acidity. MELI (400mg/kg) produced a greater decrease in UI compared to standard (40mg/kg). In aspirin induced ulcer model Ranitidine (40mg/kg) and MELI (400 mg/kg) produced a significant decrease (P< 0.01) in volume of gastric acid (P< 0.05), free acidity (P< 0.05), total acidity (P< 0.01) and increase in pH (P< 0.01). MELI (400mg/kg) produced a greater decrease in UI compared to standard (40mg/kg). Ulcers characterised by redness, hemorrhagic stripes and the damage of mucosal layer were observed in negative control group, in both pylorus ligated ulcer model and in aspirin induced ulcer model. Rats administered with MELI (400mg/kg) and the standard drugs ranitidine (40 mg/kg) showed reduced redness and mucosal uniformity.

Discussion

Although numerous drugs were available for gastric ulcers adverse reactions like gynecomastia, hematopoietic changes, acute interstitial nephritis [26], thrombocytopenia [27], anaphylaxis reactions [28], nephrotoxicity and hepatotoxicity [29] limits the use. Therefore potent and safe herbs with lesser side effects were better alternatives for gastric ulcer. Herbs with reported antiulcer activity possess active constituents like saponins, flavonoids in considerable amounts. Owing to the existence of phyto constituents like carbohydrates, flavonoids, steroids, tannins, phenols, saponins and alkaloids methanolic extract is subjected to further pharmacological screening. Saponins were proposed to act by activation of mucosal protective factors [30]. Tannins elicit activity by tanning the mucosal layer and rendering it less permeable and more resistant to chemical and mechanical injury or irritation [31]. Flavonoids act probably by increasing prostaglandin secretion of mucosal layer and reducing the deleterious effects of free radicals. *Leea indica* might have elicited antiulcer activity owing to the presence of saponins, flavonoids and tannins. According to Acute toxicity study the plant extract was found to be safe upto 2000mg/kg. Hence the doses selected for the study was 1/5th and 1/10th of the toxic dose 200mg/kg, 400mg/kg. The pylorus ligated ulcer model is reproducible and more predictable model to screen antiulcer drugs. In this model ulcers are accomplished owing to auto digestion of mucus by accumulated gastric juice, decreased mucosal blood flow, and breakdown of the mucosal barrier [32]. This model has an added advantage of endogenous ulcer development neither by utilizing

exogenous ulcerogens nor inducing exogenous interfering factors for producing ulcers. Plant extract might have elicited antiulcer activity either by decreasing gastric acid secretion or increasing mucus production proving its antisecretory effect [33]. Aspirin inhibit cyclo-oxygenase enzyme thereby inhibiting prostaglandin (PGs) synthesis. Prostaglandins (PGE₂ & PGI₂) possess antiulcer activity by stimulating the secretion of mucus and bicarbonate, causing vasodilatation and inhibiting acid secretion [34]. Antiulcer activity of *Leea indica* might be by mucosal protective effect may be due to the biosynthesis of prostaglandins. Histopathological studies reveal the protective effect of MELI (400mg/kg) against ulcers induced by pyloric ligation and aspirin induced model. Higher dose of MELI (400mg/kg) elicited greater antiulcer effect compared to MELI (200mg/kg). Marked response was seen in standard group.

Conclusion

In conclusion, obtained experimental results elucidate Antiulcer activity of *Leea indica* in pylorus ligation method and aspirin induced method. MELI (400mg/kg) elicited greater activity compared to standard. Increased efficacy and reduced side effects of herbal drugs compared to modern drug give increase prominence. Histopathology studies clearly evidenced antiulcer activity of *Leea indica*. Existence of Phytochemical constituents might have contributed to the elicited activity. Further isolation of active constituents and elucidating their mechanism gives a better antiulcer drug with lowered toxicities.

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