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, Sri Lanka, Bangladesh, Burma, Nepal, Thailand, Laos, Cambodia, China, Vietnam, New Guinea, Malaysia, 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, pthalic acid, 1-eicosanol, farnesol, soalnesol, gallic acid, three pthalic 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 (Shalg 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.](image)

**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 [21]. 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

\[
\text{Ulcer index (UI) = 10 X X; where X = total mucosal area/total ulcerated area.}
\]

% Ulcer protection = \((\text{UI of control - UI of test}) \times 100)/ \text{UI 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 toper'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 = \frac{\text{Volume of } \text{NAOH} \times \text{Normality}}{100} \text{ 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].

a) 0 = normal stomach.

b) 0.5 = red coloration.

c) 1.0 = spot ulcers.

d) 1.5 = hemorrhagic streaks.

e) 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

\text{Ulcer Index}= 10X; \text{where } X = \frac{\text{total mucosal area}/\text{total ulcerated area}}{\text{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**                | 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**  | 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 up to 2000 mg/kg b.wt. Hence 1/10th, 1/5th of dose was selected for further pharmacological screening. In pylorus ligated ulcer model Ranitidine (40 mg/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 (200 mg/kg) did not produce significant decrease in volume of gastric acid, free acidity, total acidity. MELI (400 mg/kg) produced a greater decrease in UI compared to standard (40 mg/kg). In aspirin induced ulcer model Ranitidine (40 mg/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 (400 mg/kg) produced a greater decrease in UI compared to standard (40 mg/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 (400 mg/kg) and the standard drugs ranitidine (40 mg/kg) showed reduced redness and mucosal uniformity.

Discussion

Although numerous drugs were available for gastric ulcer 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 antulcer 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 antulcer activity owing to the presence of saponins, flavonoids and tannins. According to Acute toxicity study the plant extract was found to be safe up to 2000 mg/kg. Hence the doses selected for the study was 1/5th and 1/10th of the toxic dose 200 mg/kg, 400 mg/kg. The pylorus ligated ulcer model is reproducible and more predictable model to screen antulcer 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 antulcer 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) synthesize. Prostaglandins (PGE2 & PG12) possess antulcer activity by stimulating the secretion of mucus and bicarbonate, causing vasodilatation and inhibiting acid secretion [34]. Antulcer 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 antulcer effect compared to MELI (200mg/kg). Marked response was seen in standard group.

Conclusion

In conclusion, obtained experimental results elucidate Antulcer activity of *Leea indica* in pylorus ligation method and aspirin induced method. MELI (400mg/kg) elicited greater antulcer activity compared to standard. Increased efficacy and reduced side effects of herbal drugs compared to modern drug give increase prominence. Histopathology studies clearly evidenced antulcer 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 antulcer drug with lowered toxicities.

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