

ANTIULCER ACTIVITY OF ETHANOLIC EXTRACT OF *LEPTADENIA*  
*RETICULATA* LEAVES

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**Summary**

The objective of this study was to investigate the antiulcer activity of leaves of *Leptadenia reticulata* Linn. (Asclpiadaceae) in rats. Fresh dried leaves of *Leptadenia reticulata* were extracted with 95% of ethanol by Soxhlet apparatus. Antiulcer effects of the ethanol extract at two concentrations 100 and 200 mg/kg were evaluated in rats using ethanol, indomethacin and pylorus ligation induced ulcer methods. Famotidine as Standard drug. The evaluated parameters are ulcer index, acid volume, pH and total acidity. The extract (100 mg/kg & 200 mg/kg) showed significant (P<0.001) reduction in acid volume, total acidity and ulcer index as compared to control. This present study indicates that *Leptadenia reticulata* leaves extract have potential anti ulcer activity in all the models.

**Keywords:** Antiulcer, ethanol, indomethacin, pylorus ligation, Famotidine.

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### Introduction

Gastric ulcer, one of the most widespread, is believed to be due to an imbalance between aggressive and protective factors 1. The gastric mucosa is continuously exposed to potentially injurious agents such as acid, pepsin, bile acids, food ingredients, bacterial products (*Helicobacter pylori*) and drugs 2. These agents have been implicated in the pathogenesis of gastric ulcer, including enhanced gastric acid and pepsin secretion, inhibition of prostaglandin synthesis and cell proliferation growth, diminished gastric blood flow and gastric motility 3.

The plant is *Leptadenia reticulata* belonging to family Asclepiadaceae, well known for its tonic, restorative and stimulant property in the Indian system of medicine. This plant is distributed in the southern parts of India. The main constituents reported are stigma sterol, beta-sitosterol, flavonoids, pregnane glycosides and proteins 4. Aerial parts of *Leptadenia reticulata* is reported to contain tocopherol and possess several pharmacological activities such as galactogogue, antimicrobial and anti-inflammatory activity. Seeds of *L. reticulata* are reported to contain hyperoside, aflavonoid glycoside. *L. reticulata* is claimed to have hypotensive effect in dogs. Antioxidant principles derived from plants are reported to have antitumor activity 5. Hence it was decided to illustrate the ethno botanical use the plant and the study was planned find out the unexplored antiulcer activity of the ethanolic extract of the leaves *Leptadenia reticulata*.

### Materials and methods

#### Plant material

The whole plants were collected in Jan 2011 from Calicut, Kerala, India. The plant was identified by the department of Botany. Calicut University, Calicut, India and voucher specimen was deposited in institutional herbarium (voucher no 90097).

#### Preparation of extracts

Powdered leaves of *Leptadenia reticulata* (500g) was extracted (soxhlet) with 95% ethanol in 1:10 w/v ratio for 72hrs. Qualitative chemical analysis revealed that the extract contains alkaloids, carbohydrates steroids, glycosides, flavonoids and reducing sugars. 6,7

#### Experimental animals

Healthy adult albino rats of Wistar strain weighing 150-200g of either sex were used for this study. The animals were obtained from animal house, Bharathi College of Pharmacy, Bharathinagara, Karnataka, India. The animals were maintained under controlled conditions of temperature ( $23 \pm 2^\circ\text{C}$ ), humidity ( $50 \pm 5\%$ ) and 12-h light-dark cycles. All the animals were acclimatized for seven days before the study. The animals were randomized into experimental and control groups and housed individually in sanitized polypropylene cages containing sterile paddy husk as bedding. They had free access to standard pellets as basal diet and water *ad*

*libitum*. Animals were habituated to laboratory conditions for 48 h prior to experimental protocol to minimize if any of non-specific stress. All the studies conducted were approved by the Institutional Animal Ethical Committee (IAEC) of Bharathi College of Pharmacy, Bharathinagara, Karnataka (BCP/IAEC/05/10-11), according to prescribed guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

### **Antiulcer activity**

#### **a) Ethanol induced ulcers:**

Four groups of albino Wistar rats (n=6) were selected. In this model, Group 1 served as normal control received 0.5 ml of vehicle, p. o., and group 2 received Famotidine (3 mg/kg, p.o), whereas groups 3 and 4 animals received ethanolic extract of *Leptadenia reticulata* (100 and 200 mg/kg, p.o. respectively). Animals were fasted overnight prior to start of the experiment, and water *ad libitum* 30 min after treatment, all rats received 1mL of absolute ethanol to induce gastric ulcer. After 1 h the animals were sacrificed by cervical dislocation, the stomachs were removed and opened along the greater curvature. Stomachs were gently rinsed with water to remove gastric contents and the mean ulcer index was calculated. 8

#### **b) Indomethacin induced ulcers:**

Four groups of albino Wistar rats (n=6) were selected. In this model, Group 1 served as normal control received 0.5 ml of vehicle, p. o., and group 2 received Famotidine (3 mg/kg, p.o), whereas groups 3 and 4 animals received ethanolic extract of *Leptadenia reticulata* (100 and 200 mg/kg, p.o. respectively). Animals were fasted overnight prior to start of the experiment, and water *ad libitum* Indomethacin (30 mg/kg) suspended in 1% carboxymethyl cellulose was given as a single oral dose to induce gastric ulcers after 60 min of Famotidine and ethanolic extracts of *L. reticulata* treatment. The animals were sacrificed 7-9 hours after indomethacin administration using ether anesthesia. The stomachs were removed and opened along the greater curvature. After washing with saline, the gastric lesions were quantified and to calculate the ulcer index. 9

#### **c) Pylorus ligation induced ulcers:**

Four groups of albino Wistar rats (n=6) were selected. In this model, Group 1 served as normal control received 0.5 ml of vehicle, p. o., and group 2 received Famotidine (3 mg/kg, p.o), whereas groups 3 and 4 animals received ethanolic extract of *Leptadenia reticulata* (100 and 200 mg/kg, p.o. respectively). Animals were fasted overnight prior to start of the experiment, and water *ad libitum* Pyloric ligation was applied by ligating the pyloric end of the stomach of rats under Phenobarbital anaesthesia ( 35 mg/kg) after 30 min of ethanol extract of *L. reticulata* or Famotidine treatments. Animals were allowed to recover and stabilize in individual cage and were deprived of water during postoperative method. After 6 h of surgery, rats were sacrificed with excess ether and gastric juice was collected for performing gastric secretion study and ulcer scoring was done in stomach. 10

### Statistical Analysis

The data of results obtained were subjected to statistical analysis and expressed as mean  $\pm$  SEM. The data were statistically analyzed by one-way analysis of variance (ANOVA) and  $p < 0.01$  was considered to be significant and  $p < 0.001$  was considered to be more significant.

## Results and Discussion

### Ethanol induced ulcer model:

The results indicate that *L. reticulata* leaves extract at the dose levels of 100 mg/kg and 200 mg/kg produced a significant decrease in the ulcer index (Fig: 3-4). The results of acid volume determination of *L. reticulata* leaves extract treated groups indicate that there was a more significant decrease in the volume of the gastric juice. The activity was comparable and equipotent as that of Famotidine ( $p < 0.001$ ) (Table no.1)

The results of gastric pH determination of *L. reticulata* leaves extract treated groups indicate that there was a significant increase in the pH of the gastric juice. The activity was comparable and equipotent as that of Famotidine ( $p < 0.001$ ).

The results of free acidity and total acidity estimation of gastric juice of *L. reticulata* leaves treated groups indicate that there was a significant decrease in total acidity of the gastric juice. The activity was comparable and equipotent as that of Famotidine ( $p < 0.001$ ).

### Indomethacin induced ulcer model:

The results indicate that *L. reticulata* leaves at the dose levels of 100 mg/kg and 200 mg/kg produced a significant decrease in the ulcer index ( $p < 0.001$ ) and gastric pH determination of *L. reticulata* leaves extract treated groups indicate that there was a significant increase in the pH of the gastric juice. The activity was comparable and equipotent as that of Famotidine ( $p < 0.001$ ) (Table no. 2)

The results of acid volume of *L. reticulata* extract 100mg/kg produced significant activity ( $p < 0.5$ ), and total acidity estimation of gastric juice of *L. reticulata* leaves treated groups indicate that there was a significant decrease in total acidity of the gastric juice. The activity was comparable and equipotent as that of Famotidine ( $p < 0.001$ ).

### Pylorus ligation induced ulcers:

The results suggests that *L. reticulata* leaves at the dose levels of 100 mg/kg and 200 mg/kg produced more significant decrease in the ulcer index ( $p < 0.001$ ). The Results suggests that the ulcer index of *L. reticulata* leaves extract (100mg/kg and 200mg/kg) was found to be  $7.03 \pm 0.03$  and  $5.86 \pm 0.02$  which was found to be significant when this data was compared with Standard i.e Famotidine (3 mg/ kg) with ulcer index  $4.86 \pm 0.08$  (Table no. 3)

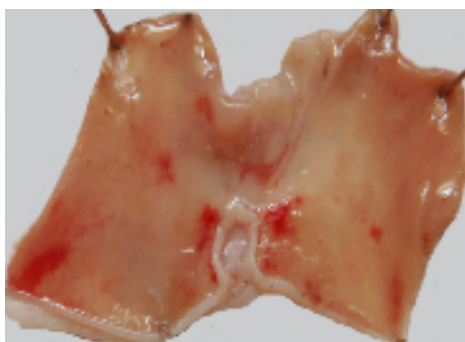


Fig: 1: Ethanol induced control

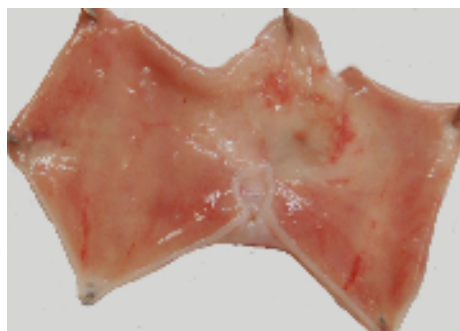


Fig: 2: Ethanol induced std. Treated



Fig: 3: Ethanol induced 100mg/kg. test drug

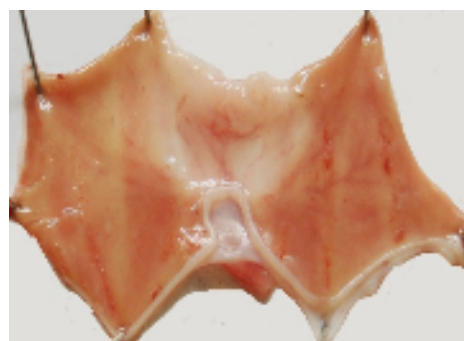


Fig: 4: Ethanol induced 200mg/kg. test rug

Table 1. Effect of *L. reticulata* ethanolic extract (LREE) on ethanol induced gastric ulcers

Gr No	Treatment	Dose mg/kg	Parameters			
			Ulcer Index	Acid Volume (ml)	pH	Total acidity (mEq/l)
I	Control (Vehicle)	0.5 ml	15.73+ 0.19	6.65+ 0.24	2.73 + 0.06	105.45 + 0.45
II	Famotidine	3	3.01+ 0.26***	2.39 + 0.24***	6.10 + 0.38***	61.40 + 0.37***
III	LREE	100	8.60+ 0.39***	4.85 + 0.03***	5.1 + 0.12***	88.09 + 1.24***
IV	LREE	200	5.04+ 0.73***	2.42 + 0.57***	5.62 + 0.41***	71.75 + 1.85***

Values are expressed as Mean + SEM (n=6 rats) P<0.05\*, P<0.01\*\* and P<0.001\*\*\* as compared to control group.

**Table 2. Effect of *L. reticulata* ethanolic extract (LREE) on indomethacin induced gastric ulcers**

Gr No	Treatment	Dose mg/kg	Parameters			
			Ulcer Index	Acid Volume (ml)	pH	Total acidity (mEq/l)
I	Control (Vehicle)	0.5 ml	18.34 ± 0.22	2.29 ± 0.15	3.29 ± 0.04	22.57 ± 0.31
II	Famotidine	3	7.60 ± 0.15***	0.83 ± 0.02***	5.39 ± 0.20***	12.73 ± 0.16***
III	LREE	100	15.52 ± 0.39***	1.92 ± 0.12*	4.72 ± 0.14***	16.49 ± 0.20***
IV	LREE	200	7.97 ± 0.11***	1.12 ± 0.02***	5.60 ± 0.10***	13.61 ± 0.15***

Values are expressed as Mean + SEM (n=6 rats) P<0.05\*, P<0.01\*\* and P<0.001\*\*\* as compared to control group.

**Table 3. Effect of *L. reticulata* ethanolic extract (LREE) on pylorus ligation induced gastric ulcers**

Gr No	Treatment	Dose mg/kg	Parameters			
			Ulcer Index	Acid Volume (ml)	pH	Total acidity (mEq/l)
I	Control (Vehicle)	0.5 ml	13.07±0.19	8.40±0.04	3.11±0.07	38.94±0.04
II	Famotidine	3	4.86 ± 0.08***	2.32 ± 0.01***	6.49 ± 0.03***	12.43±0.17***
III	LREE	100	7.03 ± 0.03***	4.56 ± 0.02***	4.60 ± 0.01**	31.77±0.12***
IV	LREE	200	5.86 ± 0.02***	3.22 ± 0.01***	5.76 ± 0.02***	20.40 ± 0.06***

Values are expressed as Mean + SEM (n=6 rats) P<0.05\*, P<0.01\*\* and P<0.001\*\*\* as compared to normal control group.

### Conclusions

From the Data of results obtained it is evaluated that the plant *Leptadenia reticulata* possesses a significant antiulcer activity compare to the standard drug. The study also helped us to identify the therapeutic values of the common plants present around us.

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