

EVALUATION OF ANTI-ULCER ACTIVITY OF *LEUCAS LAVANDULIFOLIA* ON MUCOSAL LESION IN RAT

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Abstract: The anti-ulcer activity of *Leucas lavandulifolia* (Labiatae) has been carried out in albino rats. First of all study on small group of animals was conducted to know the approximate ulcer protective activity of various solvents extracts of the plant. Finally, the methanolic extract was selected out and taken for final study. Anti-ulcer activity of methanolic extract of the herb was studied in rats, in which gastric ulcers were induced by oral administration of indomethacin (20 mg/kg) followed by pylorus ligation method. The extract was administered in the dose of 100 and 200 mg/kg intraperitoneally to the test group of animals for 3 consecutive days and on fourth day pylorus part of their stomach was ligated. After four hours of ligation, the rats were subjected for ulcer index and gastric acid evaluation. The reduction of ulcer index as well as gastric acid output in extract treated animals was found to be statistically significant with respect to control animals. The extract exhibited ulcer protection activity in dose dependent manner. Misoprostol was used as standard drug for ulcer protection.

Keywords: *Leucas lavandulifolia*, ulcer index, gastric output, misoprostol.

INTRODUCTION

A retrospection of healing power of plants, a return to natural remedies is absolute need of our time. Medicine of plant origin is based upon the premise that plants contain natural substances that can promote health and alleviate illness [1]. The present study supports the same for a folkloric herb *Leucas lavandulifolia* which is commonly used to alleviate the abdominal discomforts mainly stomach ulceration. Stomach ulcer is among the major disease of GIT, for which a large number of traditional and modern medicines are being utilized. Among these, the medicines of plant origin are more popular because of their less adverse effect [2].

Present study is based on the anti-ulcer activity of plant *Leucas lavandulifolia* of family Labiatae. The genus *Leucas* [3] contains about 100 Asiatic and African species, but the most common species found in India is *Leucas lavandulifolia* Sm [4]. It is an erect herb, 01-02 m tall, much branched from base having gray pubescent stem slender. It is commonly known as Gumma or Dronpuspi in India. In rural area of West Champaran, Bihar (India), it is used to counteract abdominal and liver diseases.

MATERIALS AND METHODS

Plant Material

The whole plant of *Leucas lavandulifolia* was collected from North Bihar (India). The plant herbarium was authenticated by Botanical Survey of India, Government of India, Kolkata Ref. no CNH/I-1(28)/2006/Tech.II/569.

Animal

Male wistar albino rats (150-200 g) and Swiss albino mice (25-30 g) were used. They were obtained from the animal house and experiments were carried out in accordance with CPCSEA guide lines [5]. The study was approved by Institutional Animal Ethics Committee.

Acute toxicity study

Swiss albino mice of either sex weighing 20-25 g were utilized for the LD₅₀ value determination. The method of Miller and Tainter (1944) was used for the experiment [6, 7]. The purified form of the methanolic extract was used for the LD₅₀ determination. Probit values against log doses were plotted and the value which corresponded to probit 5 was read as LD₅₀. The dose of 200 mg/kg body weight did not produce any toxic effect.

Anti-ulcerogenic activity

Pylorus ligation plus indomethacin induced gastric ulceration model

The modified method of Djahanguri (1969) was used for the production of gastric ulceration [8, 9]. The animals were divided into four groups (control, standard, extract 100 mg, extract 200 mg) of 6

rats in each group weighing 150-200 g. The oral suspension of Indomethacin (20 mg/kg suspension in 1% CMC) was taken as ulcerogenic drug for all groups. The control group was treated with the Indomethacin for 3 consecutive days, and on 4th day pylorus was ligated just one hour after the dosing under pentobarbitone (45 mg/kg i.p.) anesthesia. The standard groups were treated with an ulcer preventive drug misoprostol (100 mcg/kg i.p.) just one hour after Indomethacin treatment daily for 3 consecutive days and 4th day pylorus was ligated just one hour after dosing. In a similar manner the extract dose 100 mg and 200 mg per kg body weight were also given intraperitoneally to the test group of animals one hour after the Indomethacin treatment for 3 consecutive days and 4th day just one hour after dosing, rats were anaesthetized with pentobarbitone and pylorus part of their stomach was ligated. After four hours of pylorus ligation, rats were sacrificed and abdomen was opened through a mid-line incision. Gastric juice was collected after separating the stomach by surgery for the determination of total acid output. The stomach was opened through greater curvature and washed slowly under tap water to score the ulcer.

The ulcers were scored as below-

0=Normal coloured stomach

0.5=Red colouration

1.0=Spot ulcers

1.5=Hemorrhagic streaks

2.0=Ulcers $\geq 3 \leq 5$

3.0=Ulcer > 5

Mean ulcer score for each group of animals was expressed as ulcer index [10, 11]. The number of ulcer per stomach was also recorded and the percent of ulcer incidence of each group was compared with the control.

Determination of total gastric output

To determine the gastric output, the whole stomach content of the treated animal was removed in a centrifuge tube. The gastric content was centrifuged at 1000 rpm for 10 minutes. 1 ml of supernatant was taken and diluted with 9 ml of distilled water and titration was carried out against pre-standardized sodium hydroxide (0.01 N) using Topfor's reagent (dimethyl-amino-azo-benzene with phenolphthalein) as indicator.

The first end point was recorded when the solution turned to orange colour. The volume of sodium hydroxide needed was taken as corresponding to the free acid. Titration was further continued till the solution regained pink colour. The volume of sodium hydroxide required was noted and taken as corresponding to the total acid [2, 12].

$$\text{Acid strength (in m eq/L)} = \frac{\text{Volume of NaOH} \times \text{Normality} \times 100}{0.1}$$

The comparison among test, control and standard groups was done in tabular manner.

RESULTS

The LD₅₀ value of the extract was found to be 1650±24 (mg/kg ± SEM) through intraperitoneal route. It is evident from the Table 1, Table 2 and Figure 1 that the methanolic extract of the plant exhibited anti-ulcer activity in dose dependent manner. The average ulcer index of control, misoprostol (100 mcg/kg), extract (100 mg/kg) and extract (200 mg/kg) was found to be 3.92, 0.42, 2.58 and 1.58 respectively. The treatment of rats with the methanolic extract (100 and 200 mg/kg) produced a significant reduction in acid output as it is evident from Table 2. The protection was statistically significant even at 100 mg/kg dose also. Thus, results tend to confirm that the methanolic extract of the herb *Leucas lavandulifolia* has a preventive role in drug induced ulceration.

Table 1. Effect of extract against Indomethacin induced gastric ulcer (ulcer index) in rats

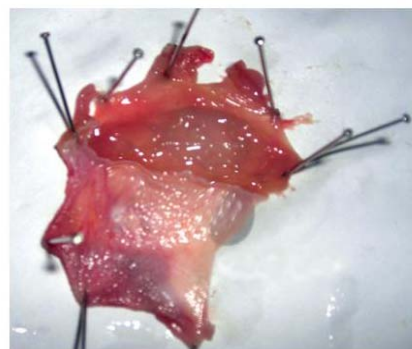
Treatment	Average ulcer index±SEM
Control (Vehicle + Indomethacin)	3.92 ± 0.54
Standard (Misoprostol+ Indomethacin)	0.42± 0.24**
Extract (100 mg/kg + Indomethacin)	2.58± 0.30*
Extract (200 mg/kg + Indomethacin)	1.58 ± 0.24**

Values are Mean ± SEM; n=6 animals in each group. *P<0.05 and **P<0.01 when compared to control (by using one way ANOVA with Dunnett t-test)

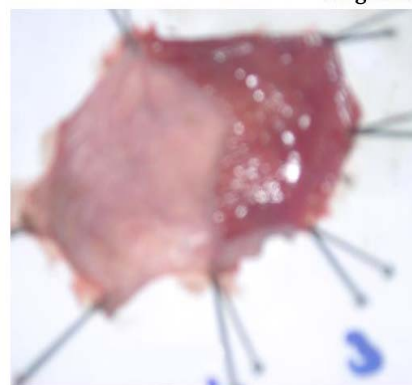
Table 2. Effect of extract against Indomethacin induced gastric ulcer (free and total acid) in rats

Design of Treatment	Free acid (m eq / L)	Total Acid (m eq/L)
Control (Vehicle+Indomethacin)	53.82 ± 3.48	65.83 ± 1.52
Standard (Misoprostol+Indomethacin)	19.50 ± 1.31**	22.5 ± 0.99**
Extract (100 mg/kg + Indomethacin)	43.66± 2.99*	58.66 ± 1.70*
Extract (200 mg/kg + Indomethacin)	29.50 ± 2.09**	56.16 ± 1.85**

n = 6 animals in each group; values are Mean ± SEM; * p<0.05, ** p < 0.01 when compared to control (by using one way ANOVA with Dunnett t-test)



(c)



(d)

Figure 1. Stomach of (a) Control (Vehicle+Indomethacin); (b) Standard (Misoprostol+Indomethacin); (c) Extract (100 mg/kg) + Indomethacin; (d) Extract (200 mg/kg) +Indomethacin

DISCUSSION

The immediate cause of peptic ulcer is disturbance in the protection of stomach mucosa against gastric acid. Risk of ulcer genesis is now greatly enhanced due to exposure of man to many noxious agents and chemicals [13]. Many drugs available in the market greatly reduce the morbidity and mortality, but may have the adverse reactions like reduction in prostaglandin synthesis by inhibiting the cyclo-oxygenase enzyme non-specifically [14]. In this study, the anti-ulcer activity of *Leucas lavandulifolia* Sm (Labiatae) was explored with the help of pharmacological experiments and statistical analysis. The extract produced a dose dependant protection against indomethacin induced peptic ulcer though not better than misoprostol. However, to establish its mode of action, several studies like radio-ligand binding assay, biochemical analysis, enzyme assay and the phyto-chemical evaluation can be done in future.

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(a)



(b)

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