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Investigation of Antiulcer Activity of *Leonotis nepetaefolia* (L.) R.Br. in Pylorus ligation induced and Ethanol induced Gastric ulcer in rats

Sumeet Dwivedi[1]*, Nidhi Bais[2], Gurmeet Chhabra[3], Diksha Joshi[4], Sujit Appasaheb Jadhav[5], Anubhav Dubey[6] and Mahavir Chhajed[7]

1, Acropolis Institute of Pharmaceutical Education and Research, Indore, Madhya Pradesh, India
2, Faculty of Pharmacy, Oriental University, Indore, Madhya Pradesh
3, Indore Institute of Pharmacy, Indore, Madhya Pradesh, India
4, Faculty of Pharmacy, Medi-Caps University, Indore, Madhya Pradesh, India
5, R.G. Sapkal College of Pharmacy, Nashik, Maharashtra, India
6, Maharana Pratap College of Pharmacy, Kanpur, Uttar Pradesh, India
7, Vikrant Institute of Pharmacy and Science, Indore, Madhya Pradesh, India

Corresponding Author
E.mail: sumeet_dwivedi2002@yahoo.com

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ABSTRACT

The current study aims to assess the antiulcer activity of hydroalcoholic extracts of *Leonotis nepetaefolia* (L.) R.Br. leaves in rats that have gastric ulcers caused by ethanol and pylorus ligation. In the pylorus ligation model, the dose-dependent anti-scretory and antiulcer impact of HAELNL was demonstrated by a significant reduction in gastric juice volume and ulcer score as well as an elevation in gastric juice pH. Compared to the control group, HAELNL demonstrated a noteworthy reduction in the ulcerogenic effect in the ethanol-induced ulcer model. The outcomes demonstrated that in both scenarios, HAELNL produced better and significant results.

Key-words: Anti-ulcer, Hydroalcoholic extract, *Leonotis nepetaefolia* (L.) R.Br. leaves

INTRODUCTION

The most common gastrointestinal ailment, ulcers are characterized by breaks in the stomach, small intestine, and occasionally the lower oesophagus' lining. The ulcer is primarily linked to

symptoms including nausea, vomiting, bloody or dark faeces, burning pain in the abdomen that radiates to the chest, loss of appetite, and unexplained weight loss. Together, stomach ulcers (also known as gastric ulcers) and duodenal ulcers (also known as duodenal ulcers) are referred to as peptic ulcers.[1] These are investigated with upper gastrointestinal series and endoscopy (gastroscopy). Proton pump inhibitors, ulcer protectants, H2 antihistaminics, and anti-H. pyloritherapy are all part of the curative treatment for peptic ulcers.[2] When taken over an extended period of time, the traditional antiulcer medications used to treat peptic ulcers may have unfavourable side effects or drug interactions. Various herbal medicines are used in traditional medicine to treat gastrointestinal disorders, with the goal of symptom relief and delaying recurrence. Drugs do not yet fulfil all of these therapeutic objectives. Thus, the most sought-after field of study is the hunt for strong, secure, and cost-effective antiulcer medicines derived from herbal sources. In traditional medicine, *L. nepetifolia* (Barchibuti) belongs to family Lamiaceae used to treat many diseases including bronchial asthma, diarrhea, fever, influenza, malaria, cough, womb prolapse, epilepsy, burns, skin ailments, and rheumatism. Constituent phytochemicals such as tannins, alkaloids, flavonoids, saponins, steroids, phenolics, glycosides, anthocyanins, and coumarins have been implicated to account for the usefulness of the plant to treat the abovementioned diseases. Importantly, the antimicrobial, immunomodulatory, anti-cancer, antioxidant, analgesic, and anti-inflammatory activities of these phytochemicals have been confirmed both in vitro and in vivo. The leaves may used in the treatment of ulcer. Various extract of plant exhibited potent anti-inflammatory action against both exudative and proliferative and chronic phases of inflammation, besides exhibiting significant anti-arthritic, antipyretic and analgesic activities. [3] Based on the facts it was an attempt to evaluate anti-ulcer potential of the leaves of selected plant.

MATERIAL AND METHODS

Collection and Authentication of the plant materials

The leaves of the plant were collected in the month of Oct. 2022 and were identified by Dr. S. N. Dwivedi, Retd. Prof. and Head, Department of Botany, Janata PG College, A.P.S. University, Rewa, (M.P.) and was deposited in our Laboratory.

Preparation of extract

The *Leonotis nepetaefolia* (L.) R.Br. leave**s** were shade dried and reduced to coarse powder in a mechanical grinder and passed through sieve No. 40. The powdered leaves were subjected to extraction by hydroalcohol as solvent. The extracts were collected in a tarred conical flask. The solvent was removed by distillation. Last traces of solvent being removed under vacuum. The extract obtained with each solvent was weighed to a constant weight and percentage w/w basis was calculated. The obtained crude extract was stored in dark glass bottles for further processing. [4-5]

Acute toxicity study of extract

The acute toxicity of extract was determined as per the OECD guideline no. 423 (Acute toxic class method) [268]. The extracts were suspended using 0.5% sodium carboxy methylcellulose and were administered orally. The concentration was adjusted in such a way that it did not exceed 1ml/kg b/w of the animal. [6]

Procurement of experimental animals

The mice were used for acute toxicity study as per OECD guidelines 423. The animals were fed with standard pellet diet (Hindustan lever Ltd. Bangalore) and water *ad libitum*. All the animals were housed in polypropylene cages. The animals were kept under alternate cycle of 12 hours

of darkness and light. The animals were acclimatized to the laboratory condition for 1 week before starting the experiment. The experimental protocols were approved by Institutional Animal Ethics Committee after scrutinization.

Test compounds

The HAELNL and standard drug ranitidine (50 mg/kg body weight) were used.

Experimental animal

Albino rats (100-120 g) used in the present studies was procured from listed suppliers from India. The animals were fed with standard pellet diet (Hindustan lever Ltd. Bangalore) and water *ad libitum*. All the animals were acclimatized for a week before use.

Pylorus ligation induced gastric ulcer in rats

Rats weighing (150-200g) of either sex were allocated into 4 groups of six animal in each group. Animals were fasted for 18hr prior to drug treatment but had free access to water.

Group 1- Control (Received vehicle- Normal saline 5ml/kg)
Group 2- Standard (Ranitidine 150 mg/kg) orally
Group 3- HAELNL (200 mg/kg)
Group 4- HAELNL (400 mg/kg)

Pylorus ligation was carried out in all groups of rats for the induction of gastric ulcers and followed by the respective treatments orally. After 6 hrs of ligation all animals were sacrificed, the abdomen was opened by using a small incision. The stomachs were dissected out and contents were drained into tubes and centrifuged for 10 minutes at 1000 rpm. Supernatants were subjected to investigation of gastric volume and pH of gastric juice. The stomachs were then cut along the greater curvature and examined for ulceration and the ulcer index (UI) was calculated. [7-8]

Ethanol induced gastric ulcer in rats

Rats weighing (150-200g) of either sex were allocated into 5 groups of six animal in each group. Animals were fasted for 18hr prior to drug treatment but had free access to water. Group I- Control (Received vehicle- Normal saline 5ml/kg)
Group II- Standard (Ranitidine 150 mg/kg) orally
Group III- HAELNL (200 mg/kg)
Group IV- HAELNL (400 mg/kg)

Animals were given test extract or standard drug. 1 hr later 1ml/200g of 99.80% alcohol was given orally to every animal. After 1 hr of treatment animals were sacrificed and stomach was incised along the greater curvature and ulceration was scored. The number of ulcers and the ulceration area were measured. Ulcer index was calculated using following formula. [7-8]

$$\mathbf{UI = UN + US + UP \times 10^{-1}}$$

Where, UI = Ulcer Index, UN = Average of number of ulcer per animal , US = Average of severity score , UP = Percentage of animal with ulcer

Statistical analysis

The statistical significance was assessed using one-way analysis of variance (ANOVA) followed by Dunnet comparison test. For comparing nonparametric ulcer scores, ANOVA followed by non-parametric Dunn post test was used. The values are expressed as mean + SEM and $p<0.05$ was considered significant.

RESULTS AND DISCUSSION

According to OECD guideline no. 423, the HAELNL was screened for an acute toxicity investigation in order to determine its LD50. According to the findings, the extracts fell into

group 5 (unclassified). Because of this, the LD50 was 2000 mg/kg and the ED50 was 200 mg/kg. As a result, 200 mg dosages were used for the current study. Table 1 displayed the findings.

Table 1: Determination of LD_{50} and ED_{50} of Leaves Extract of *Leonotis nepetaefolia* (L.) R.Br.

S/No.	No. of Animals	Extract Dose (mg/kg)	No. of death of animals
1.	3	5	0
2.	3	50	0
3.	3	300	0
4.	3	2000	0

In the Pylorus ligation approach, the volume and ulcer index of the stomach, as well as the pH of the gastric juice, increased significantly in the untreated, control pylorus-ligated rats. In the pylorus ligation model, the dose-dependent anti-scretory and antiulcer action of HAELNL was demonstrated by a significant reduction in gastric juice volume and ulcer score as well as an elevation in gastric juice pH. The proportion of protection at 200 mg/kg and 400 mg/kg dosages, respectively, was determined to be 48.37% and 34.20%. The test extract's potency was found to be lower than that of ranitidine, while HAELNL's potency at a dose of 200 mg/kg was shown to be higher. The outcomes highlighted in table 2. The percent inhibition was mentioned in graph 1.

Table 2: Effect of Extract on Pylorus ligation induced gastric ulcer in rats

Groups	Treatments	Dose (mg/k)	Vol. of gastric juice (ml)	pH	Ulcer index	%Inhibition
I	Control	-	5.44±0.11	1.95±0.10	3.82±0.34	-
II	Standard (Ranitidine)	150	2.49±0.21*	3.48±0.02**	1.12±0.03**	71.8
III	HAELNL	200	4.07±0.21**	2.32±0.02*	1.23±0.21**	48.37
IV	HAELNL	400	4.41±0.06*	2.86±0.073**	2.39±0.11*	34.20

Values are expressed as mean ±SEM, n= 6. (One way ANOVA Followed by Dunnette multiple Comparisons test). Statistically significance of *P<0.01, **P<0.001, when compared with respective control.

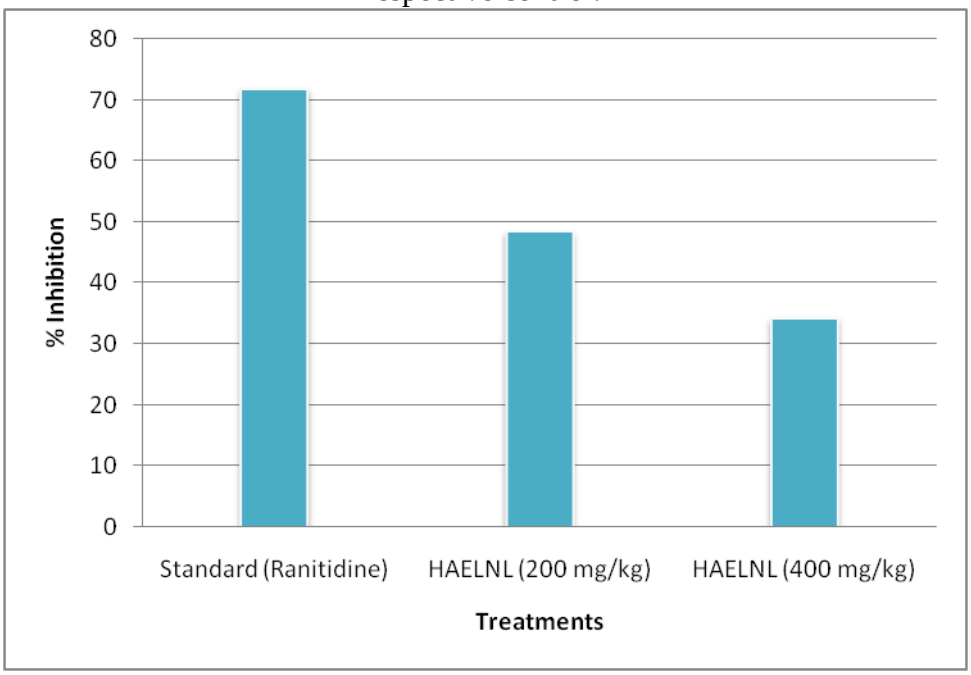
Compared to the control group, HAEMOS and HAESCS demonstrated a noteworthy reduction in the ulcerogenic effect in the ethanol-induced ulcer model. The percentage of inhibition in the animal group treated with extract at doses of 100 mg/kg was found to be 56.35%, 45.45%, and 283.9%, respectively, compared to standard, HAEMOS, and HAESCS. These data indicate that HAEMOS yields better outcomes than HAESCS. Table 3 displays the results. The percent inhibition was mentioned in graph 1. The percent inhibition was mentioned in graph 2.

Table 3: Effect of Extract on Ethanol induced gastric ulcer in rats

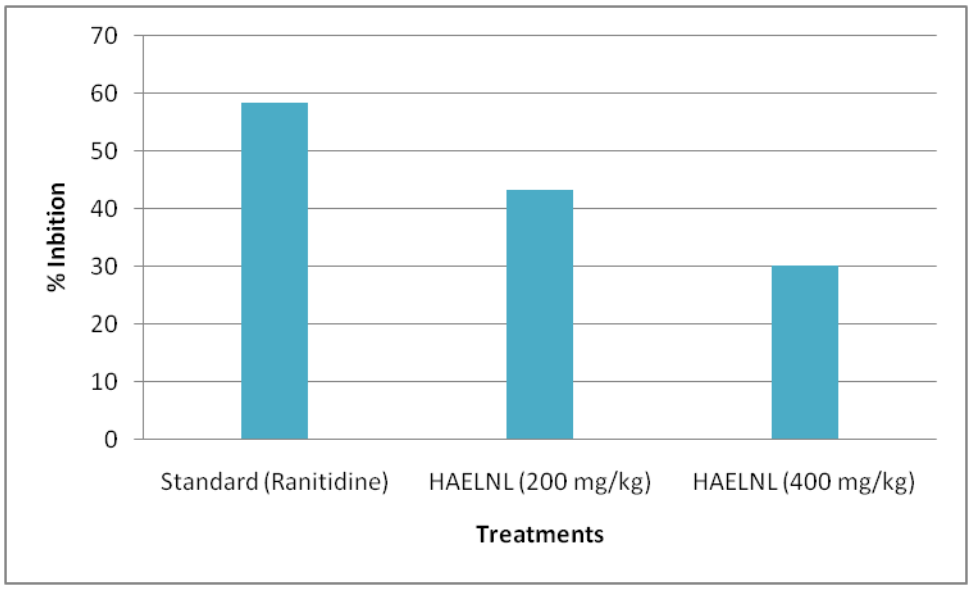
Groups	Treatments	Dose	Gastric Ulcer	% Inhibition

		(mg/kg)	**index**	
I	Control	-	5.39±0.22	-
II	Standard (Ranitidine)	150	2.44±0.10**	58.39
III	HAELNL	200	3.16±0.34**	43.2
IV	HAELNL	400	4.29±0.07*	30.1

Values are expressed as mean ±SEM, n= 6. (One way ANOVA Followed by Dunnette multiple Comparisons test). Statistically significance of *P<0.01, **P<0.001, when compared with respective control.



Graph 1: %Inhibition Extract on Pylorus ligation induced gastric ulcer in rats



Graph 1: % Inhibition of Extract on Ethanol induced gastric ulcer in rats

CONCLUSION

Rats were used to test the effects of hydroalcoholic leaves extracts of *Leonotis nepetaefolia* (L.) R.Br. on stomach ulcers caused by ethanol and bacteria ligation. The findings show that HAELNL have a dose-dependent antiulcer impact in the pylorus ligation model, as evidenced by a significant decrease in gastric juice volume and ulcer score as well as an elevation in gastric juice pH. Compared to the control group, HAELNL demonstrated a noteworthy reduction in the ulcerogenic effect in the pylorus ligation induced and ethanol-induced ulcer model.

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