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Research Article

Evaluation of Antiulcer Activity of Ethanol Extract of Leaves of *Lactuca sativa*

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ABSTRACT

In this modern era, gastrointestinal disorders are the universal problem. Peptic ulcer is one of the major diseases affecting the human population. It develops due to the imbalance between aggressive factors like acid, pepsin, H. pylori and bile salts and defensive factors like mucous, bicarbonate, blood flow, epithelial cell restoration and prostaglandins. The anti-ulcer activity of Ethanol extract of leaves of *Lactuca sativa* (EELS) was estimated using the experimental models of acute gastric lesions induced by ethanol, pylorus ligation and cold restraint stress in Wistar albino rats. Animals pre-treated with doses of 250 mg/kg, 500 mg/kg of EELS were statistically analyzed and compared to the standard and control group with the parameters like volume of gastric secretion, total acidity and ulcer index. The results suggested that EELS significantly decreased volume of gastric acid secretion, total acidity and ulcer index in comparison with standard drug Omeprazole. EELS shown significant reduction in lesion index, total affected area and percentage of lesion in comparison with control group in Ethanol induced ulcer in experimental models. The gastric mucosal protective effect of EELS is brought by inhibiting the gastric secretion, which shows it may act like a proton pump inhibitor. Thus the present study indicates that EELS has anti-ulcerogenic potency in Ethanol induced, pylorus ligation and cold restraint stress induced ulcers in rats.

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INTRODUCTION

Peptic ulcer is the most common gastrointestinal disorder in clinical practice¹. It occurs due to an imbalance between the aggressive (acid, pepsin and *Helicobacter pylori*) and the defensive (gastric mucus and bicarbonate secretion, prostaglandins, innate resistance of the mucosal cells) factors². Sometimes the gastric mucosa is continuously exposed to potentially injurious agents such as pepsin, bile acids, food ingredients, bacterial products and drugs. Factors³ such as stress, smoking, nutritional deficiency and ingestion of NSAID'S all can increase the occurrence of gastric ulcers. It is reported that prolonged anxiety, emotional stress, haemorrhagic surgical shock, burns and trauma are known to be the genesis for severe gastric irritation. Although a number of antiulcer drugs such as H₂ receptor antagonists, proton pump inhibitors and cyto-protectants are available for ulceration all these drugs have varied undesirable effects such as arrhythmias, impotence and hematopoietic changes and limitations⁴. Drug treatment of peptic ulcers is choose to either counteract aggressive factors (acid, pepsin, active oxidants, platelet aggravating factor (PAF), leukotrienes,

endothelins, bile or exogenous factors including NSAIDs) or stimulate the mucosal defences (mucus, bicarbonate, normal blood flow, prostaglandins (PG), nitric oxide⁵. There are many agents in alternative medicine, which have shown promising antiulcer activity without producing above mentioned adverse reaction. Further in the traditional medicine⁶ Ayurveda, several plants and herbs are advocated for the treatment of gastrointestinal disorders including gastric ulcers. The anti-ulcerogenic activity of many plant products is outlined due to an increase in mucosal defensive factors rather than decrease in the offensive factors⁷.

Lactuca sativa L. (lettuce) is a leafy vegetable and belongs to the *Asteraceae* family and genus *Lactuca*. It is a valuable dietary source of vitamin K, E and C as well as carotenoid⁸. Traditionally, it is well-known for its use as folk remedy for inflammation, pain, stomach problems including indigestion and lack of appetite⁹. Previously, considerable pharmacological studies have been conducted to evaluate therapeutic significance of the crude extracts of *Lactuca sativa* which showed its anticonvulsant, sedative-hypnotic and antioxidant properties¹⁰. So far, no study has been

carried out to assess the anti-ulcer activity of *L. sativa* using gastric ulceration models.

MATERIALS AND METHODS

Collection and Preparation of Plant Material

The fresh leaves of *L. sativa* were collected from the local market of Maisammaguda, Telangana state, India. The plant material was identified and authenticated by Dr. H. Ramakrishna, H.O.D, Department of Botany, Osmania University, Telangana, India. Ethanol extract was prepared using Soxhlet extraction process. Qualitative Phytochemical Evaluation for ethanol extracts were screened for the presence of various secondary metabolites like tannins, alkaloids, glycosides, terpenoids, flavonoids, amino acids and proteins using standard methods.

Animals

An ethical approval of this experimental study was obtained from the Institutional Animal Ethical Committee of Malla Reddy College of pharmacy, Hyderabad with Reg. No.1217/PO/Re/S/08/CPCSEA. Wistar albino rats with average body weight from 150 to 250 g were utilized in this study. They were procured from Teena labs, Plot no 41, SV cooperative industrial estates, Bachupally (V), Quthbullapur. The rats were housed in polypropylene cages and maintained under standard conditions (12h light and dark cycles at 25 ±3°C and 35-60 % humidity). Standard pelletized feed and tap water were provided ad-libitum.

Experimental design

Wistar albino rats were selected and divided into four groups of six animals each. Animals were fasted for 24 hour before the study, but had free access to water. Group I treated as vehicle control, received only distilled water; group II as standard group, received Omeprazole 20 mg/kg (P.O), group III and IV treated as treatment groups, received the graded dose of ethanol extract of *L. sativa* at 250 and 500 mg/kg, (P.O.) for 7 days (once in a day) respectively.

Ethanol induced mucosal damage in rats

The rats were fasted for 24 hours before the experiment. After 1 hour of administration of EELS, Omeprazole and vehicle control treatment, 1ml of absolute ethanol (0.5 ml/100g) was orally administered to each rat of every group. After 1 hour, the animals were sacrificed with excess of anaesthetic ether and the stomachs were opened along the greater curvature and washed slowly under running tap water. They were put on a glass slide and observed under 10X magnification for ulcers. The ulcers were scored. Mean ulcer score in each group was calculated and was designated as ulcer index and percentage was calculated using following formula:

$$\% \text{ Protection} = (C-T/C) \times 100$$

Where C = Ulcer index in control group;

T= Ulcer index in treated group

Pylorus ligation induced gastric ulceration

In this method, albino rats were fasted in individual cages for 24 hour EELS, reference drug and control vehicle was administered 1 hour prior to pyloric ligation. Then the pre-treated animals were anaesthetised by anaesthetic ether; the abdomen was opened by a small midline incision below the xiphoid process. The pyloric portion of the stomach was ligated without causing any damage to its blood vessels. The abdominal wall was sealed by interrupted sutures after

careful isolation of the stomach. Animals were deprived of water during the postoperative period. Four hours after ligation, the stomach was dissected out and contents were collected into clean tubes. The volume, pH and total acid content of gastric juice were determined. The contents were centrifuged, filtered and subjected to titration for estimation of total acidity. From the supernatant, aliquots (1 ml each) were taken for the determination of pH, total or free acidity activity¹¹. Each stomach was examined for lesions in the fore stomach portion and indexed according to severity¹². The numbers of ulcers were counted and scoring of ulcer was made as follows: Normal colored stomach (0), Red coloration (0.5), Spot ulcer (1), Haemorrhagic streak (1.5), Deep ulcers (2) and Perforation (3). Mean ulcer score for each animal was expressed as ulcer index¹³. Ulcer index (U_i) was measured by using following formula: $U_i = U_N + U_S + U_P \times 10^{-1}$ Where, U_i (Ulcer Index); U_N (Average number of ulcers per animal); U_S (Average number of severity score); U_P (Percentage of animals with ulcers). The percentage inhibition of ulceration was calculated and compared with control.

Statistical Analysis

The obtained results were analyzed for statistical significance¹⁴ using one way ANOVA followed by Dunnet test using the graph pad statistical software for comparison between different experimental groups. P-values < 0.001 were considered statistically significant.

RESULTS AND DISCUSSIONS

Phytochemical analysis: On phytochemical analysis of EELS, the extract has shown the presence of Carbohydrates and glycosides, Phytosterols, Phenolic compounds and tannins, saponins. Ethanol induced gastric ulcer model: The significant antiulcer activity of EELS at the both doses was noted. Ulcer index reduced at both doses, but the dose 500 mg/kg is significant one. Percentage of ulcer protection of 500 mg/kg is more when compared to 250 mg/kg and the volume of the gastric juice in ml is also reduced in EELS (500 mg/ml). Hence, it can be said that both extracts have anti-ulcer activity, but EELS at 500 mg/kg is more potent (Table 1).

Table 1: Effect of eels on ulcer index in ethanol induced and pyloric ligation gastric ulcers

Groups	Ulcer index (mean ± SEM)	
	Ethanol induced	Pyloric ligation
I	7.63±0.05	4.8±0.15
II	1.28±0.07***	2.6±0.06***
III	2.63±0.05	3.5±0.04
IV	1.59±0.04**	2.9±0.05**

N.B. Values are expressed as mean ± SEM. n=6, ***p <0.001, **p<0.01, when compared to control group. (Statistically analysed by one way Annova followed by Dunnet's t-test)

Pylorus Ligation Induced Gastric Ulceration: In pylorus ligated rats, the volume of gastric content, pH, total and free acidity are shown in (Table 2). In EELS treated groups, the volume of acid secretion and total acidity was decreased and pH of the gastric juice was increased compared to ulcer control group. The effects of ethanolic extract of *L. sativa* on acid parameters showed significant (p<0.01 and p<0.05) effect at 250 and 500 mg/kg doses compared to ulcer control animals.

Table 2: Effect of eels on various parameters in pyloric ligation model

Groups	pH of gastric juice (ml)	Gastric juice (ml)	Free acidity (mEq/ltr)	Total acidity (mEq/ltr)
I	3.1±0.25	8.8	91.61	110.34
II	5.85±0.05***	5.2***	35.53***	42.61***
III	4.51±0.13	6.2	45.21	53.13*
IV	5.52±0.15***	5.8***	40.62**	47.45**

N.B. Values are expressed as mean ± SEM. n=6, ***p <0.001, **p<0.01, *p<0.05 when compared to control group. (Statistically analysed by one way Anova followed by Dunnet's t-test)

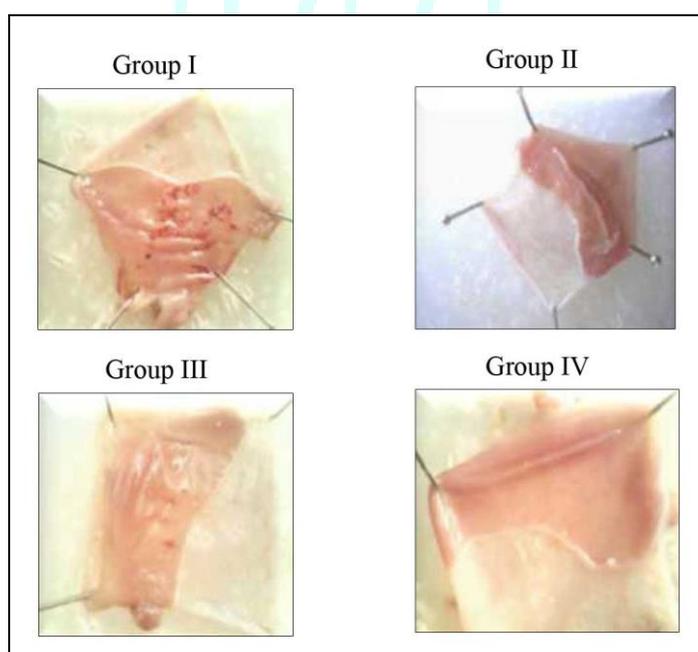
The reason behind the appearance of gastric ulcer is due to stress and increase in gastric acid (HCl) secretion. These acid secretions promote ulceration due to exposure of the unprotected lumen of the stomach to the accumulating acid. Pylorus ligation induced ulcers are shown by auto digestion of the gastric mucosa and breakdown of the gastric mucosal barrier which resulted as upper gastrointestinal damage including lesions, ulcers and life threatening perforation and haemorrhage. The pyloric ligation of the stomach is the origin for accumulation of gastric acid which leads to development of ulceration in stomach. The agents that decrease gastric acid secretion and increase mucus secretion are effective in preventing the ulcers induced by this method. Like ranitidine, omeprazole acts as anti-ulcer agent by anti-secretory mechanism via inhibition of gastric secretion. In the present study, EELS prevents the ulcer may be by anti-secretory and cytoprotective property.

Ethanol is responsible for disturbances in gastric secretion, damage to the mucosa, alterations in the permeability, gastric mucus depletion and free radical production. The generation of free radicals was produced by continuous release of superoxide anion and hydroperoxy free radicals during metabolism of ethanol. Ethanol induced gastric ulceration may be occurred due to stasis in gastric blood flow which presents the development of the haemorrhage and necrotic tissue injuries. Alcohol has ability to penetrate the gastric mucosa and causing the cellular damage which

increases the permeability to sodium and water. In other hand, the accumulation of intracellular calcium causes the pathogenesis of gastric injury that leads to cell death and exfoliation of surface epithelium. The present study observed that the EELS significantly reduced ethanol induced ulcer by cytoprotective action via antioxidant effect. The EELS extract showed cytoprotection against the ethanol and Pylorus ligation induced ulceration by reducing the gastric acid secretion.

The results of this study found that EELS established a cytoprotective action against ethanol induced cellular damage in the gastric mucosa of rats. It has also been observed that EELS significantly and dose dependently reduced the extent of gastric ulceration in pylorus ligated rats without affecting the gastric secretion. The defence potential of gastric mucosa depends upon a delicate balance between the processes affecting the synthesis and secretion of mucin constituents. EELS prevented the mucosal lesions induced by alcohol¹⁶. The modern approach towards a potent antiulcer agent involves a delicate balance of controlling the synthesis, secretion and metabolism of proteins, glycoproteins and lipids, so as to strengthen the mucosal integrity.

The images of the stomachs of the group's viz., Control, Standard and treatment groups (2 dose levels of *L. sativa*) ethanol-induced gastric lesion models are shown in figure 1.

**Figure 1: Macroscopical view of Ethanol induced Ulcer**

These above active compounds had ability to stimulate mucus, bicarbonate and prostaglandin secretion and neutralize with the deteriorating effects of reactive oxidants in gastrointestinal lumen. Therefore, EELS possess antiulcer activity, may be due to presence of tanins, flavonoids and terpenoids.

CONCLUSION

The present study concluded that the antiulcer activity of EELS may be attributed to anti-secretory, cytoprotective and antioxidant properties. The bioactivity-guided phytochemical screening of EELS revealed the presence of flavonoids, tannins and triterpenoids, which may be responsible for the anti-ulcer effect and can be further fractionated and investigated for their role and utility in any of the anti-ulcer mechanisms.

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