Research article

EVALUATION OF ANTIULCEROGENIC ACTIVITY OF OCIMUM SANCTUM LINN IN STRESS INDUCED ULCER IN RATS

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ABSTRACT:

Aims & Objectives: *Ocimum sanctum* L. (also known as Tulsi) has been used for thousands of years in Ayurveda for different properties. In this study we investigated the effect of leaf extract of Ocimum sanctum on stress induced ulcers in rats. **Materials and Method:** Albino rats were randomly, allocated to different experimental groups and aqueous leaf extract of Ocimum sanctum given for 7 days. Stress ulcers were induced by forced swim methods and results were compared with standard drug ranitidine.

Results: The aqueous extract of OS showed significant anti-ulcer effect in forced swimming method in a dose dependent manner. **Conclusion :** In the present study pretreatment with Ocimum sanctum at dose 100-200 mg/kg caused a significant anti-ulcer effect in forced swim induced models of gastric lesion in rats in comparison with control group and its effect is comparable to the standard drug ranitidine, as evidenced by the reduction in the ulcer scores.

Key words: Ocimum sanctum, Stress induced ulcers, Forced swim method.

INTRODUCTION

Peptic ulcer is a circumscribed ulceration of the gastrointestinal mucosa occurring in areas exposed to acid and pepsin and most often caused by helicobacter pylori infection¹. It is due to imbalance between the "aggressive" (acid, pepsin and H.pylori) and "defensive" factors (mucin, prostaglandin, bicarbonate, nitric oxide and growth factor)^{2,3} There is also ample evidence that stress triggers formation of ulcer. Psychological stress probably functions most often as a cofactor with H. pylori. It may act by stimulating the production of gastric ulcer or by promoting behaviour that causes risk to health⁴. Statistics from all sources indicate 10% or more of adult population are affected within their life time and 50% of healthy individuals complain of dyspepsia⁵ and it impairs the quality of life. Hence its treatment is essential. Present treatment of anti ulcer drugs consists of antacids which provide symptomatic relief without inhibiting the gastric secretion or efficiently promoting healing. The H₂ receptor blockers and proton pump inhibitors although decrease the acid secretion and promote healing of ulcer, but have not proved their worth in preventing relapse and recurrence⁶. There is also "acid rebound"

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after cessation of therapy and long term adverse effects limit their utility⁷. The anticholinergics, prostaglandin analogs and ulcer protective agents are not very effective antiulcer agents. Ulcer healing drugs like Carbenoxolone is associated with increased mineralocorticoid activity⁸. Therefore the present therapy is not satisfactory. Plants are one of the most important sources of medicines and have been used by the traditional medicinal practitioners for curing various diseases including peptic ulcer⁹. The important advantages claimed for therapeutic uses of medicinal plants in various ailments are their safety besides being economical, effective and their easily available^{10,11}. Traditionally, several ancient medicinal systems, including Ayurveda, Greek, Roman, Sidha, and Unani medicinal systems have mentioned various therapeutic properties of Ocimum sanctum (Tulsi). *Ocimum sanctum* is grown in houses, gardens and temples throughout India and has been reported to possess anti ulcer, anti-rheumatic, anti-stress and anti-bacterial properties¹²

MATERIALS AND METHODS:

Ocimum sanctum leaves extract (aqueous) was obtained from S. J. Herbals and Health Care. Bengaluru, India. The extract was stored in a cool and dry place. Accurately weighed quantity of extract was suspended in distilled water and administered orally to rats using feeding tube.

Drug: Ranitidine was obtained from Saraca Laboratories Limited. This was used as standard drug. It was suspended in distilled water and administered orally through feeding tube.

Animals: Albino rats of either sex weighing between 150-200gms were used for the study. They were randomly, allocated to different groups and placed individually in cages. Care was taken to avoid coprophagy, by keeping the animals in cages with grating floor. If coprophagy was detected such animals were excluded from the study. Animals were kept at room temperature and 12: 12 hours, light darkness cycle was maintained. The study was started after taking prior permission from Institutional Animal Ethics Committee (IAEC).

Methods of inducing Stress Ulcers in rats:

Forced swimming method -Animals were fasted for 24 hrs prior to the experiment. Stress ulcers were induced by forced swimming of rats in glass cylinder height-45 cm, diameter- 25 cm containing water to the height of 35 cm for 3 hours. The animals sacrificed and studied for gastric lesion¹³.

Grouping of animals - The animals were divided into five groups of six animals each. Group I served as control (placebo) in which distilled water (10 ml/kg). Group II, III and IV received the test drug (ocimum sanctum leaf extract) in doses of 50 mg/kg, 100 mg/kg and 200 mg/kg respectively. Group V was administered ranitidine (10 mg/ kg) served as standard for comparison. All the drugs are given orally once daily for 7 days.

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On the seventh day after giving the drug rats was kept 24 hours fasting then the rats were subjected to stress and sacrificed by stunning method. The stomachs were opened along the greater curvature and the stomach were isolated and washed in normal saline. Those stomachs were fixed on wooden board with the help of pins. The stomachs were observed with the help of magnifying lens and its external and internal surface were studied by individuals who were blinded for test drugs and control animals. The stomach were stored and fixed in 5% formalin then embedded in paraffin, solid sections were cut at 5 μ m and stained with hematoxylin and eosin. The sections were taken.

The percentage of ulcer inhibition calculated by formula as described by Njar et al. (1995) -

Percentage of ulcer inhibition =

Mean Ulcer index (Control) - Mean Ulcer index (Treated)

----- X 100

Mean Ulcer index of control

Ulcer indexing¹⁴: Evaluation of ulcers -

0 - No pathology

1 - A small ulcer	(1-2mm)
2- Medium ulcer	(3-4 mm)
4 – Large ulcer	(5-6 mm)
8 – Large ulcer	(> 6 mm)

Total severity of scores

Ulcer index = -----

Number of animals.

Statistical analysis

Data is expressed as mean \pm SEM. Data was by one-way ANOVA followed by LSD and Scheffe's multiple comparisons test. The significance of difference was accepted at p <0.01.

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RESULTS:

Table 1: Effect of Ocimum sanctum leaf extract (OSLE) in Forced swimming Stressinduced Gastric Ulcers in rats (N = 30)

Group (n=6)	Drug	Dose (mg/kg.)	Route	Ulcer index (Mean ± SD)	% of Ulcer protection
Ι	Distilled water	10 ml/kg	Oral	4.67 ± 0.82	-
II	O.S.L.E	50	Oral	$3.5 \pm 1.05^{\text{ns}}$	25.16
III	O.S L.E	100	Oral	1.67 ± 0.82**	64.23
IV	O.S L.E	200	Oral	1.83 ± 0.98**	60.81
V	Ranitidine	10	Oral	$0.83 \pm 0.75^{**}$	82.22

* P < 0.05; ** P < 0.001; ns P > 0.05



Bar diagram Comparing Ulcer Index in Forced swimming method (n= 6)

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Source	SS	Df	MS	F	Р	Sig
Between	36.83	4	9.21	10.29	P < 0.001	***
Within	17.00	19	0.89			
Total	53.83333	23				

Table no: 6 One way ANOVA for forced swimming induced ulcer

Observation of rat stomach:

Control

Ranitidine

Ocimum sanctum 50mg/kg



Ocimum sanctum 100mg/kg Ocimum sanctum 200mg/kg



Gross appearance of gastric mucosa in rats:

In the present study, the control group presented with features of ulceration. On gross examination serosal surface of stomach showed marked induration, dilated blood vessels, ecchymosis and haemorrhagic sites. Mucosal surface presented with features of severe

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degree of hyperemia, congestion and large number of pin point ulcers of varying sizes with central clots, features of perforation in the stomach. The ulcer index was high. Microscopic features were suggestive of acute gastric ulceration with de-epithelialization.

Animal pre treated with ocimum sanctum at the dose 100 & 200 mg/kg showed few signs of mucosal injury. Serosal surface revealed very few dilated blood vessels and peticheal haemorrhages. Mucosal surface revealed few ulcers of varying sizes. The percentage of damage was less compared to control group correspondingly the ulcer index also was reduced. These features were suggestive of anti ulcer activity of Ocimum sanctum.

Animals treated with ranitidine maintained near normal pattern. Serosal surface looked amber coloured with few signs of dilated blood vessels and haemorrhagic suffusions. Mucosal surface retained the normal rugae pattern with minimal signs of mucosal injury. The ulcer index was markedly reduced.

DISCUSSION:

Peptic ulcer and gastritis have been associated with multipathogenic factors and Stress is one of the etiological factors¹⁵. The relationship between psyche, stress and ulcers was first demonstrated by Pavlov¹⁶. Stress ulcers of stomach are associated with trauma, head injury, shock and sepsis. It is as a result of interaction between mucosal, vascular and neuro-humoral factors. The autonomic nervous system, central nervous system and more importantly brain gut axis are implicated in stress ulcers. Stress induced ulcers are produced by subjecting the animal to various form of stresses, either in combination or singly, restraining the animals in small cage, 3rd degree burns, shocks, cold environment and fasting have been most popular¹⁷.

In present study, we have studied anti-ulcerogenic activity of Ocimum sanctum in forced swimming method as these are well accepted models and these models provide both emotional stress as well as physiological stress to the animal¹⁸. The cause of stress induced ulcer is not known, but several factors have been incriminated. Among the most likely increased components are acid concentrations and reduced mucosal blood flow, reduced mucus secretion, reduced gastric epithelial cell turnover, activation of the hypothalamic pituitary - adrenal axis¹⁹ and generation of free radicals etc. Moreover, stress-induced ulcers can be prevented partially or entirely by vagotomy. Therefore vagal over-activity has been suggested to be the principal factor in stress-induced ulceration^{20, 21}

The present available antiulcer drugs are associated with side effects hence focus has been shifted towards natural products as new sources of antiulcer agents. Ocimum sanctum has been one of the most noteworthy plant mentioned in various medicinal systems. In addition to other therapeutic properties^{14,22} Ocimum sanctum is also reported to possess anti-ulcerogenic activity. It has histamine antagonistic, anticholinergic and antisecretory effects. The stem and leaves of Ocimum sanctum

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contain a variety of phytochemical constituents like saponins, flavonoids, triterpenoids, and tannins²³ that may have biological activity²⁴. In addition, the following phenolic actives - Rosmarinic acid, apigenin, cirsimaritin, isothymusin and isothymonin have been identified, which also exhibit antioxidant and antiinflammatory activities. Also Ocimum sanctum has been reported to possess adaptogenic activity, its anti-ulcerogenic effect may be due to its effect on neural pathways controlling acid secretion thereby strengthening the animal's physiological capabilities to decrease stress and hence ulcers³.

Analysis of data revealed the percentage of ulcer protection in forced swimming in both the test Ocimum sanctum 100mg/kg &200mg/kg and standard drug ranitidine were almost comparable and there is significant ulcer inhibition in a dose dependant manner when compared to control. Therefore, the results were suggestive of anti ulcerogenic activity of Ocimum sanctum at the dose100 -200 mg/kg.

However, further study is required to know the exact mechanism of action and to isolate the active molecule responsible for the anti-ulcer activity.

CONCLUSION:

Ocimum sanctum (Tulsi) at a dose of 100 - 200 mg/kg showed significant (p < 0.05) antiulcer effect in Forced swimming method and its effect is comparable to the standard drug ranitidine, as evidenced by the reduction in the ulcer scores. However more experimentation and clinical studies and detailed analysis are required for a definitive conclusion.

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