



International Journal of Medical Research & Health Sciences

www.ijmrhs.com

Volume 4 Issue 2

Coden: IJMRHS

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ISSN: 2319-5886

Received: 26th Feb 2015Revised: 20th Mar 2014Accepted: 26th Mar 2015

Research article

EFFECT OF *OCIMUM SANCTUM* LINN. IN STRESS INDUCED GASTRIC ULCERS IN RATS

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ABSTRACT

Aims & Objectives: *Ocimum sanctum* L. popularly known as Tulsi is a medicinal plant that has been used for curing various diseases since ages. In the present study we used leaf extract of *Ocimum sanctum* for its anti-ulcer property by inducing stress ulcers on rats. **Materials and Method:** Albino rats were randomly allocated to different experimental groups and aqueous leaf extract of *Ocimum sanctum* was given for 7 days. Stress ulcers were induced by restraint and ethanol administered methods and results were compared with standard drug ranitidine. After that animal was sacrificed and stomach was dissected out and stomachs were observed for the ulceration with the help of magnifying lens and studied its external, internal surface and ulcer index was evaluated according to the severity of ulcers. The stomach were stored and fixed in 5% formalin and studied for histopathological changes. **Results:** The ulcer index was high in control group. Animal pretreated with *Ocimum sanctum* at the dose 100 & 200 mg/kg showed few signs of mucosal injury and the percentage of damage were less compared to control group. **Conclusion:** In the present study pretreatment with *Ocimum sanctum* at dose 100-200 mg/kg caused a significant anti-ulcer effect 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, peptic ulcer.

INTRODUCTION

Stress has become a very common problem in every household and it leads to many diseases. One among them is peptic ulcer^[1, 2] Psychological stress not only causes peptic ulcer but also can exacerbate it^[3]. Peptic ulcer impairs the quality of life and is associated with increased morbidity and mortality hence its treatment is essential. Although there are many drugs available for the treatment of peptic ulcer they are associated with side effects hence there is always a need for a better drug. Plants are one of the most important sources of medicines and many drugs are derived from it. *Ocimum sanctum* commonly called as Tulsi grown easily in household is a medicinal plant used since ages for various properties^[4]. It has been used as antiasthmatic, antifungal, antiulcerogenic^[5], antipretic, antiviral, antibacterial, insecticidal and

antimalarial. It possess antioxidant, Anti-inflammatory, Immunostimulant and antistress^[6, 7] properties^[8]. It is considered to be an adaptogen, balancing different processes in the body and helpful for adapting to stress^[9]. In present study we have used leaves of Krishna tulsi for its antiulcer activity against restraint induced and ethanol treated peptic ulcer.

MATERIALS AND METHODS:

Study design: An experimental animal based study

Ethical Approval: The study was approved by the animal ethics of our institute and the procedures are followed the format given in form-B of Committee for the Purpose of Control and

Supervision of Experimentation on Animals (CPCSEA).

Animals: Albino rats of either sex weighing 180-200 g were procured and the animals were kept in wire bottomed cages. They were randomly allocated to different experimental groups and placed individually in cages and cages were kept under standard condition of illumination with a 12 - h light-dark cycle at $25 \pm 1^\circ \text{C}$, 45-70% relative humidity. They were provided with tap water *ad libitum* and balanced diet.

Ocimum sanctum leaves extract (aqueous) obtained from S. J. Herbals and Health Care, Bengaluru, Karnataka, India. The extract was stored in cool and dry place.

Extract of *Ocimum sanctum* leaves suspended in distilled water and administered orally (intragastric) Once a day (OD) for 7 days (Pretreated) to rats by using feeding tube. (Group II to IV, VII to IX)

Ranitidine: Obtained from Saraca Laboratories Limited and used as standard drug. It was suspended in distilled water and administered orally (intragastric) Once a day (OD) for 7 days (Pretreated) to rats by using feeding tube. (Group V, X)

Experimental design: To produce the ulcer we have selected two types of stress models.

1. Restraint stress (Group I-V)
2. Ethanol induced Stress (Group VI-X)

Each stress model subdivided into 5 groups (n=6 in each group) as follows (Table 1)

Table 1: Grouping and dose of drugs

Model of stress	Drug and doses
Restraint stress	
Group I	Control(received D/W)
Group II	OSLE 50 mg/kg
Group III	OSLE 100 mg/kg
Group IV	OSLE 200 mg/kg
Group V	Ranitidine 10 mg/kg
Ethanol induced Stress	
Group VI	Control (received D/W)
Group VII	OSLE 50
Group VIII	OSLE 100
Group IX	OSLE 200
Group X	Ranitidine 10

O.S.L.E = *Ocimum sanctum* leaf extract, D/W: Distilled water

After 7 days pretreatment in both the stress model the animals were kept fasting for 24 hours. After this the animals were subjected to stress

Restraint stress ^[10]: Both upper and lower extremities along with tail are tied together. The rats are placed in galvanized steel window screen; the screen is moulded around the animal and kept for 24 hours. Then animals are sacrificed by cervical dislocation.

Ethanol induced oxidative stress ^[11, 12]: Absolute ethanol was administered by orogastric tube at a dose of 5 ml/kg. After 1 hour of ethanol administration the animals are sacrificed.

The abdominal cavity was opened through a midline abdominal incision to expose stomach. The stomach dissected out, opened along the greater curvature and washed in normal saline. Those stomachs were fixed on wooden board with the help of pins. The stomachs were observed for the ulceration with the help of magnifying lens and studied its external, internal surface and ulcer index was evaluated according to the severity of ulcers. The stomach were stored and fixed in 5% formalin and studied for histopathological changes.

The percentage of ulcer inhibition calculated by formula as described by Njar et al. (1995) ^[13]

$$\% \text{ of Ulcer inhibition} = \frac{\text{Mean UI (control)} - \text{Mean UI (treated)}}{\text{Mean ulcer index of control}} \times 100$$

[UI= Ulcer index]

Evaluation of ulcers score ^[14]: 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)

$$\text{Ulcer index (UI)} = \frac{\text{Total severity of score}}{\text{Number of animals}}$$

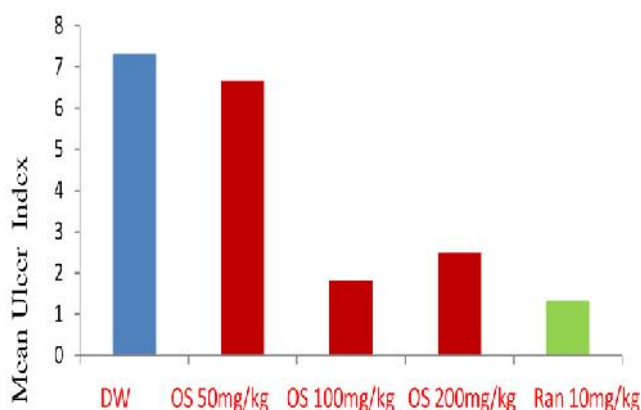
Statistical analysis: Data is expressed as mean \pm SEM. Data was by one-way ANOVA followed by LSD and Scheffe's multiple comparisons test. The significance of difference was accepted at $P < 0.01$.

RESULTS

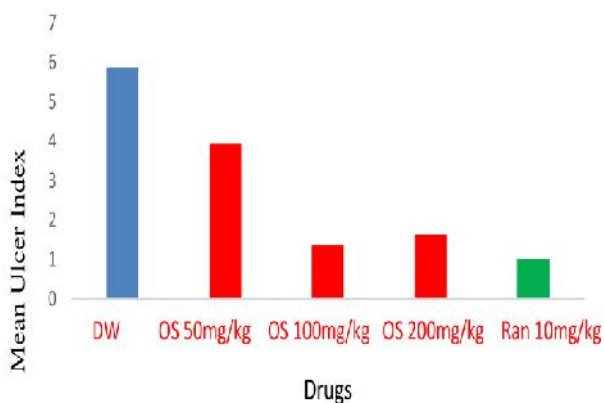
Table 2: Effect of Ocimum sanctum leaf extract (OSLE) against Restraint and ethanol induced Gastric Ulcers in rats (N = 30)

Treatment	Dose (mg/kg)	Restraint		Ethanol treated	
		Ulcer index	% of Ulcer Protection	Ulcer index	% of Ulcer Protection
Distilled water	1ml/kg	7.33 ± 2.07	-	5.88 ± 1.04	-
O.S.LE	50	6.67 ± 1.63	09.0	3.94 ± 1.42	20.95
O.S.LE	100	1.83 ± 0.75**	75.03	1.38 ± 0.88**	84.65
O.S.LE	200	2.50 ± 0.98**	65.89	1.63 ± 0.21**	79.86
Ranitidine	10	1.33 ± 0.84**	81.85	1.01 ± 0.55**	89.23

Data presented as Mean ± SD, * P < 0.05; ** P < 0.001; ^{ns} P > 0.05



DW - Distil water, OS – Ocimum sanctum, Ran – Ranitidine
Fig 1 : Bar diagram Comparing Ulcer Index in Restraint



method
Fig 2: Bar diagram Comparing Ulcer Index in ethanol induced method

Gross appearance of gastric mucosa in rats:

On gross examination the gastric mucosa in restraint and ethanol administered group showed dilated blood vessels, haemorrhagic sites and large number of pin point ulcers of varying sizes with central clots, features of perforation in the stomach. The ulcer index was high in control group. Animal pretreated

with Ocimum sanctum at the dose 100 & 200 mg/kg showed few signs of mucosal injury and the percentage of damage were 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. The ulcer index was markedly reduced.

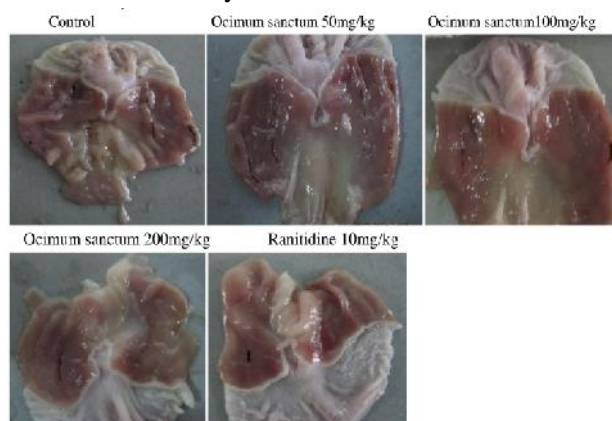


Fig 3: Gross appearance of gastric mucosa in rats

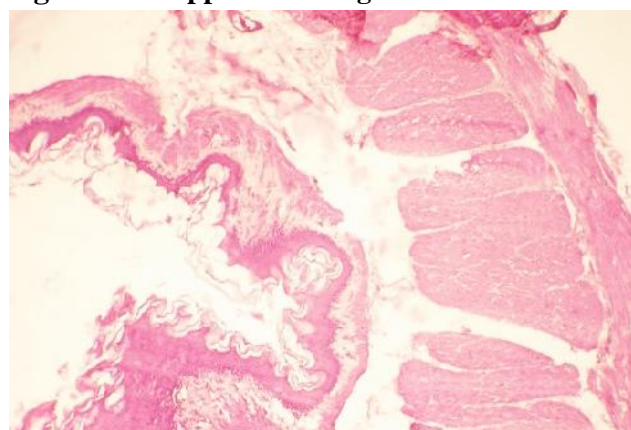


Fig 4: Microscopic observation of normal rat stomach in 10x

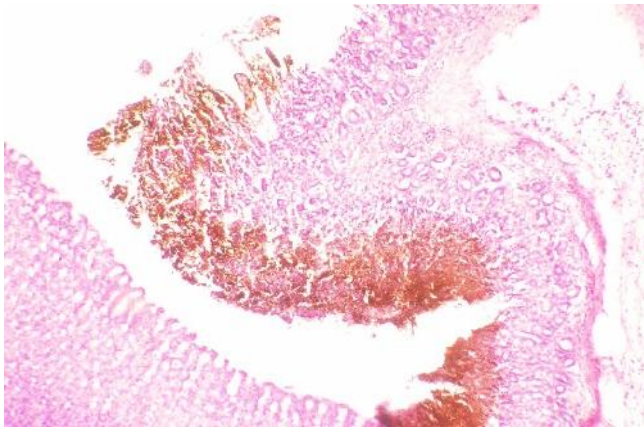


Fig 5: Microscopic observation of restraint induced ulcer rat stomach pre-treated with Ocimum sanctum 100mg/kg [10Xs]

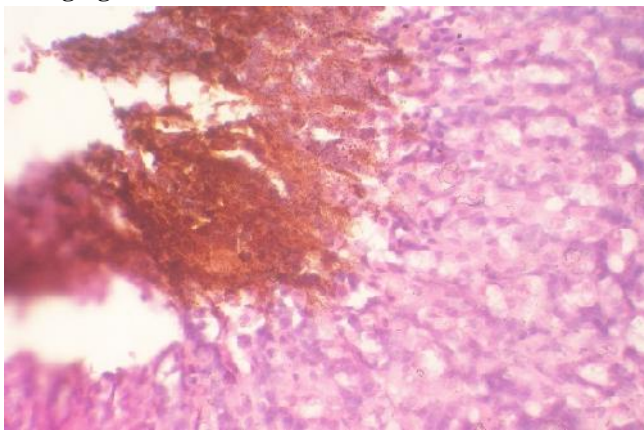


Fig 6: Microscopic observation of restraint induced ulcer rat stomach pre-treated with Ocimum sanctum 100mg/kg [100Xs]

DISCUSSION

Psychological stress has been reported to be an important contributing factor in the causation of peptic ulcer. Mechanism by which stress causes ulceration is by histamine release with increase in acid secretion and reduction in mucous production. Stress causes both sympathetic (causes direct arteriolar vasoconstriction) and parasympathetic (induces an increased motility and muscular contraction) stimulation of stomach leading to local hypoxia and near or actual ischemia". Formation of excessive free radicals due to stressful conditions is a major internal threat to cellular homeostasis of aerobic organisms^[15]. These free radicals are extremely reactive and unstable and react with most of the intracellular molecules^[16]. They enhance the process of lipid peroxidation^[17]. The products of lipid peroxidation are themselves reactive species and lead to extensive membrane organelles and cellular damage^[18]. Lipid peroxidation causes loss of

membrane fluidity, impaired ion transport and membrane integrity and finally loss of cellular functions. Many studies have implicated oxygen free radicals and lipid peroxidation, in ageing and various diseases^[19, 20] including peptic ulcer^[21]. The present study is undertaken to produce gastric ulcers by restraint and ethanol induced models of stress in albino rats.

Most of the available drugs effective in treating peptic ulcer were found to cause some potential side effects. Efforts were made to find a suitable agent in natural products of plant origin. *Ocimum sanctum* has been used since ages and has been reported to possess potent anti-ulcer and ulcer healing properties. *Ocimum sanctum* contains a variety of chemical constituents that have biological activity, including saponins, flavonoids, triterpenoids, and tannins^[22, 23] which exhibit antioxidant and anti-inflammatory and adaptogenic activities. *Ocimum sanctum* has effect on neural pathways controlling acid secretion thereby strengthening the animal's physiological capabilities to decrease stress and hence ulcers.

In the present study, we examined the effects of *Ocimum sanctum* in restraint stress and ethanol induced stress. Our data suggest that *Ocimum sanctum* treatment has significant percentage of ulcer protection in a dose dependent manner. Antiulcerogenic activity of *Ocimum sanctum* was seen at the dose 100mg/kg & 200mg/kg and was almost comparable to the standard drug ranitidine.

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

Pretreatment of rats with *Ocimum sanctum* produced a dose dependent protection in all models of stress induced ulcer. Antiulcer effect of *Ocimum sanctum* is statistically insignificant in comparison to ranitidine. However, the protection index varies between different models. *Ocimum sanctum* (Tulsi) 100, 200 mg/kg showed significant ($p < 0.05$) anti-ulcer effect 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.

Conflict of interest: Nil

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