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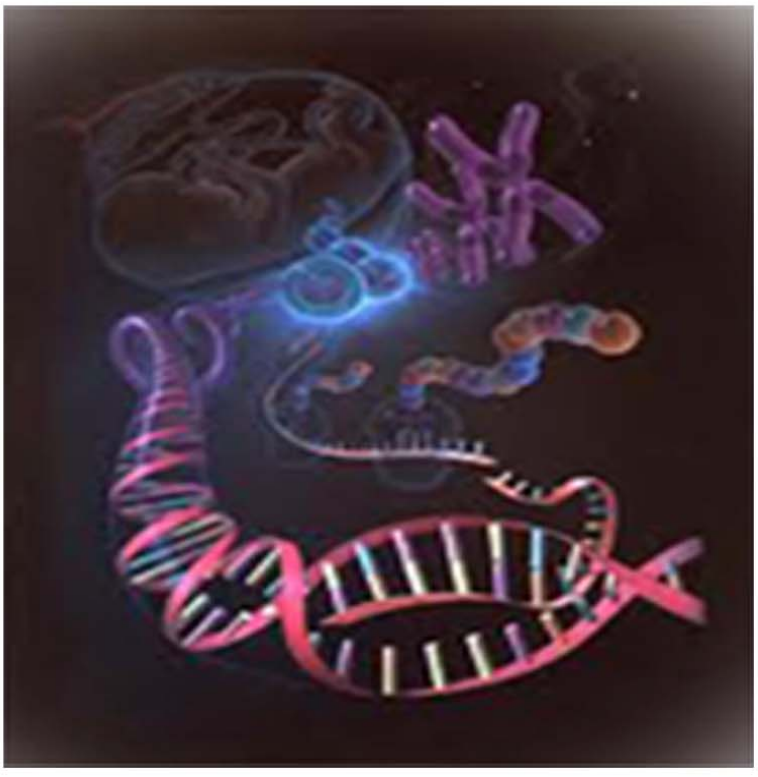

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Research Paper

EVALUATION OF THE ANTI-ULCER ACTIVITY OF *OCIMUM SANCTUM* LINN (TULSI) IN INDOMETHACIN-INDUCED GASTRIC ULCERS IN ALBINO RATS

Mrutyunjay M Mirje[1] and Sameer Uz Zaman[2]

Corresponding Author: Mrutyunjay M Mirje** ✉ mirjemrutyunjay@gmail.com*

Background and Objectives: Peptic Ulcer Disease (PUD) encompassing gastric and duodenal ulcer is the most prevalent gastrointestinal disorder. Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) such as indomethacin are used in the treatment of inflammation, fever and pain. However, NSAIDs cause gastric damage as a major adverse reaction. In this study, the gastro protective effect of *O. sanctum* was also studied and compared with omeprazole in model of indomethacin-induced ulceration. Materials and Methods: This study was conducted at Navodaya Medical College and Research Centre for a period of two years. The gastro protective effect of aqueous extract of *O. sanctum* was studied using model of indomethacin-induced gastric damage and compared with omeprazole. *O. sanctum* (200 mg/kg or 400 mg/kg) or omeprazole (10 mg/kg) were administered alone in separate group of rats. Results: *O. sanctum* showed significant ($p < 0.05$) protective effect against indomethacin-induced gastric ulcer when compared to control. The gastro protective effect of *O. sanctum* was comparable with that of omeprazole, the standard drug. Conclusion**:** *O. sanctum* showed significant gastro protective effect against indomethacin-induced gastric damage possibly due to its 5-lipoxygenase inhibitory effect, mucoprotective activity and its antisecretory effect.

Keywords: *Ocimum sanctum* Linn (Tulsi), Indomethacin; Gastric damage, Omeprazole, Short Running Title, Anti-ulcer activity of *Ocimum sanctum*

INTRODUCTION

Peptic Ulcer Disease (PUD) encompassing gastric and duodenal ulcer is the most prevalent gastrointestinal disorder. The pathophysiology of PUD involves an imbalance between offensive (acid, pepsin, *Helicobacter pylori*) and defensive factors (mucin, prostaglandin, bicarbonate, nitric oxide, growth factor). An estimated 15,000 deaths occur each year as a consequence of PUD. In India PUD is common (Dharmani and Palit, 2006).

Commercial nonsteroidal anti-inflammatory drugs cause gastric erosion as an important

[1] Department of Pharmacology, Institute of Medical Science and Research, Mayni, Tal- Katva, Dist- Satara. 415102.

[2] Kamineni Academy of Medical Science and Research Centre, L B Nagar, Hyderabad 500068.

adverse effect by inhibiting prostaglandin biosynthesis and suppression of the formation of prostaglandin. Indomethacin, which is NSAID, has been widely used to reduce inflammation, pain and fever in humans. However it causes injury, as other NSAIDs do, on the gastric mucosa, mainly due to inhibition of the COX (cyclooxygenase) enzymes and suppression of prostaglandin synthesis (Odabasoglu *et al.,* 2008).

Recently, there has been a rapid progress in the understanding of the pathogenesis of peptic ulcer. Most of the studies focus on newer and better drug therapy. These have been made possible largely by the availability of the proton pump inhibitors, histamine receptor blockers, drugs affecting the mucosal barrier and prostaglandin analogues. However the clinical evaluation of these drugs showed development of tolerance and incidence of relapses and side effects that make their efficacy arguable. This has been the rationale for the development of new antiulcer drugs, which include herbal drugs (Dharmani and Palit, 2006).

Indian medicinal plants and their derivatives have been an invaluable source of therapeutic agents to treat various disorders. Tulsi is known as the "Queen of plants" and "The mother medicine of nature". Tulsi, i.e., *Ocimum sanctum* is a plant with enormous properties for curing and preventing diseases. It is regarded as a deity in Indian subcontinent (Dharmani and Palit , 2006). It is omni present in all Indian fields. It is an erect, sweet scented herb. The name "Tulsi" in Sanskrit means "the incomparable one" (Odabasoglu *et al*., 2008). Whole plant is used as a source of remedy. The genus *Ocimum* is a group of about 150 species of aromatic plants distributed mainly in tropical and subtropical regions of the world. Charaka describes holy basil [*O. sanctum*] as a curative in many disorders (Jyoti *et al*., 2004). It may be because of their manifold use to cure a variety of human sufferings that the early Indians considered *Ocimum* plants as highly sacred and hence worshiped them and offered them to Gods. Some ancient Hindi poets described *Ocimum* plants as highly sacred saying that one who carefully grows and worships them daily is protected from misfortune, sanctified and entitled to go to Heaven. The story goes further by saying that this plant is the transformed nymph, Tulsi, a beloved of Lord Krishna of Indian mythology and for this reason it is also known as Krishna tulsi (Umadevi P Ganasoundari, 1999).

A perusal of chemical, pharmacological and clinical investigations have shown that *O. sanctum* and others in the species contains various unidentified active substances belonging to the alkaloid, glycoside, and tannin or saponin derivatives. More intensified research activities in this group of species may show many interesting results from a medicinal point of view (Pushpangadan and Sobti, 1977).

MATERIALS AND METHODS

In the present study the aqueous extract of *O. sanctum* leaves was screened primarily for its gastro protective effect. The results obtained were compared with control and also with known antiulcer agent omeprazole. The materials used and the methods adopted during the present investigation are being described briefly.

The whole fresh leaves of *O. sanctum* were collected, air dried under shade for two days and then powdered. 100 g of powder was taken and kept for maceration for two days in 1000 ml of distilled water, frequently stirred and about 5 to 10 drops of chloroform per day was added. Then

it was filtered using a muslin cloth, so as to remove insoluble material. The filtrate was again filtered by double layered muslin cloth and then poured into ordinary, cleaned and already weighed plates for drying. Finally, the chocolate-colored semisolid residue was weighed and pooled together in an air and water proof container kept in a refrigerator at 4°C. From this fresh preparations were made whenever required.

Ethical clearance from the Institutional Animal Ethical Committee was obtained. All the drugs were administered orally with the help of a sterile, nontoxic tube made up of polyvinyl chloride.

A total of 24 albino rats of either sex weighing 150-250 g were selected. They were divided into 4 groups, each group containing 6 rats. They were starved for 24 h but given access to water *ad libitum* prior to drug administration.

a) Group I – distilled water – 2 ml/kg body weight.

b) Group II - omeprazole – 10 mg/kg body weight.

c) Group III - test rats – receives *O. sanctum* 200 mg/kg body weight.

d) Group IV - test rats – receives *O. sanctum* 400 mg/kg body weight.

Indomethacin (NSAIDs)-Induced Gastric Damage in Rats

30 min after the drug administration all the animals of all groups were treated with indomethacin in a dose of 25 mg/kg body weight to induce gastric damage. The animals were sacrificed after 6 h using ether anaesthesia. Stomach were removed and placed on saline soaked filter paper until inspection. A longitudinal incision along the greater curvature was made with fine scissors. The stomach was inverted over the index finger and the presence or absence of gastric irritation was determined by the use of a magnifier. The presence of single or multiple lesions were noted. Erosions, ulcers, perforations and hyperemia were considered to be positive indicators of gastric damage. The number and depth of the ulcers and the occurrence of hyperemia and prominence of stomach rugae were noted.

Evaluation (Medhi and Prakash, 2010)

The number of animals with one or more lesions in the stomach and the percentage of the animals showing gastric ulceration were calculated.

The ulcer index was determined using the following method:

a) First the ulcers were graded; and

b) Ulcer index was determined.

Grades of ulcer severity

0 = No ulcer.

1 = Superficial ulcer.

2 = Deep ulcer.

3 = Perforation.

Calculation of ulcer index

Ulcer index [UI] = UN + US + UP X 10^{-1}

where,

UN – Average number of ulcers per animal;

US – Average severity scores;

UP – Percentage of animals with ulcers.

Ulcer index was compared between the treatment and control groups.

STATISTICAL ANALYSIS

Data were subjected to one-way analysis of variance (ANOVA) using SPSS 11.0 software.

The results of gastro protective effect were expressed as "mean total severity score ± SD". Analysis of variance (one way ANOVA) was followed by Dunnett's t-test for control, standard and test group comparisons were used for statistical evaluation. P values <0.05 were considered as significant and P<0.001 as highly significant.

RESULTS

The results obtained were compared with control and with the standard drug omeprazole. The gastro protective effect was examined by noting the number of ulcers, calculating the mean of severity score, the ulcer index and the percentage inhibition of gastric ulcer formation in the model of indomethacin-induced gastric ulceration in rats. The results obtained are shown in Table 1 and

Table 1: Gastro Protective Effect of *O. sanctum* and Omeprazole in Indomethacin-Induced Gastric Damage in Rats

Treatment Group	Total Severity Score (Mean ± SD)	Percentage Inhibition	Ulcer Index
Group I (D/W 2 ml/kg)	22.67 ± 3.38	-	53
Group II (omeprazole 10 mg/kg)	3.66 ± 2.42**	66.15	18
Group III (OS 200 mg/kg)	8.33 ± 2.58**	49.22	27
Group IV (OS 400 mg/kg)	7 ± 2.82**	58.63	22
ANOVA			
F-value	4.01		
P-value	< 0.001		

Note: Each value represents the mean ± SD (N = 6) df = (3,20). Statistical analysis by One-way ANOVA followed by Dunnett's multiple comparisons. P value; **< 0.001 is highly significant. Abbreviations: bw = body weight; D/W, distilled water; IND, indomethacin; OS, *Ocimum sanctum*.

Figure 1: Effects of the *O. sanctum* and Omeprazole on Total Severity Score in Model of Indomethacin Induced Gastric Damage in Rats

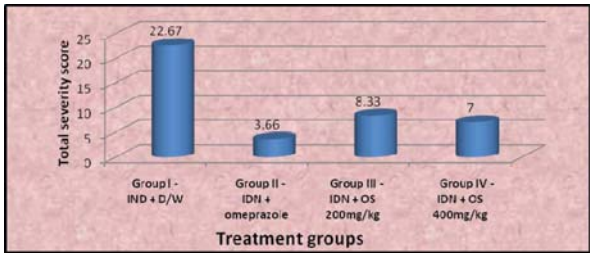


Figure 2: Percentage of Inhibition Produced by *O. sanctum* and Omeprazole in Indomethacin-Induced Gastric Damage in Rats

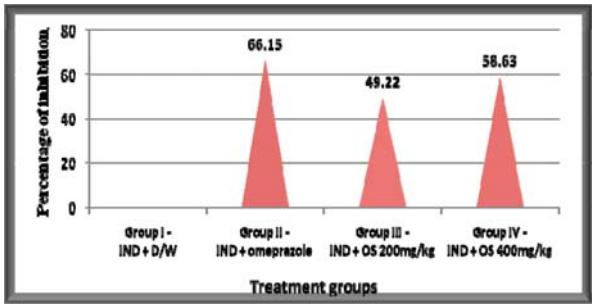
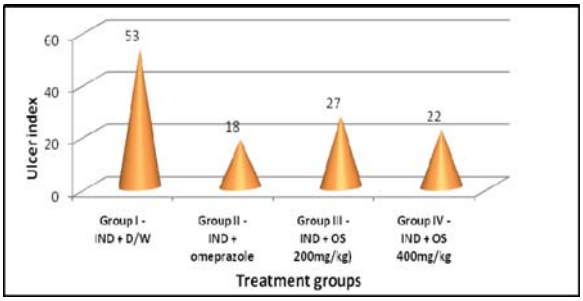


Figure 3: Effects of the *O. sanctum* and Omeprazole on Ulcer Index in Models of Indomethacin-Induced Gastric Damage in Rats



Figures 1 to 3. The administration of indomethacin caused gastric damage with a mean total severity score of 22.67 ± 3.38. The administrations of omeprazole 10 mg/kg, *O.*

sanctum 200 mg/kg, and *O. sanctum* 400 mg/kg, along with indomethacin limited the mean total severity score to 3.66 ± 2.42, 8.33 ± 2.58 and 7 ± 2.82, respectively. The percentage inhibition of gastric ulcers was 49.22%, 58.63% and 66.15% by *O. sanctum* 200 mg/kg and *O. sanctum* 400 mg/kg and omeprazole respectively. Calculation of ulcer indices returned values of 53 for indomethacin, and 27, 22 and 18 for the groups in which indomethacin was administered with *O. sanctum* 200 mg/kg, and *O. sanctum* 400 mg/kg and omeprazole respectively. These results show that *O. sanctum* 200 mg/kg, and *O. sanctum* 400 mg/kg significantly inhibited the gastric damage induced by indomethacin and their efficacy as a gastro protective agent was comparable to that of the proton pump inhibitor omeprazole.

DISCUSSION

This study is to examine the gastro protective effect of aqueous extract of *O. sanctum* on indomethacin-induced gastric damage in rats. Results of the present study clearly indicate that the aqueous extract of *O. sanctum* showed significant gastro protective effect when compared with control. The indomethacin-induced gastric damage was indicated by the total severity score and ulcer index in gastric tissue, while administration of *O. sanctum* or omeprazole along with indomethacin limited the gastric damage. The mean number of ulcer was reduced when indomethacin was administered with *O. sanctum* or omeprazole. The percentage inhibition of gastric ulcers by *O. sanctum* was comparable to omeprazole. The results thus indicate that *O. sanctum* has the potential to prevent the gastric damage resulting from indomethacin administration. A similar protective effect of the *O. sanctum* in the NSAIDs induced gastric damage has been reported elsewhere (Dharmani and Palit, 2006).

NSAIDs like indomethacin are known to induce gastric ulceration; the reason being attributed principally to inhibition of "cytoprotective prostaglandins" e.g., PGE's and PGI_2 (by inhibition of cyclooxygenase pathway of arachidonic acid metabolism) resulting in overproduction of leukotrienes and other products of 5-lipoxygenase pathway. Hence, the protective action of *O. sanctum* against indomethacin-induced gastric lesions could possibly be due to its 5-lipoxygenase inhibitory effect (Rainsford, 1987). The ulcer healing property of *O. sanctum* seems to be based on its mucoprotective activity and its antisecretory effect. The ulcer base may have healed quickly because the basic fibroblast growth factor was protected from acid, which is considered to be chiefly responsible for epithelial regeneration (Dharmani and Palit, 2006).

Eugenol (1-hydroxy-2-methoxy-4-allylbenzene) a naturally occurring phenolic compound is a major component of basil oil and exists to a lesser extent in oils of several other plants (Nagababu *et al.,* 1995). It possesses antiulcer, antiseptic, analgesic, antibacterial, anti-inflammatory and antianaphylactic properties (Prakash and Gupta, 2005; and Thakur and Pitre, 2009). The therapeutic potential effect of the fresh leaves of *Ocimum sanctum* L. has been found to be largely due to eugenol. The therapeutic use of *O. sanctum L.* in treatment of gastric ulcer has been attributed to antiulcerogenic action of eugenol and essential oil extracted from *O. sanctum* leaves (Prakash and Gupta, 2005).

The principle constituent of *O. sanctum* is eugenol (71.3%). So this study suggest that *O.*

sanctum protects against gastric damage, i.e., it has gastro protective effect.

CONCLUSION

From the above study it was concluded that *O. sanctum* possesses significant gastro protective effect against indomethacin-induced gastric damage and its effect was comparable to that of the standard drug omeprazole.

Although the exact chemical compounds responsible for the gastro protective effect of *O. sanctum* extract still remain speculative, experimental evidence obtained in the present study indicates that *O. sanctum* extract possesses gastro protective property. More detailed studies on *O. sanctum* using different doses and covering longer periods of observation are needed before reaching a clear cut conclusion. Future research to refine the extraction procedure of *O. sanctum* could lead to improved pharmaceutical products.

REFERENCES

1. Dharmani P and Palit G (2006), "Exploring Indian medicinal plants for antiulcer activity", *Indian J Pharmacol*, Vol. 38, pp. 95-99.
2. Jyoti S, Sushma S, Shashi S and Anjana T (2004), "Evaluation of hypoglycaemic and antioxidant effect of *Ocimum sanctum*", *Indian J Clin Biochem*, Vol. 19, No. 2, pp. 152-155.
3. Medhi B and Prakash A (2010), *Practical manual of experimental and clinical Pharmacology*, 1st Ed., New Delhi: Jaypee Brothers medical publishers(P) Ltd, pp. 217-221.
4. Nagababu E, Sikeran B and Lakshmaiah N (1995), *Free Radic Ros,*Vol. 23, pp. 617.
5. Odabasoglu F, Halici Z, Cakir A, Halici M, Aygun H, Suleyman H *et al.* (2008), "Beneficial effects of vegetable oils (corn, olive and sunflower oils) and α-tocopherol on anti- inflammatory and gastro intestinal profiles of indomethacin in rats", *Eur J Pharm*., Vol. 591, pp. 300-306.
6. Prakash P and Gupta N (2005), "Therapeutic uses of *ocimum sanctum linn* (tulsi) with a note on eugenol and its pharmacological actions: a short review", *Indian J Physiol Pharmacol.,* Vol. 49, No. 2, pp. 125-131.
7. Pushpangadan P andSobti S N (1977), "Medicinal properties of *Ocimum* species and some recent investigations of their efficacy", *Indian Drugs*, Vol. 14, No. 1, pp. 207-208.
8. Rainsford K D (1987), "The effects of 5-lipoxygenase inhibitors and leukotriene antagonists on the development of gastric lesions induced by non-steroidal anti-inflammatory drugs in mice", *Agents Actions*, August, Vol. 21, Nos. 3-4, pp. 316-319.
9. Thakur K and Pitre K S (2009), "Anti-inflammatory activity of extracted eugenol from *ocimum sanctum L*. leaves", *Rasayan J Chem*., Vol. 2, No. 2, pp. 472-474.
10. Umadevi P Ganasoundari (1999), "A modulation of glutathione and antioxidant enzymes by *Ocimum sanctum* and its role in protection against radiation injury", *Indian J Exp Biol.,* March, Vol. 37, No. 3, pp. 262-268.

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