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Original Research Article

Evaluation of the anti-inflammatory activity of *Ocimum sanctum* Linn (Tulsi) in albino rats

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A B S T R A C T

Inflammation continues to be an area of great interest for research, probably due to the non availability of a safer and more effective anti-inflammatory agent. This has led to increase in demand for natural products with anti-inflammatory activity having fewer side effects. 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 anti-inflammatory activity of *O. sanctum* alone and in combination with indomethacin was studied using Carrageenan-induced rat paw edema. Aqueous extract of *O. sanctum* (200mg/kg or 400mg/kg) was administered alone and in combination with indomethacin (25mg/kg) to separate group of rats and paw volume was measured by plethysmometer and compared with control group. All the test groups showed significant ($P<0.05$) anti-inflammatory effect in Carrageenan-induced rat paw edema. The reduction of edema by *O. sanctum* was better than that of the standard anti-inflammatory drug, indomethacin and on co-administration marginally improved the anti-inflammatory profile of indomethacin. *O.sanctum* possesses significant anti-inflammatory activity probably due to inhibition of both cyclooxygenase and lipooxygenase pathways of arachidonic acid metabolism (dual inhibitory property).

K e y w o r d s

Ocimum sanctum Linn (Tulsi); Carrageenan; Inflammation; Indomethacin.

Introduction

Inflammation is a complex reaction to injurious agents such as microbes and damaged, usually necrotic cells that consist of vascular response leading to accumulation of fluids, migration and activation of leucocytes and systemic reactions. Inflammation is fundamentally a protective response, the ultimate goal of which is to rid the organism of both the initial cause of cell injury like microbes, toxins and consequence of such injury like necrotic cells and tissue. Though the process of inflammation is brought about by vascular as well as cellular events, the

former appear to contribute maximum for the pathogenesis of acute inflammation. This complex phenomenon involves endogenous chemical mediators such as histamine, 5-hydroxytryptamine, various chemotactic factors, bradykinin, leukotrienes and prostaglandins (Vinay *et al*., 2010).

Many non steroidal anti-inflammatory drugs (NSAID's) like aspirin, phenylbutazone, indomethacin etc. are in clinical use but all these are not completely devoid of adverseeffects (Laurence *et al*., 2011). Hence the search for safer and better anti-inflammatory agents continues to be an area of great interest due to the non availability of a safer and more effective anti-inflammatory agent. This has led to increase in demand for natural products with anti-inflammatory activity having fewer side effects. In the traditional system of medicine many plant based drugs and formulations are in use since ancient times. Many herbs and plant products have been claimed to have a significant anti-inflammatory action. However, not much work has been reported on the pharmacological evaluation of such plant-based products for the anti-inflammatory effects claimed in traditional medicine.

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 (Singh *et al*., 2010). It is omnipresent in all Indian fields. It is an erect, sweet scented herb. The name "Tulsi" in Sanskrit means "the incomparable one" (Bansod and Rai, 2008). Whole plant is used as a source of remedy. The genus *Ocimum* is a group of about 150species 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 (Nadkarni, 1954). 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 (Pushpangadan and Sobti, 1977). The present study is therefore under taken to evaluate the effect of aqueous extract of *O.sanctum* L. on the anti-inflammatory profile in response to indomethacin administration.

Materials and Methods

In the present study the aqueous extract of *O. sanctum* leaves was screened primarily for its anti-inflammatory activity. 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 1000ml 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-coloured semisolid residue was weighed and pooled together in an air and water proof container kept in a refrigerator at 4^0C. 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.

Albino rats of either sex weighing between 150 and 250 g were used. 36 rats were selected and divided into 6 groups of 6 each.

Group I: Control rats- receive vehicle (distilled water) only
Group II: Standard rats – receive indomethacin 25 mg/kg
Group III: Test rats – receive *O.sanctum* 200 mg/kg
Group IV: Test rats – receive *O.sanctum* 400 mg/kg
Group V: Test rats – receives indomethacin 25mg/kg + *O.sanctum* 200 mg/kg
Group VI: Test rats – receives indomethacin 25mg/kg + *O.sanctum* 400 mg/kg

Carrageenan-induced edema in rat hind paw

This method is based on the plethysmometeric (IITC 520) measurement of edema produced by subplantar injection of Carrageenan, in the right hind paw of rat. The hind paw volume obtained at 1 h, 2 h, 3 h and 4 h after Carrageenan injection both in control and test animals. By comparing the edema produced in control rats and in those treated with drugs, percentage inhibition of edema was calculated as follows.

$$\text{Percentage inhibition} = \frac{Vc - Vt}{Vc} \times 100$$

Where

Vc = Volume of paw edema in control animals.
Vt = Volume of paw edema in treated animals.

Statistical analysis

Data were subjected to one-way analysis of variance (ANOVA) using SPSS 11.0 software. The results of anti-inflammatory activity were expressed as "mean increase in paw volume ±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 and Discussion

The results obtained were compared with control and also with known anti-inflammatory agent, indomethacin.

The mean paw volumes at hourly interval in millilitre (ml) of each group are represented in Table 1 and Graph 1.

In control group there was a progressive increase in mean paw volume, where as in standard and test groups there was progressive decrease in mean paw volume from 1h to 4h.

Anti-inflammatory activity is expressed as percent inhibition. The results obtained are shown in (Table 2) and also depicted in

bar diagram (Graph 2). The mean paw volume of the control group reached its peak at about 4h after the administration of Carrageenan. Groups treated with indomethacin, *O.sanctum* 200mg/kg, *O.sanctum* 400mg/kg displayed values of 80.48%, 92.68% and 95.12% percentage inhibition of paw edema at 4h respectively. The administration of indomethacin with *O.sanctum* 200mg/kg and indomethacin with *O.sanctum* 400mg/kg decreased the paw volume by 95.12% & 97.56% respectively. The anti-inflammatory effect of the above mentioned treatment groups were significant i.e.($p<0.05$) at time intervals of [2h] and highly significant ($p< 0.001$) at time intervals of [3h and4h] but not at the 1h interval ($p>0.05$).

Inflammation is a protective attempt by the organism to remove the injurious stimuli and to initiate the healing process. Inflammation can be classified as either acute or chronic. Acute inflammation is the initial response of the body to harmful stimuli and is achieved by the increased movement of plasma and leukocytes (especially granulocytes) from the blood into the injured tissues. A cascade of biochemical events propagates and matures the inflammatory response, involving the local vascular system, the immune system, and various cells within the injured tissue. Prolonged inflammation, known as chronic inflammation, leads to a progressive shift in the type of cells present at the site of inflammation and is characterized by simultaneous destruction and healing of the tissue from the inflammatory process. Anti-inflammatory drugs inhibit different stages of inflammation.

Results of the present study in comparison with control clearly indicate that the *O.sanctum* showed significant anti-inflammatory activity when administered alone, but when administered in combination with indomethacin, it only marginally enhanced the efficacy of indomethacin for reducing paw edema in acute model induced by Carrageenan. The edema results from the action of inflammatory mediators such as histamine, serotonin, kinins and prostaglandins at the site of a local inflammatory insult (Wallace, 2002). The early phase of edema, beginning from 1h after administration of the irritant, is due to the release of histamine and serotonin, while the later phase, occurring from 3 h to 5 h after administration of the irritants induced by bradykinin, protease, prostaglandin and lysosome (Wallace, 2002).

Linolenic acid could significantly inhibit the edema induced by PGE2, LTB4 and arachidonic acid (Singh *et al*., 2007). Linolenic acid present in fixed oil of *O. sanctum* could account for the anti-inflammatory activity of the oil by dual inhibition of arachidonic acid metabolism. The fixed oil of *O. sanctum* possesses significant anti-inflammatory, analgesic, antiarthritic, antipyretic, antiulcer, antimastitic and antimicrobial properties without any noticeable toxicity (Singh and Majumdar, 1999; Singh *et al*., 1996).

The lipids present in *O. sanctum* contain relatively large amount of gammalinolenic acid (GLA), an omega-6 (18:3, n-6) fatty acid (all cis-6, 9, 12 octadecatrienoic acid) which contains the first double bond at 6th carbon atom from the methyl (ω) end of the fatty acidchain. GLA is rapidly converted to dihomogammalinolenic acid (DGLA) (20:3, n-6) (a precursor of anti-inflammatory prostaglandin E1) which competes with arachidonate for oxidative enzymes thereby reducing production of

Table.1 Effect of *O.sanctum* and indomethacin administered alone and in combination at various time intervals of Carrageenan-induced paw edema in rats

Treatment group	**Paw volume(edema) in ml (mean ± SD)**			
	1h	**2h**	**3h**	**4h**
Control group (D/W 2 ml/kg)	1.18 ± 0.12	1.25 ± 0.07	1.31 ± 0.07	1.36 ± 0.08
Group 2 (IND 25mg/kg)	1.20 ± 0.07	1.12 ± 0.10	1.06 ± 0.10**	0.99 ± 0.10**
Group 3 (OS 200mg/kg)	1.18 ± 0.12	1.10 ± 0.09*	1.03 ± 0.08**	0.94 ± 0.03**
Group 4 (OS 400mg/kg)	1.14 ± 0.06	1.06 ± 0.06*	1.00 ± 0.05**	0.93 ± 0.08**
Group 5 (IND 25mg/kg + OS 200mg/kg)	1.26 ± 0.07	1.18 ± 0.06	1.09 ± 0.04**	1.01 ± 0.04**
Group 6 (IND 25mg/kg + OS 400mg/kg)	1.18 ± 0.11	1.11 ± 0.12	1.00 ± 0.06**	0.94 ± 0.07**
One-way ANOVA				
F-values	1.01	3.19	14.91	30.86
P- values	>0.05	<0.05	<0.001	<0.001

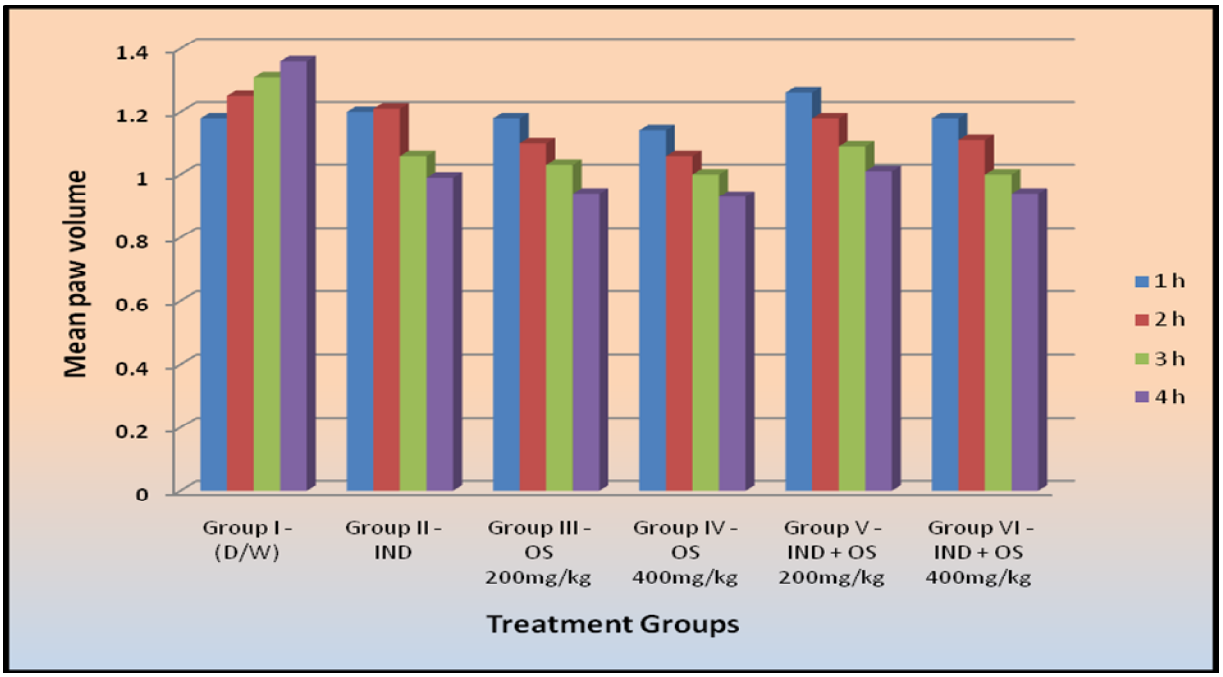
Each value represents the mean ± SD (N = 6) df = (5, 30). Statistical analysis by One-way ANOVA followed by Dunnett's multiple comparisons. P value * < 0.05 is significant; **< 0.001 is highly significant. Abbreviations: bw, body weight; D/W, distilled water; IND, indomethacin; OS, *Ocimum sanctum*.

Table.2 Percentage inhibition of edema produced by *O.sanctum* and indomethacin alone and in combination at various time intervals of Carrageenan-induced rat paw edema

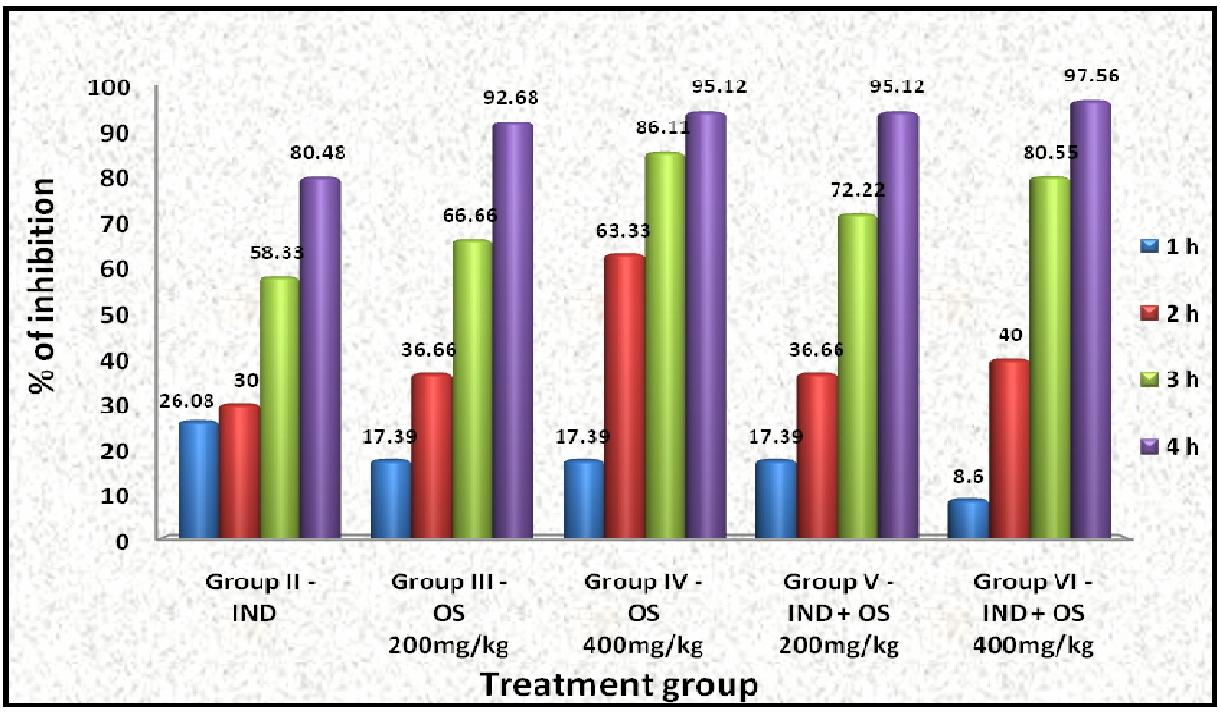
Treatment groups	**IP of paw edema**			
	1h	**2h**	**3h**	**4h**
Group 2 (IND 25mg/kg)	26.08	30	58.33	80.48
Group 3 (OS 200mg/kg)	17.39	36.66	66.66	92.68
Group 4 (OS 400mg/kg)	17.39	63.33	86.11	95.12
Group 5 (IND 25mg/kg + OS 200mg/kg)	17.39	36.66	72.22	95.12
Group 6 (IND 25mg/kg + OS 200mg/kg)	8.6	40	80.55	97.56

Abbreviations: IP, inhibition percentage; IND, indomethacin; OS, *Ocimum sanctum.*

Graph.1 Effect of *O.sanctum* and indomethacin administered alone and in combination at various time intervals of Carrageenan-induced paw edema in rats.



Graph.2 Percentage inhibition of edema produced by *O.sanctum* and Indomethac in alone and in combination at various time intervals of Carrageenan-induced paw edema in rats.



cyclooxygenase products derived from arachidonate. In addition, DGLA is converted by 5-lipoxygenase to 15-hydroxy DGLA which possesses 5-lipoxygenase inhibitory activity (Ziboh and Chapkin, 1987). The results of the different studies shows that linolenic acid could inhibit both cyclooxygenase and lipoxygenase pathways of inflammation (dual inhibitory property) (Singh S, Majumdar, 1999 a,b). Linolenic acid, a ω - 3 (18:3, n-3) fatty acid (allcis-9, 12, 15 octadecatrienoic acid), is progressively metabolized in the body to 6, 9, 12, 15octadecatetraenoic acid (18:4, n-3), stearadonic acid (20:4, n-3) and eicosapentaenoic acid (20:5, n-3). The end product, eicosapentaenoic acid, has the capacity to competitively inhibit the formation of prostaglandins and leukotrienes derived from arachidonate while serving as a substrate for synthesis of prostaglandins with three double bonds and leukotrienes with five double bonds, which are anti-inflammatory. This could be possible mechanism for the anti-inflammatory activity of linolenic acid (Lee *et al*., 1984).

Eugenol (1-hydroxy-2-methoxy-4-allylbenzene) a naturally occurring phenolic compoundis a major component of basil oil and exists to a lesser extent in oil of several other plants (Nagababu *et al*., 1995). It possesses antiulcer, antiseptic, analgesic, antibacterial, anti-inflammatory and antianaphylactic properties (Nagababu *et al*., 1995; Prakash and Gupta, 2005). The therapeutic potential effect of the fresh leaves of *Ocimum sanctum L.* has been found to be largely due to eugenol.

The principle constituents of *O. sanctum* are both eugenol (71.3%) and linolenic acid (43–64%). So this study suggest that *O.sanctum* helps in the reduction of edema i.e. it has anti-inflammatory activity. In view of the present results, it can be inferred that aqueous extract of *O.sanctum* possesses anti-inflammatory activity in acute model of inflammation.

From the above study it was concluded that *O.sanctum* possesses significant anti-inflammatory activity. The anti-inflammatory activity of *O.sanctum* is better than that of the standard drug, indomethacin in the model of Carrageenan induced paw edema and on co-administration marginally improved the anti-inflammatory profile of indomethacin. Although the exact chemical compounds responsible for the anti-inflammatory activity of *O.sanctum* extract still remain speculative. 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. Due to its easy availability, efficacy, safety and potency it can be used as an adjuvant with the available anti inflammatory agents, with reduced dosage and minimal side effects for the therapy of inflammation. Hence if the general public is made aware of this information, tulsi can be used in its native fresh form whenever required for disorders associated with inflammation.

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