

Anti-inflammatory activity of *Ocimum sanctum* (Linn) in formalin induced acute models of albino rats

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The inflammatory process is the response to an injurious stimulus. It can be evoked by a wide variety of noxious agents (e.g., infections, antibodies, physical injuries). Many nonsteroidal anti-inflammatory drugs (NSAIDs) like aspirin, phenylbutazone, indomethacin etc. are in clinical use but all these are not completely devoid of adverse effects. In this study, the anti-inflammatory activity of *O. sanctum* alone and in combination with indomethacin was studied using formalin-induced rat paw edema. Aqueous extract of *O. sanctum* (200mg/kg, 300mg/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 formalin-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 lipoxygenase pathways of arachidonic acid metabolism (dual inhibitory property).

KEYWORDS: Nonsteroidal anti-inflammatory drugs (NSAIDs); Indomethacin; Plethysmometer.

INTRODUCTION

Inflammation, despite having undergone years of intense study, continues to evoke great interest in the field of research. The inflammatory process is the response to an injurious stimulus. It can be evoked by various noxious agents (e.g., infections, antibodies, physical injuries). The ability to mount an inflammatory response is essential for survival in the face of environmental pathogens and injury; in some situations and diseases, the inflammatory response may be exaggerated and sustained without apparent benefit and even with severe adverse consequences. The inflammatory response is characterized mechanistically by transient local vasodilation and increased capillary permeability, infiltration of leukocytes and phagocytic cells, and tissue degeneration and fibrosis¹. Prostanoid biosynthesis is significantly increased in inflamed tissue. Inhibitors of the COXs, which depress prostanoid formation, are effective and widely used anti-inflammatory agents, highlighting the general role of prostanoids as pro-inflammatory mediators¹. Many nonsteroidal anti-inflammatory drugs (NSAIDs), like aspirin, phenylbutazone, indomethacin, etc., are in clinical use, but all these are not completely devoid of adverse effects¹. Hence the search for safer and better anti-inflammatory agents continues to be an area of great interest. This has led to increased demand for natural products with anti-inflammatory activity having fewer side effects.

Many herbs and plant products have been claimed to have significant anti-inflammatory action. 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 the Indian subcontinent². The name "Tulsi" in Sanskrit means "the incomparable one"³. Recent studies suggest that *Ocimum sanctum* (Tulsi) may be a COX-2 inhibitor, like many modern painkillers, due to its high concentration of eugenol (1-hydroxy-2-methoxy-4-allylbenzene)⁴.

The present study is, therefore, undertaken to evaluate the effect of an 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 described briefly.

The whole fresh leaves of *O. sanctum* were collected, air dried under shade for two days, and then powdered. The Tulsi leaf extract was prepared by using soxhlet apparatus. Extract kept in a refrigerator at 4°C in an air and waterproof container. 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 300 g were used. 48 rats were selected and divided into 8 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* 300 mg/kg

Group V : Test rats - receive *O.sanctum* 400 mg/kg

Group VI : Test rats - receives indomethacin 25mg/kg + *O.sanctum* 200 mg/kg

Group VII : Test rats - receive indomethacin 25mg/kg + *O.sanctum* 300 mg/kg

Group VIII : Test rats - receive indomethacin 25mg/kg + *O.sanctum* 400 mg/kg

Formalin-induced edema in rat hind paw:

This method is based on the plethysmometric (IITC 520) measurement of edema produced by sub-plantar injection of Formalin in the rat's hind paw. The hind paw volume was obtained at 1 h, 2 h, 3 h, 4 h, and 5 h after Formalin injection, both in control and test animals. The percentage inhibition of edema was calculated by comparing the edema produced in control rats and in those treated with drugs.

Percentage inhibition = $V_c - V_t \times 100/V_c$

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 \pm SD". Dunnett's t-test for control followed analysis of variance (one-way ANOVA), and standard and test group comparisons were used for statistical evaluation. P values <0.05 were considered significant, and $P<0.001$ was highly significant.

RESULTS

The results were compared with the control and a known anti-inflammatory agent, indomethacin.

The mean paw volumes at the hourly interval in milliliters (ml) of each group are represented in Table 1.

	Paw volume(edema) in ml (mean \pm SD)				
	1h	2h	3h	4h	5h
Control group (D/W 2 ml/kg)	1.08 \pm 0.10	1.15 \pm 0.03	1.29 \pm 0.09	1.34 \pm 0.05	1.40 \pm 0.01
Group 2 (IND 25mg/kg)	1.16 \pm 0.04	1.08 \pm 0.10	1.01 \pm 0.10**	0.89 \pm 0.10**	0.82 \pm 0.04**
Group 3 (OS 200mg/kg)	1.12 \pm 0.12	1.04 \pm 0.07*	0.99 \pm 0.03**	0.88 \pm 0.01**	0.78 \pm 0.05**
Group 4 (OS 300mg/kg)	1.24 \pm 0.99	1.17 \pm 0.03*	1.03 \pm 0.09**	0.94 \pm 0.99**	0.79 \pm 0.07**
Group 5 (OS 400mg/kg)	1.18 \pm 0.05	1.09 \pm 0.05*	0.97 \pm 0.02**	0.92 \pm 0.06**	0.86 \pm 0.02**
Group 6 (IND 25mg/kg + OS 200mg/kg)	1.32 \pm 0.05	1.24 \pm 0.09	1.12 \pm 0.06**	1.04 \pm 0.03**	0.88 \pm 0.07**
Group 7 (IND 25mg/kg + OS 300mg/kg)	1.38 \pm 1.00	1.24 \pm 0.06	1.10 \pm 0.03**	0.98 \pm 0.01**	0.87 \pm 0.08**
Group 8 (IND 25mg/kg + OS 400mg/kg)	1.22 \pm 0.09	1.09 \pm 0.08	0.96 \pm 0.02**	0.86 \pm 0.08**	0.79 \pm 0.04**
One-way ANOVA					
P- values	>0.05	<0.05	<0.001	<0.001	<0.001

Table 1. Effect of *O. sanctum* and indomethacin administered alone and in combination at various time intervals of Formalin-induced paw edema in rats. (Each value represents the mean \pm 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*.)

In the control group, there was a progressive increase in mean paw volume; in the standard and test groups, there was a progressive decrease in mean paw volume from 1h to 5h.

Treatment groups	IP of paw edema				
	1h	2h	3h	4h	5h
Group 2 (IND 25mg/kg)	22.04	28.03	48.23	78.56	84.67
Group 3 (OS 200mg/kg)	14.12	34.44	60.00	89.75	94.09
Group 4 (OS	17.15	40.46	67.87	92.13	95.65

300mg/kg)					
Group 5 (OS 400mg/kg)	17.39	63.33	86.11	95.12	97.25
Group 6 (IND 25mg/kg + OS 200mg/kg)	17.39	36.66	72.22	95.12	97.01
Group 7 (IND 25mg/kg + OS 300mg/kg)	16.48	49.72	69.12	95.87	98.04
Group 8 (IND 25mg/kg + OS 400mg/kg)	8.6	40	80.55	97.56	98.23

Table 2. Percentage inhibition of edema produced by *O. sanctum* and indomethacin alone and in combination at various time intervals of Formalin-induced rat paw edema. (Abbreviations: IP, inhibition percentage; IND, indomethacin; OS, *Ocimum sanctum*.)

Anti-inflammatory activity is expressed as percent inhibition. The results obtained are shown in (Table 2). The mean paw volume of the control group reached its peak at about 5h after the administration of formalin. The anti-inflammatory effect of the above-mentioned treatment groups was significant i.e. ($p < 0.05$) at time intervals of [2 h] and highly significant ($p < 0.001$) at time intervals of [3 h, 4 h and 5h] but not at the 1 h interval ($p > 0.05$).

DISCUSSION

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 injuries like microbes and toxins and the consequences of such injuries like necrotic cells and tissue. Though vascular and cellular events bring about the process of inflammation, the former appear to contribute the maximum to the pathogenesis of acute inflammation. This complex phenomenon involves endogenous chemical mediators such as histamine, 5-hydroxytryptamine, various chemotactic factors, bradykinin, leukotrienes and prostaglandins⁵. Inflammation can be classified as either acute or chronic. In this study, only acute models of albino rats are used.

Results of the present study in comparison with control clearly indicate that the *O. sanctum* showed significant anti-inflammatory activity when administered alone. Still, when administered in combination with indomethacin, it only marginally enhanced the efficacy of indomethacin for reducing paw edema in acute models induced by formalin. The edema results from the action of inflammatory mediators such as histamine, serotonin, kinins and prostaglandins at the site of a local inflammatory insult⁶. The early phase of edema, beginning from 1 h 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 lysosome⁶. Eugenol and Linolenic acid present in the fixed oil of *O. sanctum* could account for the oil's anti-inflammatory activity by dual inhibiting arachidonic acid metabolism.⁷⁻⁸

In view of the present results, it can be inferred that an aqueous extract of *O. sanctum* possesses anti-inflammatory activity in the acute model of inflammation. From the above study, it was concluded that *O. sanctum* possesses significant anti-inflammatory activity. More detailed studies on *O. sanctum* are needed before reaching a clear-cut conclusion.

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