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ANTI-INFLAMMATORY ACTIVITY OF OCIMUM SANCTUM (LINN) IN FORMALIN INDUCED ACUTE MODELS OF ALBINO RATS

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ABSTRACT

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[2]. 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 lipooxygenase 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 a wide variety of 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 a transient local vasodilation and increased capillary permeability, infiltration of leukocytes and phagocytic cells, and tissue degeneration and fibrosis. 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 mediators[1]. 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[1]. Hence the search for safer and better anti-inflammatory agents continues to be an area of great interest. This has led to increase in demand for natural products with anti-inflammatory activity having fewer side effects.

Many herbs and plant products have been claimed to have a 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 Indian subcontinent[2]. 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 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. The Tulsi leaf extract was prepared by using soxhlet apparatus. Extract kept in a refrigerator at 4^0C in air and water proof 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 – receives indomethacin 25mg/kg + *O.sanctum* 300 mg/kg
Group VIII : Test rats – receives indomethacin 25mg/kg + *O.sanctum* 400 mg/kg

Formalin-induced edema in rat hind paw:

This method is based on the plethysmometeric (IITC 520) measurement of edema produced by sub plantar injection of Formalin, in the hind paw of rat. The hind paw volume obtained at 1 h, 2 h, 3 h, 4 h and 5 h after Formalin 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.

$$Percent\ inhibition = \frac{\mathrm{Vc} - \mathrm{Vt}}{Vc} X100$$

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

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.
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 5h.

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

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

P value * < 0.05 is significant; **< 0.001 is highly significant. Abbreviations: bw, body weight; D/W, distilled water; 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.

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.

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 300mg/kg)	17.15	40.46	67.87	92.13	95.65
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

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

The anti-inflammatory effect of the above mentioned treatment groups were 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 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[5]. 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, but when administered in combination with indomethacin, it only marginally enhanced the efficacy of indomethacin for reducing paw edema in acute model 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 insult[6]. 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 lysosome[6]. Eugenol and 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. [7,8]

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. More detailed studies on *O.sanctum* are needed before reaching a clear cut conclusion.

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REFERENCES

1) Laurence, L.B., Bruce, A.C, and Björn C.K.2011. Goodman & Gilman's The pharmacologicalbasis of therapeutics.12th ed China: McGraw- Hill Publishers.1067.
2) Singh V, Amdekar S , Verma O. Ocimum sanctum (tulsi): Bio-pharmacological activities. WebmedCentral Pharmacology 2010; 1(10):WMC001046:01-07.
3) Bansod S, Rai M. Antifungal activity of essential oils from Indian medicinal plants against human pathogenic Aspergillus fumigatus and A. niger. World J Med Sci 2008; 3(2): 81-88.
4) Nadkarni AK. Ocimum sanctum in Indian Materia Medica. Vol. 1, 3rd ed Mumbai: Popular book depot:1954. 865-867.
5) Vinay K, Abdul KA, Nelson F, Aster JC. Robbins and Cotran's Pathologic basis of diseases. 8th ed Philadelphia: Elsevier Publishers; 2010. 43-77.
6) Pushpangadan P, Sobti SN. Medicinal properties of Ocimum species and some recent investigations of their efficacy. Indian Drugs 1977; 14(1): 207-208.
7) Singh S, Majumdar DK. Effect of Ocimum sanctum fixed oil on vascular permeability and leucocytes migration, Indian J Exp Biol 1999 Nov; 37(11): 1136- 1138.
8) Singh S, Majumdar DK, Yadav MR. Chemical and pharmacological studies on fixed oil of Ocimum sanctum, Indian J Exp Biol 1996; 34:12.