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Research Article

Anti-inflammatory and analgesic properties of *Ocimum sanctum*: a comparative study using animal models

A. Umamageswari*, B. L. Kudagi

Department of Pharmacology, Narayana Medical College & Hospital, Nellore, Andhra Pradesh, India

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***Correspondence to:**
Dr. A. Umamageswari,
Email: leeuma6@gmail.com.

ABSTRACT

Background: *Ocimum sanctum* commonly known as Tulsi has been used for thousands of years in the Ayurveda for its diverse healing properties. This study was conducted to evaluate the anti-inflammatory and analgesic activity of *O. sanctum* aqueous leaf extract in thermal and chemical induced pain and inflammatory animal models.
Methods: Wistar albino rats (150-200 g) and swiss albino mice (25-30 g) were randomly divided into 4 groups of 6 animals each. The control group, test group, and standard drugs group received normal saline, *O. sanctum* extract (100 mg/kg), aspirin, and celecoxib respectively, by oral feeding. The anti-inflammatory effect was assessed by carrageenan induced rat paw edema and cotton pellet induced granuloma in rats. Analgesic effect was assessed by hot plate method and acetic acid induced writhing method in mice.
Results: In carrageenan induced rat paw edema, maximum inhibition by *O. sanctum*, aspirin, and celecoxib were 13.43%, 30%, and 32%, respectively, and time to reach maximum inhibition for *O. sanctum* was 2 hrs. In cotton pellet induced granuloma, percentage inhibition by *O. sanctum*, aspirin, and celecoxib were 23.85%, 45.84%, and 42.77%, respectively. In hot plate method, maximum inhibition by *O. sanctum*, aspirin and celecoxib were 143.92%, 288.18%, and 260.59%, respectively. In acetic acid induced writhing method, percentage protection by *O. sanctum*, aspirin, and celecoxib were 50.2%, 71.4%, and 66.5%, respectively.
Conclusion: The current study demonstrates statistically significant anti-inflammatory and analgesic activity of *O. sanctum*.

Keywords: *Ocimum sanctum*, Carrageenan, Granuloma, Writhing, Hot plate

INTRODUCTION

Ocimum sanctum (Tulsi), the Queen of herbs, the legendary "Incomparable one" of India, is one of the holiest and most cherished of the many herbs of the orient. The sacred basil, Tulsi, is renowned for its religious and spiritual sanctity.[1] Tulsi extracts are used in the Ayurvedic remedies for common colds, headaches, inflammation, heart disease, stomach disorders, various forms of poisoning, and malaria.

Tulsi is an erect, branched sub-shrub 30-60 cm tall, with simple opposite green or purple leaves that are strongly scented and hairy stems. Leaves have petiole and are ovate, up to 5 cm long, usually somewhat toothed. Flowers are purplish in elongate racemes in close whorls. *O. sanctum* belongs to the family Lamiaceae is native throughout the world tropics and widespread as a cultivated plant.[2,3] The stem and leaves of Tulsi contain a variety of constituents such as saponins, flavonoids, triterpenoids, and tannins. These active constituents have been attributed the therapeutic activity of the plant. Tulsi possess numerous therapeutic uses including analgesic,[4] anti-inflammatory,[5] antidiabetic,[6] gastroprotective,[7] cardioprotective[8] antioxidant,[9] immunomodulatory,[10] antifertility,[11] etc.

Therefore, this work was aimed at the scientific validation of the ethnopharmacological claim about the anti-inflammatory and analgesic properties of the aqueous leaf extract by comparing with standard drugs using animal models.

METHODS

Preparation of aqueous extract

The fresh leaves of *O. sanctum* were collected, identified and authenticated by a Pharmacognocist of Narayana Pharmacy College, Nellore. The leaves were thoroughly washed with tap water, dried in shade and grounded

to make powder. 100 g of dried *O. sanctum* powder was boiled with 100 ml of distilled water in the flask for 24 hrs. The flask was kept on heating mantle for boiling until the content was reduced to half, and then cooled and filtered using muslin cloth so as to remove the insoluble materials. The filtrate was again filtered through an ordinary filter paper and poured in a cleaned and already weighed petridish. It was placed on a hot plate for complete evaporation. Then the extract was cooled at room temperature and weighed to calculate extractability percentage and finally stored in desiccators in cool and dry place.[12]

Phytochemical screening test

Aqueous extract of *O. sanctum* leaves was subjected to phytochemical screening test to evaluate the presence of constituents.

Dose of the study

Estimated by a pilot study with different doses 25 mg/kg, 50 mg/kg, 100 mg/kg, and 150 mg/kg. Both analgesic and anti-inflammatory activity of *O. sanctum* were significant at dose of 100 mg/kg.

Chemicals

Carrageenan (Sigma), celecoxib (Cipla), aspirin (Cipla).

Animals

Wistar albino rats (150-200 g) and Swiss albino mice (25-30 g) of either sex were procured from the central animal house of the institute. They were housed in standard polypropylene cages and were kept under controlled room temperature at 25±2°C in a 12 hrs light/dark cycle. Animals were given dry pellets and water *ad libitum*. The animals were accustomed during the day time to the new environment for at least 2 days prior to the experiment. Institutional Animal Ethics Committee approval was taken prior to the start of study. The ethical guidelines for the investigation of animals used in experiments were followed in all tests.

Evaluation of anti-inflammatory activity

Albino rats were divided into 4 groups of 6 each.
Group I: 0.9% Normal saline (control)
Group II: *O. sanctum* aqueous extract 100 mg/kg. (test drug)
Group III: Aspirin 150 mg/kg
Group IV: Celecoxib 20 mg/kg (standard drugs).

All the groups received drugs by the oral route. Following models were used to screen the anti-inflammatory activity of *O. sanctum*.

Rat paw edema induced by carrageenan[13]

1 hr after oral administration of drugs, acute inflammation was produced by sub plantar injection of 0.1 ml of freshly prepared 1% (w/v) suspension of carrageenan in normal saline in all the animals. The paw volumes were measured at 0, 1, 2, 3, and 4 hrs after carrageenan injection using mercury plethysmograph (INCO Chennai) by noting the displacement of mercury when the paw is dipped in mercury column up to a predetermined mark on the paw. A mark was made on the leg at lateral malleolus to facilitate uniform dipping and recording of paw volumes. Edema was expressed as an increase in the volume of paw, and the percentage inhibition (PI) of edema was calculated as:

$$\text{Percentage of inhibition} = \frac{V_c - V_t \times 100}{V_c}$$

Where, V_c=Mean edema volume in the control group, V_t=Mean edema volume in the treated group.

Cotton pellet induced granuloma[14]

Pellets of cotton weighing 30±1 mg were sterilized in a hot air oven at 120°C for 2 hrs, implanted bilaterally in regions of axilla and groin of rat, under ether anesthesia. Animals were administered drugs throughout the experimental period of 7 days. On the 8th day, the pellets were dissected out under the light anesthesia, dried overnight at 70°C and weighed after cooling. The increase in the dry weight of the pellets was taken as a measure of granuloma formation.

Evaluation of analgesic activity

Swiss albino mice were divided into 4 groups of 6 each.
Group I: 0.9% Normal saline (control)
Group II: *O. sanctum* aqueous extract 100 mg/kg (test drug)
Group III: Aspirin 150 mg/kg
Group IV: Celecoxib 20 mg/kg (standard drugs).

All the groups received drugs by the oral route. Following models were used to screen the analgesic activity of *O. sanctum*.

Eddy's hot plate[15]

The temperature of hot plate was set at 55±1°C, with the help of thermostat. Animals were placed on the hot plate with the paws touching the plate. The cut off time was considered as 15 sec to prevent injury to the animal. The reaction time which was defined as licking of paw and jumping out from hot plate was noted. The reaction time was recorded at every 30 mins up to 4 hrs. All the drugs were administered 1 hr prior to the commencement of estimation of reaction time.

Acetic acid induced writhing[16]

Albino mice were placed individually in the glass container immediately after acetic acid injection 0.1 ml/10 g intraperitoneally. All drugs were administered 1 hr prior to the acetic acid administration. The mice were observed for 15 mins, and a number of writhing was recorded for each animal. For scoring, a writhe is indicated by stretching of the abdomen with simultaneous stretching of at least one hind limb. The PI was calculated by,

$$\frac{\text{Average writhes in the control group} - \text{writhes in the test group} \times 100\%}{\text{Writhes in the control group}}$$

Statistical analysis

Data were expressed as mean±standard deviation and percentages. Percentage change as compared to control was analyzed. ANOVA followed by tukeys multiple comparison tests was used for analysis of data between the four. For all inferential statistical tests, a two-tailed p=0.05 considered significant. All the statistical methods were carried out through Sigma graph pad prism version-5.

RESULTS

Preliminary phytochemical screening of aqueous leaf extract of *O. sanctum* revealed the presence of saponins, triterpenoids, flavonoids, tannins, and eugenol.

Rat paw edema induced by carrageenan

O. sanctum, aspirin, and celecoxib treated groups significantly inhibited carrageenan induced rat paw edema ($p<0.001$). The time to reach maximum inhibition was observed at 2 hrs, 1 hr, and 1 hr for *O. sanctum*, aspirin, and celecoxib, respectively. Anti-inflammatory activity is expressed as PI. The PI with *O. sanctum*, aspirin, and celecoxib were 13.43%, 30%, and 32%, respectively (Table 1 and Figure 1).

Cotton pellet induced granuloma

When compared to control, increase in granuloma weight was significantly less in *O. sanctum*, aspirin, celecoxib ($p<0.0001$) which also corresponds to 23.85%, 45.84%, and 42.77% PI of anti-inflammatory activity (Table 2).

Eddy's hot plate

When compared to control, mean reaction time was significantly increased in all the groups. Maximum inhibition for *O. sanctum*, aspirin and celecoxib were at 2 hrs, 1 hr, and 1 hr, respectively. *O. sanctum* exhibited significant longer retention time as compared to control ($p<0.001$), however it was shorter as compared to aspirin ($p<0.001$) and celecoxib ($p<0.001$) (Table 3 and Figure 2).

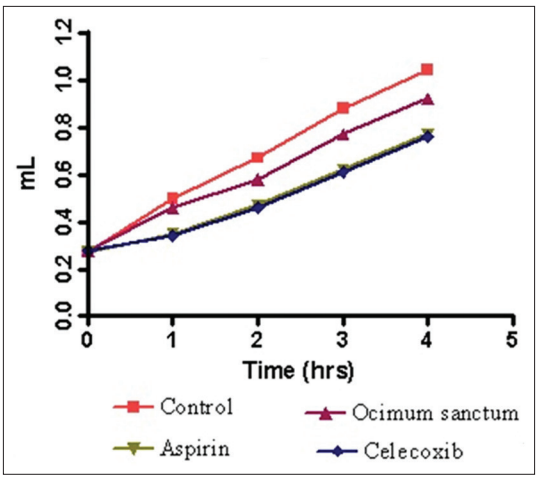

Figure 1: Carrageenan induced rat paw edema.

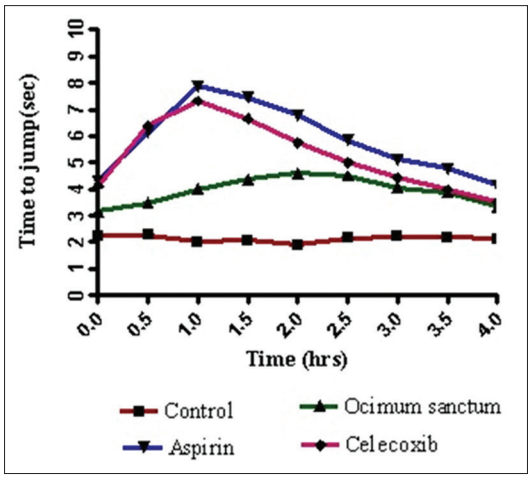

Figure 2: Analgesic activity of Eddy's hot plate method.

Table 1: PI of rat paw edema.

Groups	1 hr	2 hrs	3 hrs	4 hrs	Maximum inhibition (%)	Time to maximum inhibition (hrs)
Group I control	-	-	-	-	-	-
Group III *O. sanctum* 100 mg/kg	8.0	13.43	12.50	11.53	13.43	2.0
Group III aspirin 150 mg/kg	30.0	29.85	29.54	25.96	30.0	1.0
Group IV celecoxib 20 mg/kg	32.0	31.34	30.68	26.92	32.0	1.0

PI: Percentage inhibition, *O. sanctum: Ocimum sanctum*

Acetic acid induced writhing

When compared to control, *O. sanctum*, aspirin, celecoxib groups significantly ($p<0.0001$) reduced the mean number of writhing movements which corresponds to PI by 50.2%, 71.4%, and 66.5%, respectively. On evaluation, *O. sanctum* showed the significantly fewer number of writhing movements as compared to control group ($p<0.001$). However, it was more when compared to aspirin ($p<0.01$) and celecoxib ($p<0.05$) (Table 4).

DISCUSSION

O. sanctum is an annual herb commonly found in India. Tulsi has been suggested to possess anticancer,[17] antifungal, antimicrobial, antispasmodic, and adaptogenic actions. In the present study, the analgesic and anti-inflammatory effects of *O. sanctum* was compared with aspirin and celecoxib. This extract was found to have significant antinociceptive and anti-inflammatory properties.

Carrageenan induced edema involves the synthesis or release of mediators at the injured site.[18] It is believed to be biphasic, the first phase (1 hr) involves the release of serotonin and histamine while the second phase (over 1 hr) is mediated by prostaglandins, the cyclooxygenase (COX) products, and the continuity between the two phases is provided by kinins.[19] In our study, 100 mg/kg of aqueous leaf extract of *O. sanctum* (13.43% inhibition at 2 hrs) showed significant anti-inflammatory activity ($p<0.001$). Thakur[20] also showed a significant anti-inflammatory effect of essential oil extract of *O. sanctum* leaf (Eugenol) in the dose of 100 mg/kg with 33% of inhibition. Anti-inflammatory activity of *O. sanctum* may be due to the presence of amino acids resembling creatine and isoleucine. Singh in his studyreported that linoleic acid present in *O. sanctum* has the capacity to block both the COX and lipoxygenase pathways of arachidonate metabolism and could be responsible for the anti-inflammatory activity.[21]

Table 2: Effect of drugs in cotton pellet granuloma.

Groups	Mean granuloma weight (mg)±SD	PI
Group I control	237.3±4.0	-
Group II *O. sanctum* 100 mg/kg	180.7±4.6*	23.85
Group III aspirin 150 mg/kg	128.5±3.8*	45.84
Group IV celecoxib 20 mg/kg	135.8±3.7*	42.77

*$p<0.001$, SD: Standard deviation, *O. sanctum: Ocimum sanctum,* PI: Percentage inhibition

Cotton pellet granuloma method has been widely employed to assess the transudative, exudative, and proliferative components of chronic inflammation.[22] In our study, 100 mg/kg of *O. sanctum* extract treated group showed extremely significant inhibition ($p\leq0.0001$) of granuloma formation with the PI of 23.85%. Varde et al. showed that the oral administration of 2 ml of the boiled coconut oil extract of *O. sanctum* produced 13.6% inhibition of cotton pellet granuloma formation.[23] The result of the cotton pellet implantation model further supports the anti-inflammatory activity of the extract of *O. sanctum*. Several flavonoids isolated from this medicinal plant have been discovered to possess significant anti-inflammatory activity.

In our hot plate method, 100 mg/kg of *O. sanctum* extract exhibited significant longer retention time as compared to control ($p<0.001$), however it was shorter as compared to standard drugs aspirin and celecoxib. Godhwani et al. showed that oral administration of aqueous suspension of *O. sanctum* in the dose of 100, 250, and 500 mg/kg has percentage increase in reaction time of 21, 33, and 89%, respectively.[24] According to Kumar et al. repetitive afferent input enhances the response of the animal to a given noxious stimulus. Activation of prostanoid receptors increases the opening of voltage sensitive Ca^{2+} channels and enhances primary afferent peptide release.[25] It might be possible that the extract exert its therapeutic action by antagonizing prostanoid receptor or inhibiting the synthesis of prostaglandins. Eugenol isolated from the *O. sanctum* leaves, available in appreciable quantity may account for the analgesic activity of *O. sanctum*.

In our analgesic model using acetic acid-induced writhing test, 100 mg/kg of *O. sanctum* extract showed a significant reduction in the number of writhing movements with the PI of 50.2%. Khanna and Bhatia[4] showed that oral administration of *O. sanctum* in the dose of 100 mg/kg had PI of 69.9%. The abdominal constriction response induced by acetic acid is a sensitive procedure to establish acting analgesics peripherally. This response is thought to involve local peritoneal

Table 3: Percentage inhibition in Eddy's hot plate method.

Groups	0.5 hr	1 hr	1.5 hr	2 hrs	2.5 hrs	3 hrs	3.5 hrs	4 hrs
Group I control	-	-	-	-	-	-	-	-
Group II *O. sanctum* 100 mg/kg	53.07	97.04	111.59	143.92	107.37	83.33	77.98	57.55
Group III aspirin 150 mg/kg	168.42	288.18	259.90	258.73	168.66	130.63	119.27	95.75
Group IV celecoxib 20 mg/kg	180.26	260.59	220.77	204.76	130.88	99.55	82.57	65.09

O. sanctum: Ocimum sanctum

Table 4: Effect of drugs in acetic acid induced writhing.

Groups	Mean number of writhes±SD	PI
Group I control	49.83±5.70	-
Group II *O. sanctum* 100 mg/kg	24.83±3.54*	50.2
Group III aspirin 150 mg/kg	14.16±2.92*	71.4
Group IV celecoxib 20 mg/kg	16.67±3.61*	66.5

*p<0.001, SD: Standard deviation, *O. sanctum: Ocimum sanctum,* PI: Percentage inhibition

receptors.[26] Acetic acid induces pain by the release of endogenous mediators such as prostacyclines which involves the sensory C-fibres activation through the activity of the enzyme COX. Our results, therefore, supports *O. sanctum* has peripheral analgesic properties similar to nonsteroidal anti-inflammatory drug probably due to inhibition of COX activity and further inhibition of the release of other endogenous pain mediators. Since, *O. sanctum* was effective in both the models, it implies that the active constituent in the extract exhibit analgesic activity centrally as well as peripherally.

CONCLUSION

In the present study, *O. sanctum* extract was found to have significant anti-inflammatory and analgesic properties. The preliminary data of the present investigation provide some evidence for the effectiveness of *O. sanctum* and supports its use in the treatment of arthritis, rheumatism, pain, and fever as claimed in the Ayurvedic system of medicine.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Animal Ethics Committee

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