RESEARCH ARTICLE

Evaluation of efficacy of *Ocimum sanctum*, *Azadirachta indica*, and their combination in comparison with aspirin on acute inflammatory pain

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ABSTRACT

Background: Aspirin, a potent drug used for acute inflammatory pain, is also associated with various adverse effects, whereas some Indian herbs such as *Ocimum sanctum* and *Azadirachta indica* are known to have efficacy against such pain with less serious adverse effects. Aims and Objectives: This study aims to evaluate efficacy of *Ocimum sanctum*, *Azadirachta indica*, and their combination in acute inflammatory pain in comparison with aspirin. Materials and Methods: Wistar albino rats of either sex (150–250 g) were divided into five groups with six rats in each group. To induce inflammation, formalin (2.5%, 0.1 ml) was injected into subplantar region of the left hind paw of rats. The study groups were administered orally with distilled water (3 ml), aspirin (200 mg/kg), *O. sanctum* (400 mg/kg), *A. indica* (500 mg/kg), and their combination (combination of *O. sanctum* and *A. indica* having 400 mg/kg *O. sanctum* + 500 mg/kg *A. indica*) half an hour before administration of formalin. Effect of test drugs was assessed by the formalin test, where rats were observed for a period of 40 min to record any response after formalin injection. Two responses were observed – time spent in paw licking and number of paw lifting in early/neurogenic and late/inflammatory phases. Results (mean ± SD) were analyzed using one-way ANOVA test followed by Tukey’s HSD test. Results: *A. indica* had produced a comparable effect to aspirin on inflammatory phase of pain. Conclusion: *A. indica* could be used as an add-on therapy to conventional drugs for acute inflammatory pain. However, clinical studies on humans would be required to test its accurate efficacy.

KEY WORDS: Formalin Test; Biphasic Response; Neurogenic Phase; Inflammatory Phase; Paw Licking and Paw Lifting

INTRODUCTION

Inflammation is an initial response produced by a human body in response to an insult (noxious stimuli) of physical, chemical, or microbiological origin. It eliminates the cause of cell injury and necrotic cells and tissues resulting from any such insult. Its protective mechanism occurs by diluting, destroying, or neutralizing harmful agents which further leads to healing and repairing of sites of injury. Without it, infections would not get checked and wounds would never heal.[¹ ²] Apart from destroying and eliminating microbes and dead tissues, inflammatory reactions are also capable of injuring normal tissues which can cause are redness, pain, heat, swelling, and loss of function of injured site or part of body.[³ ⁴ ⁵]

A noxious stimulus causes activation of phospholipase A₂ and causes release of arachidonic acid from membrane phospholipid of injured cell which leads to generation of many inflammatory mediators due to metabolism of arachidonic acid either by cyclooxygenase (COX) or by lipoxygenase enzymatic pathways. Prostaglandins are one
of the major inflammatory mediators synthesized from COX enzyme pathway of arachidonic metabolism which is majorly responsible for pain sensation arising due to inflammation from any such stimulus. Pain is one of the most common reasons for which patients seek medical care. Pain due to inflammation can be acute or chronic, depending on the nature of stimulus and effectiveness of reaction and duration of inflammation.

Aspirin, a prototype nonsteroidal anti-inflammatory drug (NSAID), causes irreversible inhibition of COX enzyme by causing acetylation at a serine of COX, thus effective in reducing prostaglandins synthesis and thereby used as a most common drug for the treatment of acute painful conditions. Although effective, it is associated with various adverse effects such as that of peptic ulcer, increasing bleeding loss in stools, and hypersensitivity reactions.

India is a biggest repository of medicinal plants in the world and produces raw materials for crude drugs or bioactive compounds used in the formulation of pharmaceuticals and cosmetics. Some known herbal plants with anti-inflammatory activity are Ocimum sanctum (Tulsi) and Azadirachta indica (Neem). These herbs are known to produce effective anti-inflammatory response with very few or no adverse effects.

The formalin test is one of the oldest and reliable models to evaluate efficacy of drugs against acute inflammatory pain. In 1985 and 1986, Hunskaar and Hole performed this test in mice. Time spent in licking the injected paw or leg was recorded. On the basis of the response pattern, two distinct phases (biphasic response) of intensive licking activity were identified. Early phase was recorded 0–5 min after late phase was recorded 20–30 min after the injection of phlogistic agent. In early phase, activity in C-fibers evokes due to direct chemical stimulation of nociceptors. It is followed by a period of 10–15 min where animals show very little activity which suggests nociception. Late phase starts 15–20 min after formalin injection and lasts for 20–40 min and has inflammatory origin. Few experimental studies have shown that substance P and bradykinin are involved in early phase, while histamine, serotonin, prostaglandins, and bradykinin are involved in late phase. Hunskaar et al. (1985) reported that acetylsalicylic acid had a dose-dependent antinociceptive effect during early and late phase, as the effect was significant for 300 mg/kg and higher during the early phase and for 200 mg/kg and higher during the late phase.

Hence, with the view of evaluating these herbs for their efficacy in acute inflammatory pain in comparison with aspirin, the present study was conducted where chemical inflammation was produced in rats using formalin test and reduction in response to inflammation due to herbs and aspirin was observed and compared as a measure of the efficacy of these agents.

MATERIALS AND METHODS

The study was commenced after Institutional Animal Ethics Committee approval was granted and was conducted as per Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines. Rats were housed under standard conditions as per CPCSEA guidelines and were deprived of food 24 h before initiating the experiment and water was given ad libitum.

Extract Preparation

The following extract was prepared:

i. Aqueous extract of leaves of O. sanctum
ii. Ethanolic extract of leaves of A. indica
iii. Combination of O. sanctum leaves and A. indica bark (COA)

Study Design

Wistar albino rats (200–250 g) of either sex were procured from animal house of Dr. D. Y. Patil Medical College, Hospital and Research Centre, Pimpri, Pune. They were divided into five groups. Six rats were included in each group on a random basis. Aspirin and extracts were dissolved into 3 ml of distilled water and were given to rats orally. Groups were given:

- Group I: Control (only 3 ml of distilled water)
- Group II: Aspirin (200 mg/kg)
- Group III: O. sanctum (400 mg/kg)
- Group IV: A. indica (500 mg/kg)
- Group V: COA (400 mg/kg of O. sanctum + 500 mg/kg of A. indica).

The Formalin Test

0.1 ml of freshly prepared formalin (2.5%) was injected into subplantar region of the left hind paw of rats half an hour after administration of drugs and then rats were placed cages having two mirrors one at floor and other at one lateral side of cage opposite from the side of observer. Rats were observed for the duration of 40 min. Two main responses—time spent in paw licking and number of paw lifting, were observed in periods of 0–5 min (early/neurogenic phase) and 15–40 min (late/inflammatory phase) for all the groups. Time in seconds was recorded using stopwatch for paw licking and number of paw lifting was counted and recorded for each rat in the first 5 min and 1–40 min after formalin injection.

Mean and standard deviation for paw licking and paw lifting for each group in two different phases were calculated and appropriate statistical test was applied to assess anti-inflammatory efficacy of treatment groups.

Percentage for inhibition of time spent in paw licking and number of paw lifting were calculated using the formula:

\[
\text{Percentage Inhibition} = \left(1 - \frac{\text{Mean value of treatment group}}{\text{Mean value of control group}}\right) \times 100
\]
Where, X = mean observation in rats of control groups at early phase and late phase
Y = mean observation in rats of respective test group at early phase and late phase.

**Statistical Analyses**

Data were compiled in Microsoft Excel 2016 spreadsheet and were analyzed using IBM SPSS version 26 statistical software. Data passed the Shapiro–Wilk normality test for distribution. Results were analyzed using one-way ANOVA test followed by post hoc Tukey’s HSD test.

**RESULTS**

Tables 1-4 show mean and standard deviations of all the parameters observed along with their percentage reduction in each group and Figures 1-4 show graphical comparison and statistical difference between each group as per the parameters.

![Figure 1: Comparison between the groups for time spent in paw licking in early phase. *P < 0.05 as compared to control. †P < 0.05 as compared to aspirin. ‡P < 0.05 as compared to Ocimum sanctum](image1)

![Figure 2: Comparison between the groups for time spent in paw licking in late phase. *P < 0.05 as compared to control, †P < 0.05 as compared to Ocimum sanctum](image2)

![Figure 3: Comparison between the groups for number of paw lifting in early phase. *P < 0.05 as compared to control. †P < 0.05 as compared to aspirin. ‡P < 0.05 as compared to Ocimum sanctum](image3)

![Figure 4: Comparison between the groups for number of paw lifting in late phase. *P < 0.05 as compared to control](image4)

**DISCUSSION**

The present study reveals that in early phase, all the test groups, that is, *O. sanctum*, *A. indica*, and COA showed significant reduction in time spent in paw licking as compared to control and aspirin group; *A. indica* and COA showed significant reduction in time spent in paw licking as compared to *O. sanctum* but *A. indica* and COA did not show significant reduction in time spent in paw licking when compared with each other. In the late phase, all the treatment groups, that is, aspirin, *O. sanctum*, *A. indica*, and COA showed significant reduction time spent in paw licking as compared to control group; aspirin, *A. indica*, and COA showed significant reduction in time spent in paw licking as compared to *O. sanctum*; aspirin, *A. indica*, and COA did not show significant reduction in time spent in paw licking when compared with each other. Reduction in number of paw lifting in early and late phase of formalin test was also evaluated in the present study. In the early phase, all the test groups showed significant reduction in number of paw lifting as compared to control and aspirin; *A. indica* and COA showed significant reduction in number of paw lifting as compared to *O. sanctum.* *A. indica* and COA did not showed significant reduction in number of paw lifting when compared with each other. In the late phase, all the treatments showed significant...
The present study supports finding in previous two studies that *O. sanctum* and *A. indica* have antinociceptive activity in both early and late phases of formalin test and aspirin like other NSAIDs lacks efficacy against neurogenic phase of inflammation. As far as we know, no previous studies were conducted which evaluated reduction in number of paw lifting using *O. sanctum* and *A. indica*.

This study was different that it also evaluated and compared efficacy of combination of *O. sanctum* and *A. indica* with aspirin in acute inflammatory pain but it was done in small number of animals and evaluation for adverse effect profile of herbs was lacking. Hence, a study to evaluate their safety and efficacy in acute inflammatory pain in large sample of animals is required.

From this test, it can be said that aspirin does not possess any activity in early (neurogenic) phase of inflammation while all test groups have activity in early phase also but efficacy of *A. indica* and COA is better than *O. sanctum* also. In late (inflammatory) phase, all the treatment groups possess significant activity against acute inflammatory pain activity and aspirin, *A. indica* and COA also have better efficacy compared to each other.

**CONCLUSION**

This study revealed that both *O. sanctum* and *A. indica* and their combination (COA) have significant effect on acute inflammatory pain. *A. indica* and COA proved to be a better agent compared to *O. sanctum* and showed comparable effect to aspirin in acute inflammatory pain. From above

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<th>Table 1: Time spent for paw licking in early phase of formalin test and its percentage reduction in different treatment groups</th>
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It can be considered that COA is having its effect on acute inflammatory pain mainly due to its A. indica as its constituent. Therefore, it was concluded that A. indica could be used as an add-on therapy for the treatment of acute inflammatory pain but more studies are required to validate its effect in clinical settings.

REFERENCES


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