Acute anti-inflammatory activity of ethanolic extract of leaves of Leucas indica by carrageenan induced paw oedema in wistar albino rats

Chandrashekar R.*, S. N. Rao

ABSTRACT

Background: Inflammation is basically a defense phenomenon but can lead to serious pathological conditions. It is treated by various agents with good to moderate success because of both considerable toxicity and side effects. There are various mediators to cause an inflammatory reaction that can contribute to the associated symptoms and tissue injury. Even though non steroidal anti-inflammatory drugs are the most commonly prescribed drugs in the world, their use as anti-inflammatory agents continues to be principally limited by their undesired side effects. Hence, the traditional medical practitioners and scientists are turning towards Indian System of Medicine (ISM).

Methods: Dried powdered leaves of Leucas indica were subjected to solvent extraction by using 90 % ethanol. Based on acute oral toxicity study according to Organization for Economic Cooperation and Development (OECD) guidelines No. 423, three doses of the test drug 75, 150 & 300mg/kg were selected and subjected to preclinical anti-inflammatory screening by carrageenin induced paw oedema in Wistar Albino rats.

Results: Oral administration of Ethanolic Extract Of Leaves Of Leucas Indica (EELLI) at doses of 150 mg/kg and 300mg/kg showed significant anti-inflammatory activity 52.58% (p<0.01) and 36.87% (p<0.05) respectively compared to control.

Conclusion: Even though oral administration of EELLI has shown significant anti-inflammatory activity, further studies are required to evaluate its comprehensive analysis including quantitative / semi quantitative analysis, characterize its chemical structure and assess its pharmacotherapeutic activities with exact mechanism of action as an anti-inflammatory agent.

Keywords: Anti-inflammatory activity, Leucas indica, Carrageenin, Indomethacin

INTRODUCTION

Inflammation is a biological response of living tissue to harmful stimuli, pathogens, irritants characterized by redness, warmth, swelling and pain. Inflammation is a common manifestation of infectious diseases like leprosy, tuberculosis, syphilis, asthma, inflammatory bowel syndrome, nephritis, vasculitis, celiac diseases, auto-immune diseases etc. Anti-inflammatory drugs like Non Steroidal Anti-Inflammatory Drugs (NSAIDs) are used to reduce pain and inflammation. But these agents carry the risk of gastro-intestinal, cardiovascular and other toxicities on prolonged use. For this reason, there is still need for newer anti-inflammatory drugs having less severe side. Recently more interest is shown in alternative and natural drugs for treatment of various diseases, but there is lack of scientific data. Hence the background and objective of this study was to carry out preclinical screening acute anti-inflammatory activity of Ethanolic Extract of Leaves of Leucas indica (EELLI) by carrageenan induced paw oedema in Wistar Albino rats.

METHODS

Institutional Animal Ethical Committee approval was obtained from Yenepoya University, Derlakatte, Mangalore, Karnataka, India, before conducting the experiments dated 6th May 2010, Ref Ph.D 1/ 2010).

Plant material

The whole plant was collected from rural region of Manjanady, in Mangalore region, Karnataka, India, in the month of June – August 2010. It was authenticated by
(Prof) Dr. Krishna Kumar G., Chairman, Dept of Applied Botany, Mangalore University, Mangalore, Karnataka, India. The herbarium of the plant (voucher specimen no YU/LI/2010) has been deposited at Museum of Department of Pharmacology, Yenepoya Medical College, Yenepoya University, Mangalore, Karnataka, India.

**Extraction**

Leaves of *Leucas indica* were carefully separated, cleaned, shade dried, mechanically ground and coarsely powdered. The coarse powder was subjected to solvent extraction in Soxhlet extractor using 90% ethanol. The ethanolic extract was concentrated by vacuum distillation to dryness; the yield obtained was 15.5% w/w with respect to dried leaf. The obtained yield was stored in a desiccator. A suspension of the extract prepared in 1% gum acacia was used in present study.

**Sample Size, Grouping and Dose of the Drugs**

Animals were divided into 5 groups (Control, Standard & 3 groups of Test drug) containing 10 animals, making a total number of 50 animals.

**Drugs and Chemicals**

The Standard Indomethacin was obtained from our institutional pharmacy and 1% gum acacia from Department of Pharmacology, YMC, Mangalore.

**Anti-Inflammatory activity of EELLI by carrageenan induced paw oedema in wistar albino rats**

In this method, rats were divided into five groups of 10 animals each (Equal number of Male & Female). All the animals were pretreated with drugs / vehicle 60 minutes before injection of 1% carrageenan (0.1 ml). Swellings of carrageenan-injected foot were measured at zero, first, second, third, fourth and sixth hour using digital plethysmometer. The right hind paw was injected with 0.1 ml of vehicle. The animals which received indomethacin (10 mg/kg, p.o.) served as reference standard and those which received 1% gum acacia 3ml/kg served as control. Three doses of the test drug were selected (75, 150 & 300 mg/kg, p.o.) for test group of rats, and all the groups were subjected to screening for anti-inflammatory activity. The peak effect of the carrageenan induced oedema was observed at the 3rd hr after the injection. Increase in the paw volume at 3rd hr was calculated as percentage compared with the volume measured immediately after injection of carrageenan for each animal. The data was compared between groups.²

**Statistical analysis**

Results were expressed as mean ± SEM. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Dunnnett's Multiple Comparison test. P < 0.05 was considered statistically significant.

**RESULTS**

Oral administration of EELLI at doses of 150 mg/kg and 300mg/kg showed significant anti-inflammatory activity with a p value of p<0.01 and p<0.05 respectively by carrageenan induced paw oedema in wistar albino rats compared to control. The percentage inhibition of the oedema at 3rd hour was 52.58% and 36.87% at the dose of 150 mg/kg and 300 mg/kg respectively compared to control and standard groups (Table 1 & Figure 1).

<table>
<thead>
<tr>
<th>Table 1: Anti-inflammatory effect of ethanolic extract of leaves of <em>Leucas indica</em> (EELLI) by carrageenan induced paw oedema in rats (Acute study).</th>
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</thead>
<tbody>
<tr>
<td>Drugs</td>
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<tr>
<td>Control (1% Gum acacia (3ml/kg), p.o)</td>
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<tr>
<td>Indomethacin 10mg/kg, p.o</td>
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<td>EELLI 75 mg/kg, p.o</td>
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<td>EELLI 150mg/kg, p.o</td>
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<td>EELLI 300mg/kg, p.o</td>
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n=10. The observation are mean ± S.E.M. *p>0.05, **<0.05, *** p< 0.01 as compared to control (ANOVA followed by Dunnett’s multiple comparison test); EELLI- Ethanolic Extract of the leaves of *Leucas indica*; p.o – per orally.
Phytochemical studies on EELLI showed the presence of phytosterols, triterpenoids, flavonoids, lactones, fats and fatty acid, glycoside, phenolic compound and tannins. Phenolic compounds possess biological properties such as antiapoptosis, antiaging, anticancer, anti-inflammatory, antiatherosclerosis, improvement of endothelial function, inhibition of angiogenesis and cell proliferation activities. Presence of phenolic compounds could be the reason for its anti-inflammatory activity of EELLI.

However, further studies are required to evaluate its comprehensive analysis including quantitative / semi quantitative analysis, characterize its chemical structure and assess its pharmacotherapeutic activities with exact mechanism of action as an anti-inflammatory agent.

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

**REFERENCES**
