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RESEARCH ARTICLE

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ANTI INFLAMMATORY ACTIVITY OF FRESH TULSI LEAVES (*Ocimum Sanctum*) IN ALBINO RATS.

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ABSTRACT

Objective: To evaluate the anti-inflammatory activity of fresh ocimum sanctum (tulsi) leaves paste, suspended in gum acacia in experimentally induced acute anti-inflammatory animal models.

Methodology: Adult Albino rats of either sex weighing 150-250gms were randomly divided into 3 groups (n=6); control (vehicle), Standard (Indomethacin 100mg/kg) and fresh tulsi paste (500mg/kg). Each rat was fed orally with the respective drug 1hr prior to the administration of the phlogistic agent. The in vivo anti-inflammatory activity was studied using carrageenan induced paw edema.

Results: Anti-inflammatory activity is expressed as Percent Inhibition. The Percent Inhibition with the control, Standard (Indomethacin) and the test compound (Tulsi), in the carrageenan induced paw edema model were 0%, 76% and 67% respectively. The anti-inflammatory response of 500 mg/kg of the tulsi paste was found to be 88.15% as that of the response observed with 100 mg/kg of indomethacin.

Conclusion: The fresh tulsi leaf in its paste form also shows considerable anti-inflammatory activity in comparison to Indomethacin, with minimal side effects. Hence fresh tulsi leaves can be used as a potential adjuvant with the conventional anti-inflammatory drugs for the therapy of inflammation.

KEYWORDS

Ocimum sanctum, Tulsi, Anti inflammatory, Carrageenan.

INTRODUCTION

Inflammation is a protective attempt by the tissue, in response to harmful stimuli, such as pathogens, damaged cells, or irritants[1] through a complex biological response of vascular and non vascular tissues, which help in localization of infection or inflammation. The most common presentation of patient to the doctor is pain and inflammation. Treatment of inflammation is a debate as the conventional NSAIDS are commonest to cause Adverse Drug Reactions. Hence there is ongoing research to develop safer and more effective drugs for the therapy of inflammation.

Ocimum sanctum natively known as tulsi, or Holy Basil is an aromatic plant belonging to the family Lamiaceae. It is mentioned in the Charaka Samhita, an ancient Ayurvedic text [2] by Charaka for its medicinal importance. Its extracts are used in ayurvedic remedies for common colds, headaches, stomach disorders, heart disease, various forms of poisonings, malaria, in the management of neurological (e.g. convulsions & epilepsy), inflammatory [3] and allergic disorders. It is considered to be an adaptogen [4] and is regarded in Ayurveda as a kind of "elixir of life" and is believed to promote longevity [5]. The methanol extract and the aqueous extract of *Ocimum sanctum* has shown to inhibit acute as well as chronic inflammation in rats [6]. But no study has been done using a crude preparation of fresh tulsi leaves. Hence the current study was done to evaluate the anti-inflammatory activity of fresh ocimum sanctum (tulsi) leaves' paste.

MATERIAL AND METHODS

Preparation of extract- Fresh tulsi leaves were taken and triturated in a mortar with a pestle and made into a fine paste. This was then suspended in gum acacia and at a dose of 500 mg/kg [6] (a pilot study was done to assess the dose) was administered orally to the animals with the help of oral feeding tube.

Chemicals used- Indomethacin (100mg/kg), 1% Carrageenan - 0.05ml.

Animals- Adult albino rats of either sex weighing between 150 to 250 grams were randomly selected from central animal facility, JSS Medical College, Mysore. Animals were housed in groups of 3 at an ambient temperature of 25± 1ºC with ad libitum access to food and water. The study protocol was approved by Institutional Animal Ethics Committee.

METHODOLOGY: [7]

Animals were randomly divided into 3 groups of 6 rats each; I group: Control (1ml of 2% gum acacia suspension); II group: Standard drug (indomethacin 100mg/kg); III group: Test drug (fresh tulsi leaves paste 500mg/kg). Each rat was fed orally with the respective drug 1hr prior to the administration of the phlogistic agent. The in vivo anti-inflammatory activity was studied using carrageenan induced paw edema.

CARRAGEENAN INDUCED RAT PAW EDEMA MODEL: [7]

0.05 ml of 1% Carrageenan was injected aseptically into the sub plantar surface of right hind paw of each rat. Paw edema was measured by mercury plethysmograph (VGO Basile, Italy) at '0' hour and at the end of '4' hours. The difference between the '0' and '4th' hour reading gives the actual edema. Percentage inhibition (protection) against edema formation was taken as an index of acute anti inflammatory activity.

It was calculated by:
The percent inhibition of edema
$= 100 \left(\frac{vc - vt}{vc}\right)$
Where, V_c = mean paw edema volume in the control group.
V_t = mean paw edema volume in the drug treated group.

STATISTICAL ANALYSIS:
The effects of different drugs under study was presented by calculating the mean and S.D of the outcome parameters.
One way Analysis of Variance (ANOVA) and independent samples T test is applied to see the difference between any two groups at a time.
Tests of significance will be carried out at 5% level.
SPSS for windows (version 16) was applied in the statistical analysis.

RESULTS

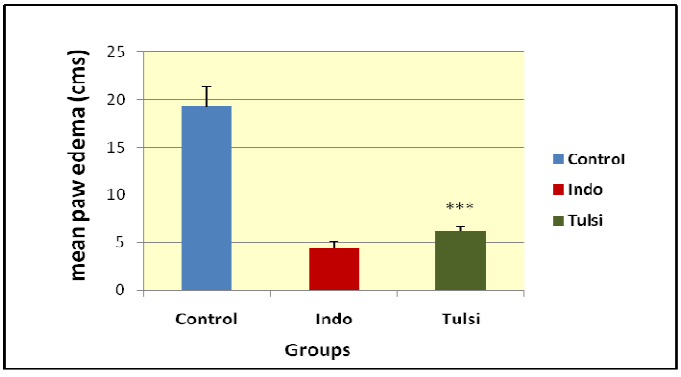
Anti-inflammatory activity is expressed as Percent Inhibition. The Percent Inhibition with the control, Standard (indomethacin) and the test compound (Tulsi), in the carrageenan induced paw edema model were 0%, 76% and 67% respectively. Results are displayed in table 1 and figure 1

Table-1
Effect of fresh Tulsi leaves paste (Ocimum sanctum) in Carrageenan Induced rat paw edema

Group	N	Mean paw edema(cm)± SD	Percent inhibition (%)
I control (1ml of vehicle)	6	19.2083±2.12965	0.0%
II indomethicin (100 mg/kg)	6	4.4583±0.62082***	76%
III fresh tulsi paste (500mg/kg)	6	6.1667±0.49160***	67%

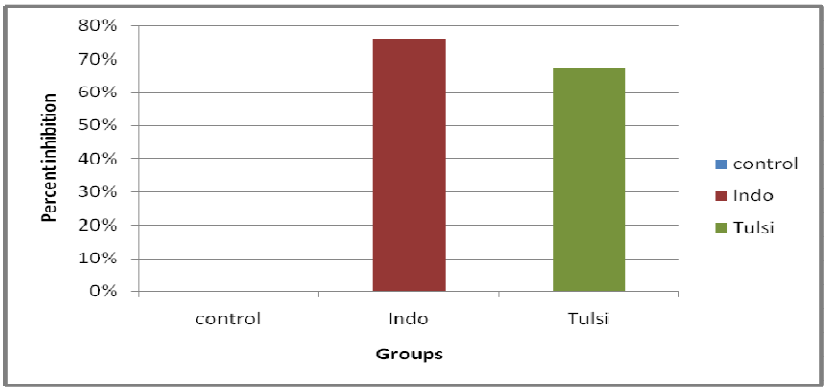
*Values are expressed as mean ± SD, n=6 animals per group, *p<0.05, **p<0.01, ***p<0.001 as compared to control (one-way ANOVA).*

Graph 1
Effect of fresh Tulsi leaves paste (Ocimum sanctum) in Carrageenan Induced rat paw edema



Graph 2

Percent inhibition (%) with the control, Standard (indomethacin) and the test compound (tulsi), in the carrageenan induced paw edema



DISCUSSION

Inflammation is a protective attempt by the organism to remove the injurious stimuli and to initiate the healing process. Inflammation can be classified as either acute or chronic. Acute inflammation is the initial response of the body to harmful stimuli and is achieved by the increased movement of plasma and leukocytes (especially granulocytes) from the blood into the injured tissues. A cascade of biochemical events propagates and matures the inflammatory response, involving the local vascular system, the immune system, and various cells within the injured tissue. Prolonged inflammation, known as chronic inflammation, leads to a progressive shift in the type of cells present at the site of inflammation and is characterized by simultaneous destruction and healing of the tissue from the inflammatory process. Anti-inflammatory drugs inhibit different stages of inflammation. Tulsi known as “Queen of plants”, “The mother medicine of nature” is a plant with enormous properties for curing and preventing diseases [8]. Various studies have been performed with Ocimum sanctum (tulsi) for its antibacterial, antioxidant, antiulceric, antimalarial, antidiabetic, anti-inflammatory, antilipidemic, anticancer and immunomodulatory properties [8].

Eugenol (l-hydroxy-2-methoxy-4-allylbenzene), the active constituent present in Ocimum sanctum *L.,* has been found to be largely responsible for the anti inflammatory property of tulsi[9]. It demonstrated 97% cyclooxygenase-1 inhibitory activity when assayed at 1000 µM concentration (pn) [10]. In studies conducted previously, the fixed oil of Ocimum sanctum was found to possess significant anti-inflammatory activity against carrageenan and other mediator-induced paw edema in rats [11]. The results of anti-inflammatory activity of Ocimum sanctum support the dual inhibition of arachidonate metabolism as indicated by its activity in inflammation models that are insensitive to selective cyclooxygenase inhibitors[11]. Linolenic acid present in O.sanctum fixed oil has the capacity to block both the cyclo-oxygenase and lipoxygenase pathways of arachidonate metabolism and could be responsible for the anti inflammatory activity of the oil [12].

Carrageenan induced paw edema has been used to study acute and sub acute phases of inflammation. It is a widely used irritant or a phlogistic agent. Chemically it is a sulfated polysaccharide from seaweeds. The inflammation induced by it is biphasic in nature. The first phase is attributed to the release of histamine, 5-HT and kinins, while the second

phase is related to the release of prostaglandins[13]. In our study fresh tulsi leaves paste (500 mg/kg) suspended in 2% gum acacia, p.o. significantly reduced edema induced by the Carrageenan. The percent inhibition of paw edema by indomethacin was 76% while that of tulsi paste is 67%. Hence fresh tulsi paste showed 88.15% anti inflammatory activity as that of standard indomethacin under the present experimental condition. The results obtained in this study using fresh paste of tulsi leaves are in concurrence with the previous studies undertaken using various extract forms. Thus the probable mechanism of anti-inflammatory effect of fresh tulsi paste may be due to dual inhibition of cyclo-oxygenase and lipoxygenase pathways of arachidonate metabolism through eugenol.

CONCLUSION

From the above results we can conclude that fresh tulsi leaves show considerably good anti inflammatory activity in comparison to indomethacin. Due to its easy availability, efficacy, safety and potency it can be used as an adjuvant along with the available anti inflammatory agents, with reduced dosage and minimal side effects for the therapy of inflammation. In this study fresh tulsi leaves have been used in its native form without any extraction. Hence if the general public is made aware of this information, tulsi can be used in its native fresh form when ever required for disorders associated with inflammation.

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