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Evaluation of *in-vivo* anti-inflammatory activity of *Syzygium aromaticum* oil in male wistar rats

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Abstract
Objective: The aim of the present study was to evaluate *in vivo* anti-inflammatory activity of *Syzygium aromaticum* oil (clove oil) following single dose oral administration (100, 250 and 500 mg/kg) in male wistar rats.
Methods: The *in-vivo* anti-inflammatory assay of clove oil was carried out using carrageenan induced paw edema model in male rats. Indomethacin was administered @ 10 mg/kg in standard drug control rats. Rats of control groups were kept untreated and other groups were treated with clove oil @ 100, 250 and 500 mg/kg b. wt., respectively.
Results: Clove oil showed dose dependent anti-inflammatory effect at various doses in wistar rats. The anti-inflammatory effect of clove oil was highest at 3h (35.77%) at the dose of 500mg/kg. It was lower than anti-inflammatory effect of standard drug indomethacin at 3h (41.75%).
Conclusion: Clove oil showed dose dependent anti-inflammatory activity in wistar rats.

Keywords: *Syzygium aromaticum*, clove oil, anti-inflammatory, paw edema

1. Introduction
Essential oils (EO) are complex mixtures of low molecular weight (usually less than 500 daltons) compounds extracted by steam distillation, hydrodistillation or solvent extraction [1]. Most of the time the bioactivities of a particular EO is decided by either one or two of its main components [2]. Plant essential oils possess various applications mainly in health, agriculture, cosmetic and food industries. Use of EOs in traditional systems of medicine is being practiced since ancient times in human history. These naturally occurring antimicrobials have extensive histories of their use in foods and can be identified from various components of the plants leaves, barks, stems, roots, flowers and fruit. Plant essential oils possess various applications mainly in health, agriculture, cosmetic and food industries. Use of EOs in traditional systems of medicine is being practiced since ancient times in human history. These naturally occurring antimicrobials have extensive histories of their use in foods and can be identified from various components of the plants leaves, barks, stems, roots, flowers and fruits [3-4-5]. Researchers from all over the world are trying to characterize a range of biological properties of EOs which includes anti-inflammatory, antimicrobial, antiviral, ant mutagenic, anticancer, antioxidant, immunomodulatory and antiprotozoal activities [2].
Plants of the genus Eugenia (Syzygium), comprising of about 100 species, grow in tropical climate in which *Syzygium aromaticum* or *Eugenia caryophyllata* plant is a high (up to 15 m), evergreen tree of the familyMyrtacae commonly called Clove in English and Laung in hindi [6]. Clove oil can be obtained from distillation of buds, leaf or stem, each resulting in an oil having different characteristics of oil. Clove bud oil is a colorless or yellow liquid. Clove buds contain 15 to 20 % of oil by weight. The main oil constituents are eugenol (70–95 %), eugenol acetate (up to 20 %) and β-caryophyllene (12–17 %). Various activities of clove oil were reported like an anti-inflammatory, analgesic, antiseptic, deworming, disinfectants and antibacterials because it inhibits the growth or kills most pathogens [8]. Clove oil is recommended for the treatment of sore throat, colds, catarrh and inflammation of the mucous membranes of the mouth. It is also helps to deal with breathing problems, general weakness and neuralgia [7]. Hence in the present study research was done to evaluate the anti-inflammatory activity of clove oil in carrageenan induced paw edema model in male rats.

2. Materials and Methods

2.1 Experimental animals

The study was conducted on adult healthy male wistar rats. Twenty five male rats (335 to 355 g) of 8-10 weeks of age were procured from Cadila Healthcare Ltd. (R & D Centre), Ahmedabad, Gujarat. The experimental protocol was approved by Institutional Animal Ethics Committee (Project No. IAEC/279/VPT/2018) at College of Veterinary Science and Animal Husbandry, Anand, Gujarat and protocols were followed according to the guidelines of committee for the purpose of control and supervision of experiments on animals (CPCSEA). The animals were housed in standard polypropylene cages and maintained under controlled room temperature (22 ± 2 ^{0}C) and humidity (55 ± 5%) with 12 h light and12 h dark cycle. All the rats were fed normal pellet diet and deionized water was provided *ad libitum* throughout the course of the experiment. All the rats were kept under acclimatization for 5 days prior to grouping and initiation of experiment. Rats were kept under constant observation during entire period of study. All necessary manage mental procedures were adopted to keep the rats free from stress.

2.2 Drugs and chemicals

Carrageenan (Non-gelling, mixture of λ & κ carrageenan) was purchased from Sigma-Aldrich, India. Indomethacin was purchased from local medical store of Anand district (Gujarat). Clove essential oil (Natural, Functional grade) was purchased from Sigma-Aldrich, India.

2.3 Preparation of carrageenan and indomethacin solution

For the preparation of 10% w/v carrageenan suspension, 0.5 gm carrageenan was weigh using digital analytical weighing balance which was dissolved in 5 ml of normal saline. For the preparation of Indomethacin suspension each 25mg capsule was dissolved in 5 ml of distilled water so each ml contains 5 mg/ml.

2.4 Induction of paw edema

The *in-vivo* anti-inflammatory assay of clove oil (*Syzygium aromaticum)* was carried out using rat paw edema method as described by [9]. All rats were injected subcutaneously with 0.1 ml of 10% w/v carrageenan suspension (0.1 ml of a 1% suspension in 10% saline) in the sub-planter region of the left hind limb as a local acute edema inducer after 30 minutes of oral administration of clove oil as well as indomethacin.

2.5 Experimental design

The present study was conducted on 25 male rats were dividing them in 5 various groups, having 5 rats in each group. Control rats were given *ad libitum* drinking water. Standard control rats were treated with Indomethacin (10 mg/kg, orally). Five rats in each group were treated with clove oil at the dose of 100, 250 and 500 mg/kg b. wt. orally, respectively.

2.6 Measuring of paw edema volume & Percent inhibition of inflammation

Edema was expressed as the increase in paw volume (ml). The paw volume was measured up to the tibiotarsal articulation. Volume of edematous paw was measured by using plethysmometer (PLM-01 plus, Orchid Scintific Instrument, India) at 0 h (before treatment), 1, 2, 3, 4, 6 and 24 hours after treatments. Percent inhibition of paw edema volume in male wistar rats was calculated.

$$(\%)\ \text{Inhibition} = \frac{\text{Mean paw volume (control)} - \text{Mean paw volume (treated)}}{\text{Mean paw volume (control)}}$$

2.7 Statistical analysis

All the data have been presented as mean ± SE. Statistical comparisons of the results were made using one way analysis of variance (ANOVA) using software SPSS (Version 25). Significant differences (p<0.05) between different experimental groups were determined by Duncan's test.

3. Results

The present study was conducted to evaluate *in-vivo* anti-inflammatory activity of clove oil @ 100, 250 and 500 mg/kg b.wt.in male rats. The result of anti-inflammatory effect was presented as change in paw volume (Table 1 and Figure 1) and percentage inhibition (Table 2 and Figure 2) in male wistar rats. The results revealed that clove oil showed anti-inflammatory effect with all three doses. The anti-inflammatory effect of indomethacin was highest at 3h (41.75%) as compare to other doses of clove oil. The anti-inflammatory effect of clove oil was highest at 3h (35.77%) at the dose of 500mg/kg. The anti-inflammatory activity of clove oil was found dose dependent in male wistar rats.

Table 1: Effect of oral administration of clove oil on carrageenan-induced paw edema (ml) in male wistar rats (Mean ± SE, n=5)

Group	0h	1h	2h	3h	4h	6h	24h
Control	0.72 ± 0.02	0.95 ± 0.01^{c}	1.14 ± 0.01^{d}	1.79 ± 0.03^{e}	1.72 ± 0.02^{d}	1.52 ± 0.02^{c}	0.91 ± 0.02^{b}
Indo	0.71 ± 0.02	0.82 ± 0.02^{a}	0.88 ± 0.03^{a}	1.04 ± 0.03^{a}	1.15 ± 0.02^{a}	1.16 ± 0.01^{a}	0.81 ± 0.04^{a}
SA-100	0.70 ± 0.01	0.88 ± 0.01^{b}	1.03 ± 0.02^{c}	1.38 ± 0.02^{d}	1.34 ± 0.01^{c}	1.29 ± 0.03^{b}	0.86 ± 0.04^{ab}
SA-250	0.71 ± 0.01	0.86 ± 0.01^{ab}	0.98 ± 0.01^{bc}	1.24 ± 0.01^{c}	1.22 ± 0.01^{b}	1.20 ± 0.01^{a}	0.83 ± 0.01^{ab}
SA-500	0.69 ± 0.02	0.84 ± 0.01^{ab}	0.94 ± 0.02^{b}	1.15 ± 0.02^{b}	1.16 ± 0.02^{a}	1.16 ± 0.04^{a}	0.81 ± 0.02^{a}

Mean value with dissimilar superscript in a column vary significantly at p<0.05
Indo = Indomethacin @ 10 mg/kg b.wt in male wistar rats
SA-100 = *Syzygium aromaticum* @ 100 mg/kg b.wt in male wistar rats
SA-250 = *Syzygium aromaticum* @ 250 mg/kg b.wt in male wistar rats
SA-500 = *Syzygium aromaticum* @ 500 mg/kg b.wt in male wistar rats

Table 2: Percent inhibition of paw edema in wistar male rats treated with clove oil

Group	1h	2h	3h	4h	6h	24h
Indo	13.84	23.04	41.75	33.06	24.09	11.87
SA-100	7.64	10.06	22.87	21.86	15.49	5.55
SA-250	9.56	13.88	30.78	29.20	20.92	8.62
SA-500	11.64	17.17	35.77	32.56	23.71	10.78

Indo = Indomethacin @ 10 mg/kg b.wt in male wistar rats

SA-100 = *Syzygium aromaticum* @ 100 mg/kg b.wt in male wistar rats
SA-250 = *Syzygium aromaticum* @ 250 mg/kg b.wt in male wistar rats
SA-500 = *Syzygium aromaticum* @ 500 mg/kg b.wt in male wistar rats

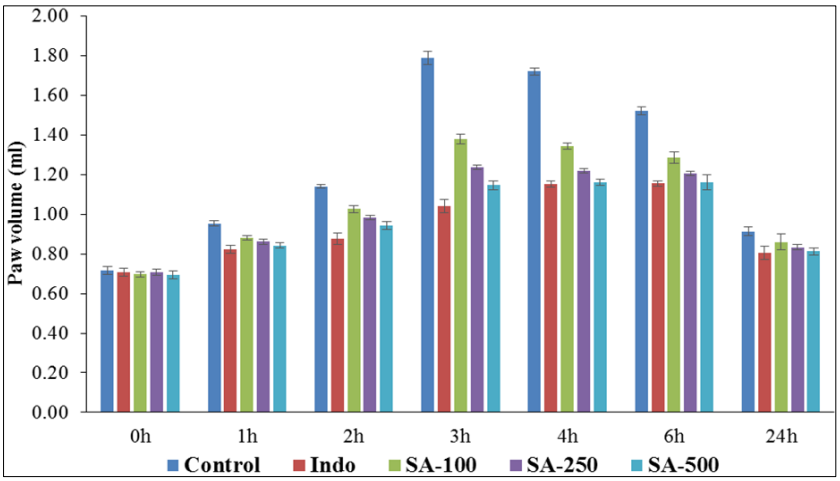

Fig 1: Effect of oral administration of clove oil on carrageenan-induced rat paw edema (ml) in male wistar rats (Mean ± SE, n=5)

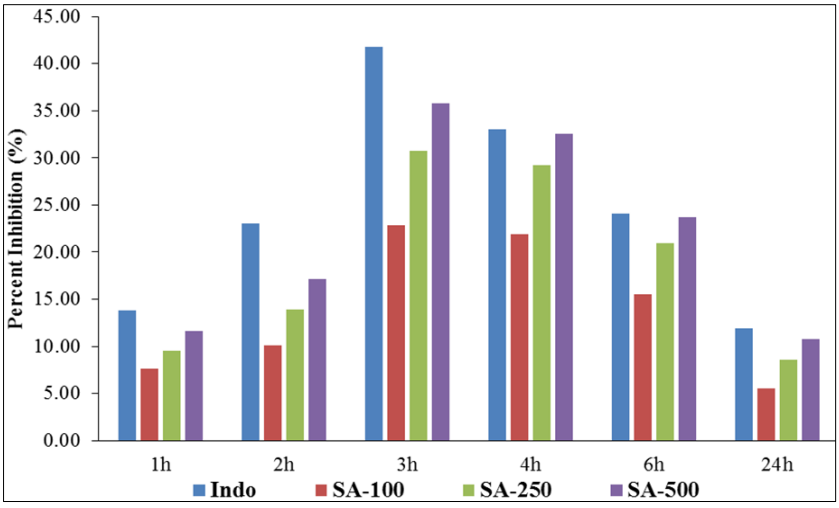

Fig 2: Percent inhibition of paw edema in male wistar rats treated with clove oil

4. Discussion

In the present study, the significant decrease in paw edema volume was observed in carrageenan induced male rats treated with indomethacin (10 mg/kg) and clove oil @ 100, 250 and 500mg/kg b. wt. treated rats. Clove oil showed dose dependent anti-inflammatory activity. Similar observations were reported for the anti-inflammatory activity of *Eugenia caryophyllata* oil at 0.025, 0.050, 0.100 and 0.200 ml/kg b. wt. in carrageenan induced paw edema in rats revealed 46.55, 90.15, 66.94 and 82.78% inhibition of inflammation, respectively [10]. Likewise anti-inflammatory activities of ethanolic extract of *syzygium aromaticum* flower bud in wistar rats at doses of 50, 100 and 200 mg/kg body weight were reported 42, 45 and 52% inhibition of inflammation at 5h observation [11]. Similar results were also reported for eugenol oil by inflammatory exudates volume in carrageenan-induced paw edema in rats at 100, 200 and 400 mg/kg body weight and result revealed that the oral administration of eugenol significantly inhibited paw edema 22.2, 40 and 41.1% at 2-4 h after carrageenan injection and the inhibition rate was comparable to that of indomethacin [12]. Anti-inflammatory activity of the aqueous extract of *syzygium aromaticum* in acute inflammation at 1 g/kg body weight in carrageenan induce paw edema model in rats reported 84% inhibition of paw edema as compare to control at 3h [13]. Anti-inflammatory activities of clove oil was studied in mice at a dose of 33 mg/kg body weight (i.p.) in which clove oil significantly suppressed the increased in paw thickness by 50.6% compared with control mice at 3h [14]. Similarly, anti-inflammatory effect of ethanolic extract of *syzygium aromaticum* in carrageenan induce paw edema in rats showed significant decreased in the edema size at efficacy rates of 79.41, 82.39 and 63.92% for the dose 500 mg/kg body weight at the 2nd, 4th and 6th h, respectively [15].

5. Conclusions

The present study revealed that oral administration of clove oil showed dose dependent anti-inflammatory activity in male wistar rats. The highest anti-inflammatory activity was observed at 3 hour post oral administration of clove oil in male wistar rats.

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