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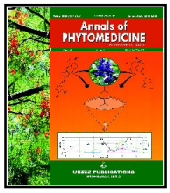

Original article

Anti-inflammatory activity of *Syzygium aromaticum* (L.) Merrill & Perry oil in carrageenan-induced paw edema in female rats

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Abstract

The present study was planned to evaluate *in vivo* anti-inflammatory activity of *Syzygium aromaticum* (L.) Merrill & Perry oil (clove oil), following single dose oral administration @ 100, 250 and 500 mg/kg in female wistar rats by using carrageenan-induced paw edema model. Twenty five rats were divided randomly into 5 groups and each group consists of five female rats. All rats were injected subcutaneously with 0.1 ml of 10% w/v carrageenan suspension subcutaneously as a local acute edema inducer after 30 min subsequent to oral administration of clove oil. Rats of control groups were kept untreated. Rats of standard control group were treated orally with indomethacin @ 10 mg/kg body weight as a reference standard drug. Rats of other three treatment groups were treated with clove oil @ 100, 250 and 500 mg/kg body weight, respectively. Edema was expressed as the increase in paw volume in ml and measured up to the tibiotarsal articulation. Volume of edematous paw was measured at 0 h (before treatment), 1, 2, 3, 4, 6 and 24 h after treatments. Increase in paw thickness was measured by using digital plethysmometer and per cent inhibition was calculated. The anti-inflammatory effect of clove oil was highest at 3 h (35.46 %) at the dose of 500 mg/kg. The anti-inflammatory effect of standard drug indomethacin (42.99 %) was higher than clove oil at 3 h. Clove oil at 3 h all doses gave higher anti-inflammatory effect. Clove oil showed dose dependent anti-inflammatory activity in female wistar rats.

Key words: *Syzygium aromaticum* (L.) Merrill & Perry, clove oil, anti-inflammatory, paw edema

1. Introduction

Plant essential oils possess various applications mainly in health, agriculture, cosmetic and food industries. Use of essential oils in traditional systems of medicine is being practiced since ancient time. Essential oils also called volatile or ethereal oil are aromatic oily liquid, obtained from different plant materials (flowers, buds, seeds, leaves, twigs, bark, wood, fruits and roots). Essential oils are complex mixtures of low molecular weight (usually less than 500 daltons) compounds extracted by steam distillation, hydrodistillation or solvent extraction procedures (Nakatsu *et al*., 2000). Researchers from all over the world are trying to characterize a range of biological properties of essential oils which includes antimicrobial, antiviral, antimutagenic, anticancer, antioxidant, anti-inflammatory, immunomodulatory and antiprotozoal activities (Bakkali *et al*., 2008).

Plants of the genus Eugenia (Syzygium), comprising of about 100 species, grow in tropical climate, in which *Syzygium aromaticum* (L.) Merrill & Perry or *Eugenia caryophyllata* Thun. plant is a high (up to 15 m), evergreen tree of the family-Myrtaceae (Gora, 2005). It is commonly called clove in English, laung in Hindi and laving in Gujarati. The clove is an aromatic, dry, fully grown, but unopened flower bud of clove tree. Clove is a tree which is growing in islands of Indonesia, Tanzania, Sri Lanka, Madagascar, India and Malaysia (Arung *et al*., 2011). In India, clove is mostly grown in the hilly tracts of Tamilnadu, Karnataka and Kerala states. Clove relieves stomach pain, nausea and vomition (Bhowmik *et al*., 2012). Clove has a deodorizing property and so used in perfumes and cosmetics (Daniel *et al*., 2009). Eugenol, a key compound found in clove is an excellent agent for prevention of metastasis related to oxidative stress (Nam, 2012).

Clove oil can be obtained from distillation of buds, leaf or stem, each resulting in oil having different characteristics. Clove bud oil is a colourless 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 %) (Guenther, 1950). Various activities of clove oil like an anti-inflammatory, analgesic, antiseptic, deworming, disinfectants and antibacterials because it inhibits the growth or kills most pathogens have been were reported (Nowak *et al*., 2012). The clove oil nanoemulsion is a potential source of natural antibacterial agents and to be used as food preservatives (Shahvi, 2016). The essential oil of clove has biocidal activity against *Aedes albopictus* (tiger mosquitos), thereby

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helping in the control of malaria (Bhat and Kempraj, 2009). Stress which is very common in every individual can also be relieved with the help of hydro-alcoholic extracts of clove oil (Singh *et al*., 2009). Clove oil is also used for the treatment of sore throat, colds, catarrh and inflammation of the mucous membranes of the mouth. It also helps to deal with breathing problems, general weakness and neuralgia (Cimanga *et al*., 2002). Clove and its essential oil has been found effective in poultry to improve growth performance, control some intestinal pathogens and stimulate digestion and also showed strong antimicrobial, antifungal, anti-inflammatory, anesthetic, anti-carcinogenic, anti-parasitic and antioxidant activities (Mitsch *et al*., 2004; Najafi and Torki, 2010). The alcoholic extract of clove buds showed significant antibacterial activity against *Propionibacterium acnes* and *Staphylococcus epidermis* (Singh *et al*., 2018). The clove oil and cinnamon oil showed promising *in vitro* antibacterial activity against both gram-positive (*Staphylococcus aureus, Listeria monocytogenes* and *Streptococcus agalactiae*) and gram-negative (*Salmonella typhimurium* and *Escherichia coli*) bacteria (Prajapati *et al*., 2018). Recently reported studies revealed promising anti-inflammatory effects of essential oils, *i.e.,* cinnamon oil showed anti-inflammatory effect in carrageenan-induced edema model in male and female wistar rats (Prajapati *et al*., 2019a; Prajapati *et al*., 2019b) and also similar study reported for clove oil in male wistar rats (Humbal *et al*., 2019). There are limited reports on the anti-inflammatory activity of clove oil. Hence, the present study was done to evaluate an anti-inflammatory activity of clove oil in carrageenan-induced paw edema in rats.

2. Materials and Methods

2.1 Experimental animals

The study was conducted on adult healthy female wistar rats. Twenty five female rats (220 to 250 g) of 8-10 weeks of age were procured from Cadila Healthcare Ltd., Ahmedabad, Gujarat. The experimental protocol was approved by Institutional Animal Ethics Committee (Project No. IAEC/279/VPT/2018) of 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°C) and relative humidity (55 ± 5 %) with 12 h light and12 h dark cycle. All the rats were fed normal pellet diet (mention name of company) and deionized water was provided *ad libitum* throughout the study period. All the rats were kept under acclimatization for 5 days prior to grouping and initiation of the experiment. All necessary managemental procedures were adopted to keep the rats free from stress.

2.2 Drugs and chemicals

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

2.3 Preparation of carrageenan and indomethacin solution

For the preparation of 10 % w/v carrageenan suspension, 0.5 g carrageenan was dissolved in 5 ml of normal saline. For the preparation of Indomethacin suspension, each 25 mg capsule was dissolved in 5 ml of distilled water to get the concentration of 5 mg/ml.

2.4 Induction of paw edema in rats

The *in vivo* anti-inflammatory assay was carried out using rat paw edema method as described by Winter *et al*. (1962). 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 min of oral administration of clove oil as well as indomethacin in respective groups as mentioned below.

2.5 Experimental design

All the rats were divided randomly in to 5 groups, having 5 rats in each group. Rats of control group (C1) were kept without ant treatment. Rats of standard control group (C2) were treated with single dose of indomethacin (10 mg/kg, orally). The rats of each treatment group (T1, T2, T3) 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

Inflammation in the form of edema was expressed as the increase in paw volume (ml). The paw volume was measured up to the tibiotarsal articulation using plethysmometer (PLM-01 plus, Orchid Scintific Instrument, India) at 0 h (before treatment), 1, 2, 3, 4, 6 and 24 h after treatments.

2.7 Percent inhibition of inflammation

Percent inhibition of paw edema volume in wistar rats was calculated using formula as (%) Inhibition = [Mean paw volume (control)–Mean paw volume (treated)]/Mean paw volume (control)

2.8 Statistical analysis

All the data have been presented as mean ± SE. Statistical comparison of the mean values in different groups was 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 body weight in female wistar rats. During the study period, no clinical signs of toxicity were observed in rats upon oral administration of clove oil. In the present study, peak inflammation was observed between 3-4 h after subcutaneous injection of carrageenan suspension (0.1 ml of a 1% suspension in 10% saline) in the sub-planter region of the left hind limb.

The result of anti-inflammatory effect is presented as change in paw volume in Table 1 and also graphically depicted in Figure 1 and percentage values of inhibition of inflammation by different treatments are presented in (Table 2 and graphically depicted in Figure 2). The results revealed that clove oil showed anti-inflammatory effect with all three doses. The anti-inflammatory effect of indomethacin was highest at 3 h (42.99%) as compared to

other doses of clove oil. The anti-inflammatory effect of clove oil was highest at 3h (35.46%) at the dose of 500 mg/kg. The anti-inflammatory activity of clove oil was found dose dependent in this experiment. In our study, the significant decrease in paw edema volume was observed in carrageenan-induced inflammation in wistar rats treated with indomethacin (10 mg/kg) and clove oil @ 100, 250 and 500 mg/kg b.wt. Clove oil showed dose dependent anti-inflammatory activity.

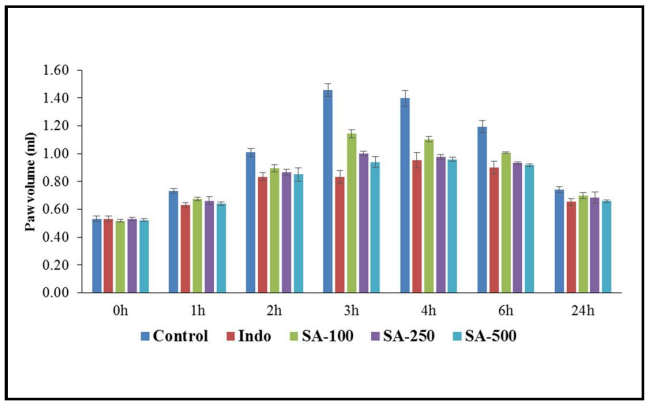

Figure 1: Effect of oral administration of clove oil on carrageenan-induced rat paw edema (ml) in female wistar rats.

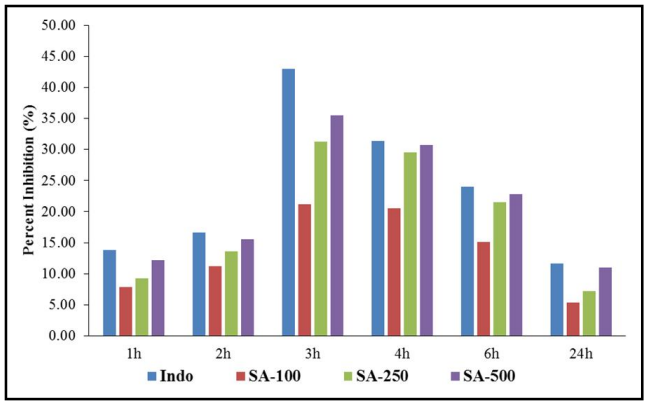

Figure 2: Percent inhibition of inflammation in wistar female rats under different treatments.

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

Group	0 h	1 h	2 h	3 h	4 h	6 h	24 h
Control(C1)	0.53 ± 0.02	0.73 ± 0.02^{b}	1.01 ± 0.03^{b}	1.46 ± 0.05^{d}	1.4 ± 0.06^{c}	1.19 ± 0.04^{c}	0.74 ± 0.02^{b}
Indo(C2)	0.53 ± 0.02	0.63 ± 0.02^{a}	0.84 ± 0.03^{a}	0.83 ± 0.05^{a}	0.95 ± 0.06^{a}	0.90 ± 0.04^{a}	0.65 ± 0.02^{a}
SA-100(T1)	0.52 ± 0.01	0.67 ± 0.01^{a}	0.90 ± 0.03^{a}	1.14 ± 0.03^{c}	1.10 ± 0.02^{b}	1.01 ± 0.01^{b}	0.70 ± 0.02^{ab}
SA-250(T2)	0.53 ± 0.01	0.66 ± 0.03^{a}	0.87 ± 0.02^{a}	1.00 ± 0.02^{b}	0.98 ± 0.02^{a}	0.93 ± 0.01^{a}	0.69 ± 0.04^{ab}
SA-500(T3)	0.52 ± 0.01	0.64 ± 0.01^{a}	0.85 ± 0.05^{a}	0.94 ± 0.04^{b}	0.96 ± 0.02^{a}	0.92 ± 0.01^{a}	0.66 ± 0.01^{a}

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

Table 2: Percent inhibition of inflammation in wistar female rats under different treatments

Group	1 h	2 h	3 h	4 h	6 h	24 h
Indo	13.78	16.67	42.99	31.41	24.00	11.66
SA-100	7.85	11.16	21.16	20.52	15.10	5.35
SA-250	9.23	13.61	31.22	29.50	21.53	7.18
SA-500	12.20	15.52	35.46	30.76	22.81	10.95

Indo = Indomethacin @ 10 mg/kg b.wt in wistar rats
SA-100 = *Syzygium aromaticum* @ 100 mg/kg b.wt
SA-250 = *Syzygium aromaticum* @ 250 mg/kg b.wt
SA-500 = *Syzygium aromaticum* @ 500 mg/kg b.wt

4. Discussion

Similar to the present study, many researcher were utilised this carrageenan-induced paw oedema model in rats to evaluate *in vivo* anti-inflammatory activity in rats. Reported studied on the *in vivo* anti-inflammatory effect of ethanolic stem bark extract of *Cordia africana* was carried out using carrageenan-induced paw edema model in rats (Tijjani *et al*., 2015), Similarly, Sridevi *et al.* (2017) also reported topical anti-inflammatory activity of an herbal gel containing solanesol extracted from tobacco scrap using carrageenan induced paw edema model in albino wistar rats. Likewise, recently the anti-inflammatory activity of rutin (100 mg/kg) and meloxicam (5 mg/kg) following its intramuscular administration was also assessed using carrageenan-induced paw edema model in rats (Modi *et al*., 2019).

In support to our findings, 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 body weight in carrageenan-induced paw edema in rats revealed 46.55, 90.15, 66.94 and 82.78% inhibition

of inflammation, respectively (Ozturk and Ozbek, 2005). Likewise, anti-inflammatory activity of ethanolic extract of *S. aromaticum* flower bud in wistar rats at 50, 100 and 200 mg/kg body weight were reported with 42, 45 and 52% inhibition of inflammation at 5 h post administration (Tanko *et al*., 2008). 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 by 22.2, 40 and 41.1% at 2-4 h after carrageenan injection and the inhibition rate was comparable to that of indomethacin (Daniel *et al*., 2009). Rodrigues *et al*. (2009) investigated the *in vivo* effect of water-soluble part of hydro-alcoholic extract of clove on pro-inflammatory cytokines (IL-1 beta and IL-6) production by macrophages in BALB/c mice. Results showed that the treatment of mice with water extract of clove was found to inhibit macrophages to produce both IL-1 beta and IL-6 which suggest an anti-inflammatory action of hydro-alcoholic extract of clove. Likewise, Grespan *et al.* (2012) reported the efficacy of eugenol, a compound obtained from the essential oil of cloves (*S. aromaticum*) in collagen-induced arthritis. Treatment with eugenol starting at the onset of arthritis (day 25) ameliorated these clinical signs of collagen-induced arthritis. Anti-inflammatory activity of the aqueous extract of *S. aromaticum* in acute inflammation at 1 g/kg body weight in carrageenan induce paw edema model in rats has been reported with 84 % inhibition of paw edema as compare to control at 3 h (Ahmad *et al*., 2012). Anti-inflammatory activity of clove oil was studied in mice at a dose of 33 mg/kg body weight intraperitoneal in which clove oil significantly suppressed the increase paw thickness by 50.6 % compared with control mice at 3 h (Taher *et al*., 2015). Similarly, anti-inflammatory effect of ethanolic extract of *S. aromaticum* in carrageenan-induced paw edema in rats has been reported with significant decrease in the edema at efficacy rates of 79.41, 82.39 and 63.92 % at the dose of 500 mg/kg body weight at 2nd, 4th and 6th h, respectively (Saeed *et al*., 2017). Nikoui *et al.* (2017) studied the anti-inflammatory effect of clove oil in thirty adult male dogs which were divided into four groups after surgical incision on abdomen. They reported that in the clove oil (25 mg/kg) treated animals, there was significant decrease in edema as compared to control dogs. Singh *et al.* (2018) evaluated anti-inflammatory effect of the clove bud oil (100, 200, 400, 800 and 1000 µg/ml) and they observed dose-dependent anti-inflammatory response with increasing concentration of the clove bud oil. However, the essential oil (1%, v/v) exhibited significant effect comparable results to that of diclofenac taken as reference standard. In support to our study, the anti-inflammatory effect of clove oil was highest at 3 h (35.77%) at the dose of 500 mg/kg in carrageenan-induced paw edema in male wistar rats and it showed dose dependent anti-inflammatory effect at various doses (Humbal *et al*., 2019). In another reported studies on essential oil, following single dose oral administration (50, 100 and 200 mg/kg) of cinnamon oil (*C. zeylanicum*) showed significant dose dependent anti-inflammatory activity in male and female wistar rats were reported (Prajapati *et al*., 2019a; Prajapati *et al*., 2019b).

5. Conclusion

The present study revealed that oral administration of clove oil showed dose dependent anti-inflammatory activity @ 100, 250 and 500 mg/kg body weight in female wistar rats. Rat paw edema model was successfully developed by injecting subcutaneously 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.

The anti-inflammatory effect of indomethacin was higher as compare all three doses of clove oil treated rats. The highest anti-inflammatory activity of all three doses of clove oil was observed at 3 h post oral administration in female wistar rats. However, further studies are required to elucidate the mechanism behind this anti-inflammatory effect.

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Conflict of interest

The authors declare that there are no conflicts of interest in the course of conducting the research. All the authors had final decision regarding the manuscript and decision to submit the findings for publication.

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