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Research Article

ANTI-INFLAMMATORY EFFECT OF *ELETTARIA CARDAMOM* OIL ON CARRAGEENAN-INDUCED PAW EDEMA USING RATS BASED ON TUMOR NECROSIS FACTOR α, INTERLEUKIN 6, AND INTERLEUKIN 1 LEVELS IN SERUM

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ABSTRACT

Objective: In this study, we evaluated the anti-inflammatory effect of *Elettaria cardamom* oil and the underlying mechanism using *in vivo* models of inflammation.

Methods: Male Sprague–Dawley rats, 4-6 weeks old, weighing 120-130 gms are used for the study. The anti-inflammatory study of *E. cardamom* oil was studied by injecting 0.1 ml of 1% carrageenan to the subplantar region of the right hind paw of rats. The development of acute inflammation was measured at the end of every 1st, 2nd, 3rd, 4th, 5th, and 6th h using plethysmometer.

Results: As results from the above study, *E. cardamom* oil at a dose of 0.175 ml/kg was less significant than that of *E. cardamom* oil at a dose of 0.280 ml/kg when given orally. A $p<0.05$ shows a significant decrease in paw edema. It also reduced the levels of pro-inflammatory cytokines such as tumor necrosis factor α, interleukin (IL) 1, and IL 6 levels in the serum. The histopathology results also showed a significant reduction of congested blood vessels with no marked impression for inflammation.

Conclusion: *E. cardamom* oil possesses anti-inflammatory activity in dose-dependent manner as they inhibit the levels of pro-inflammatory cytokines.

Keywords: Carrageenan, *Elettaria cardamom* oil, Pro-inflammatory cytokines, Orally, Subplanter, Plethysmometer, Dose dependent, Anti-inflammatory.

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INTRODUCTION

Inflammation is the local defense of the body in response to the injury or irritant typically presented with edema, pain, redness of the skin, and heat [1]. Inflammation is the common problem found in general population, and it has a great impact in affecting health of the individuals [2]. Nonsteroidal anti-inflammatory drugs are the first line anti-inflammatory drugs that are used worldwide. The side effect of the drugs includes gastric ulcer, renal damage, hepatic damage, and cardiac abnormalities [3,4]. They act by inhibiting cyclooxygenase-1 (COX-1) and COX-2, thereby preventing the synthesis of thromboxanes and prostaglandins [5,6]. the use of NSAIDS produces adverse effects of nausea, vomiting, dyspepsia, gastric ulceration and bleeding, diarrhea, kidney problems etc., [7]. *Cardamom* oil is that which is probably extracted from the seeds of *Cardamom* and whose scientific name is found to be *Elettaria cardamom* oil. *Cardamom* oil has many health benefits and plays as a vital component of overall health [8]. The health benefits of the oil are antispasmodic, antiseptic, antimicrobial, digestive, stomachic, stimulant, and diuretic [9].

MATERIALS AND METHODS

Animals

Male Sprague–Dawley rats, 4-6 weeks old, are obtained from the animal house at TANUVAS, Chennai. They were housed in an air-conditioned room and fed up with a standard laboratory diet and tap water throughout the experiments. Rats weighing 120-130 g are used. The study protocol was approved by the Institutional Animal Ethical Committee XIX/VELS/PCOL/13/2000/CPCSEA/IAEC/03.10.2016.

Drugs and chemicals

Diclofenac and carrageenan obtained from Sigma Aldrich were used in the study. Furthermore, rat tumor necrosis factor (TNF-α) Elisa kit, rat interleukin (IL-6) Elisa kit, and rat IL-1α Elisa kit were obtained from Invitrogen and used for the study. All chemicals used in the study are of analytical grade.

Experimental design

For the experiment, the animals were divided into 4 groups with 6 animals in each group.

- Group-I - Received carrageenan (0.1 ml of a 1% suspension in 0.85% saline, subplantar)
- Group-II - Received standard drug (diclofenac 10 mg/kg, p.o.) + carrageenan (0.1 ml of a 1% suspension in 0.85% saline, subplantar)
- Group-III - Received *E. cardamom* oil (0.175 ml/kg, p.o.) + carrageenan (0.1 ml of a 1% suspension in 0.85% saline, subplantar)
- Group-IV - received *E. cardamom* oil (0.280 ml/kg, p.o.) + carrageenan (0.1 ml of a 1% suspension in 0.85% saline, subplantar).

Carrageenan-induced paw edema method

This is one of the most commonly employed methods for the screening of acute inflammation. All the groups were treated with a single dose of the respective drug, and 1 h after the administration of the drugs, acute inflammation was produced by subplantar injection of 0.1 ml of freshly prepared suspension of 1% carrageenan in normal saline, to the right hind paw of the rats. The paw was marked at the level of the lateral malleolus and immersed every time up to this mark. The paw volumes were measured at 0 h, 1 h, 2 h, 3 h, 4 h, 5 h, and 6 h after the carrageenan injection using digital plethysmometer (Orchid scientific, India). The percentage inhibition of paw edema at each time interval was calculated using following formula:

Edema (%) inhibition = (1-D/C) 100

Where,

D - represents the mean of paw volume after the administration of test drugs to the rats.

C - represents the mean of increased volume in the control groups.

Estimation of TNF α, IL-1, and IL-6 levels in serum

After completion of carrageenan-induced paw edema experiment, rats were anesthetized and blood samples were collected from orbital sinus. The serum was separated by allowing blood to clot followed by centrifugation, and the samples were stored at −20°C until use. TNF α, IL-6, and IL-1 levels from each sample were measured in duplicate with highly sensitive rat TNF α Elisa kit, rat IL-6 Elisa kit, and rat IL-1α Elisa kit, respectively, specifically designed for rats, according to manufacturer's instructions.

Histopathological examination

Under deep anesthesia, biopsies of the paw were taken 6 h after injection of carrageenan. The tissue slices were fixed with 10% neutral buffered formaldehyde dehydrated by graded ethanol and embedded in paraffin. Then, 5 mμ thick section of slide was taken then stained with hematoxylin and eosin. The slides were dried and viewed using an Olympus microscope, and the photography was taken with 400 times magnification.

Statistical analysis

The statistical analysis of the evaluation of the anti-inflammatory activity of *E. cardamom* oil against the carrageenan-induced paw edema in rats was analyzed using ANOVA followed by Dunnett's multiple comparison test and expressed as mean ± SEM. Differences between the mean of control groups and treated animals were considered statistically significant at $p<0.05$.

RESULTS

As seen from the above study, in the carrageenan-induced rat paw edema in acute inflammation, the standard drug (diclofenac 10 mg/kg) showed maximum inhibition at the end of the 1st, 2nd, 3rd, 4th, 5th, and 6th h. The inhibition of paw edema was maximum at the end of 3rd h for all the drugs, and the effect of *E. cardamom* oil at a dose of 0.175 ml/kg was less significant than that of *E. cardamom* oil at a dose of 0.280 ml/kg when given orally. There is no significant difference of inhibition of paw edema between *E. cardamomum* oil at a dose 0.280 ml/kg and the standard group at the end of 5th and 6th h in Table 1.

The change in paw edema produced with the 0.175 ml/kg and 0.280 ml/kg was compared with the standard drug similarly treated. The test drug produced the anti-inflammatory effect in a dose-dependent manner.

The effect of *E. cardamom* oil was studied in rats by observing its anti-inflammatory activity induced by carrageenan. The values (Table 2) shows that administration of *E. cardamom* oil in doses of 0.175 ml/kg/p.o. and 0.280 ml/kg/p.o. are statistically significant in reducing the paw inflammation. The effect *E. cardamomum* on carrageenan-induced rat paw edema at different hours of the study was compared to that of the positive control group for the evaluation of anti-inflammatory activity on the basis of percent inhibition of paw edema volume.

The experiment showed (Table 1) that the test drug exhibited statistically significant ($p<0.05$) inhibition of paw volume in a dose-dependent manner. A significant inhibition of paw edema was observed with both doses tested till the 6th h. However, maximum inhibition of paw edema was found to be in Group II 33.48%, and although the inhibition in paw oedema found with the test doses was found to have a dose dependent effect compared with the reduction in paw volume of the standard drug diclofenac.

The histological examination of paw section of rats treated with carrageenan as shown in Fig. 1a revealed a fibromuscular tissue densely infiltered by lymphocytes with few congested blood vessels. Fig. 1b revealed fibromuscular tissue showing very few congested blood vessels with no significant inflammatory changes noticed. Fig. 1c and d also showed few congested blood vessels with significant inflammatory changes in dose-dependent manner.

The levels of pro-inflammatory cytokines TNF α, IL6, and IL1α were increased (Table 2) with the control group, and the levels started to decline after 6th h. This clearly shows that the administered *E. cardomum* oil inhibited the levels of pro-inflammatory cytokines.

DISCUSSION

The carrageenan-induced edema in rat hind paw is the most widely used primary test for screening anti-inflammatory agent and is

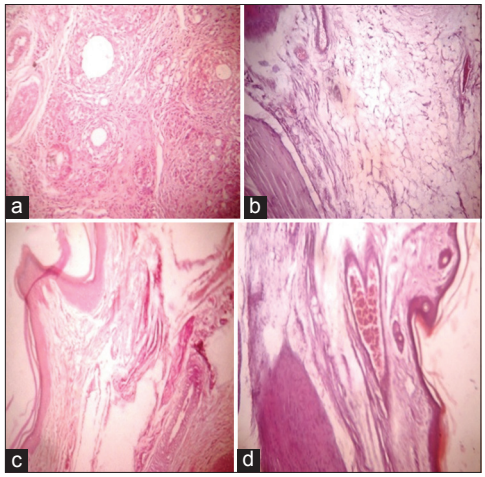

Fig. 1: Histopathological examination of paw sections at ×400, 6 h after carrageenan injection. (a) positive control group, (b) diclofenac 10 mg/kg, p.o., (c) *Elettaria cardamom* oil 0.175 ml/kg, p.o, and (d) *Elettaria cardamom* oil 0.280 ml/kg, p.o

Table 1: Mean increases in paw volume (edema) in ml (mean±SEM), (% inhibition)

Groups	Initial paw volume 0 h (ml), % inhibition	Mean increase in paw volume at various time intervals (ml) and percentage inhibition of edema in various time intervals					
		1st h	2nd h	3rd h	4th h	5th h	6th h
Group I	1.22±0.08	1.84±0.12	2.22±0.10	2.36±0.14*	2.48±0.12*	2.60±0.08**	2.78±0.04**
Group II	1.21±0.10, (0.81)	1.40±0.06 (23.91)	1.72±0.08* (22.52)	1.84±0.12* (22.03)	1.58±0.10* (36.29)	1.35±0.08* (48.07)	1.24±0.04** (55.59)
Group III	1.18±0.12, (3.27)	1.48±0.14 (19.56)	1.76±0.16 (20.72)	1.88±0.12 (20.33)	1.60±0.08* (35.48)	1.38±0.06* (46.92%)	1.24±0.02* (55.39)
Group IV	1.19±0.04, (2.45)	1.44±0.08 (21.73)	1.74±0.12 (21.62)	1.86±0.10 (21.38)	1.56±0.08** (37.09)	1.34±0.06*** (48.46)	1.22±0.04** (56.11)

Values are represented as mean±SEM of the six animals in each group. Statistically significant was studied using student t-test followed by ANOVA. Followed by Dunnet's multiple comparison test. *$p<0.05$ is significant, **$p<0.01$ is more significant, and ***$p<0.001$ is most significant

Table 2: Measurement of levels of pro-inflammatory cytokines - TNF α, IL 6, and IL 1 levels in blood serum

Groups in the study	TNF α (pg/ml)	IL 6 (pg/ml)	IL 1 (pg/ml)
Group I carrageenan (0.1 ml of a 1% suspension in 0.85% saline, subplantar region of the right hind paw) (control)	707.50±66	605.25±43	252.50±26
Group II (*E. cardamom* oil, 0.175 ml/kg, p.o.) + carrageenan (0.1 ml of a 1% suspension in 0.85% saline, subplantar region of right hind paw)	380.45±48**	250.44±22**	115±16**
Group III (*E. cardamom* oil, 0.280 ml/kg, p.o.) + carrageenan (0.1 ml of a 1% suspension in 0.85% saline, subplantar region of right hind paw)	440.24±42*	430.25±28*	180±20*
Group IV (diclofenac 10 mg/kg, p.o.) + carrageenan (0.1 ml of a 1% suspension in 0.85% saline, subplantar region of right hind paw)	480.34±56**	390.32±18*	165±38**

Values are expressed as mean±SEM of six animals for each group. Statistical significance was studied using student t-test followed by ANOVA. Followed by Dunnets multiple comparison test. *p<0.05 is significant and **p<0.001 is more significant when compared to the controlled group. *E. cardamom: Elettaria cardamom,* TNF: Tumor necrosis factor, IL: Interleukin

considered as a standard model of inflammation for screening anti-inflammatory activity of different compounds [10]. The development of edema after injection of carrageenan is a biphasic event [11-13]. The initial phase of inflammation which is observed during the 1 h is attributed to the production of histamine, leukotriene, and possibly COX product [14]. While on second phases, carrageenan-induced inflammatory response has been linked to neutrophil infiltration and the production of neutrophil-derived free radicals such as hydrogen peroxide, superoxide, and hydroxyl radicles, as well as released of other neutrophil-derived mediator [15,16-21].

The result of the studies that showed that *E. cardamom oil* significantly suppressed the rat hind paw edema induced by subplanter injection of carrageenan. In the current study, the anti-inflammatory effect of *Cardamom* oil was further evaluated to elucidate the underlying mechanism involved in this animal model.

CONCLUSION

The result of the current study suggested that *E. cardamom* oil passed anti-inflammatory effects in carrageenan-induced rat paw edema, which is comparable to diclofenac. The anti-inflammatory mechanism of *E. cardamom* oil may be related to the reduction of TNF-α, IL1, and IL6 that could result in inhibition of COX-2 expression and its product PGE2.

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