

Evaluation of anti-inflammatory activity of ethanolic extract of *Cananga odorata* Lam in experimental animals

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ABSTRACT

Background: The current study evaluates the anti-inflammatory activity of ethanolic extract of *Cananga odorata* Lam (EECO) in experimental animals.

Methods: Acute toxicity test was done following OECD guidelines. Carrageenan induced paw edema method in Wistar Albino rats were used in this study. Aspirin in the dose of 300 mg/kg was used as the standard drug and three doses of EECO (100 mg/kg, 200 mg/kg and 400 mg/kg b.w. p.o) were used as the test drug. The results were measured at 1st hr, 3rd hr and 5th hr after carrageenan injection.

Results: EECO in the doses of 100 mg/kg, 200 mg/kg and 400 mg/kg showed highly significant anti-inflammatory activity ($p < 0.001$) ($p < 0.001$) ($p < 0.001$) at 3rd hr and ($p < 0.001$) ($p < 0.001$) ($p < 0.001$) 5th hr, respectively. In doses of 100 mg/kg, 200 mg/kg, and 400 mg/kg of EECO showed the percentage of inhibition of 62.9% which is more than the standard drug aspirin, which showed 60.14% inhibition.

Conclusion: EECO has significant anti-inflammatory activity.

Keywords: Ethanolic extract, *Cananga odorata* Lam, Anti-inflammatory activity

INTRODUCTION

Inflammation is the earliest organic response before tissue damage or infection. Before a tissue injury, the local accumulation of prostaglandins, thromboxane's, and other chemical mediators cause a change in the threshold nociceptors, resulting in hyperalgesia.^{1,2} The inflammatory process is part of a mechanism of host defense against stimuli that cause injuries, but when this process is not controlled, can damage the health of the individual.³ The cardinal signs that identify the inflammation are heat, flushing (redness), tumor (swelling), pain, and loss of function, of which the first four were described by Cornelius Celsus.⁴

Cananga odorata belongs to Annonaceae (Custardapple family); it is called as Ylang-Ylang in English, ban champak in Hindi. It is a fast growing tree that attains a height of 12 m. It grows in full or partial sun and prefers acidic soil. It distributed in both tropical and subtropical regions. The flower, seeds, and leaves yield highly fragrant essential oil.⁵

The distilled oils are used in perfumes, shampoos, creams, and lotions, but also in ice creams, candies and baked goods flavours.^{5,6} Ylang-Ylang oil is used in the food industry as the flavoring ingredient. It is approved as a food additive by Food and Drug Administration.⁷

It is used for asthma, malaria, fever, cholera, typhoid, dermatitis, ulcers and wounds. Aroma therapist claim that oil is useful for depression, distressed breathing, hypertension, anxiety, as an aphrodisiac.⁸ The phytochemical bioactive components are alkaloids, carbohydrates, glycosides, saponin, tannin, phenol, flavonoids, steroids.⁷ It was shown to have anti-inflammatory effects which are mediated by inhibition of cyclooxygenase-2 enzyme,⁷ lipoxygenase inhibitory effect, and inhibition of leukotrienes.⁹

Likewise, studies have shown that *C. odorata* possesses analgesic and anti-inflammatory activity. Thus, an effort will be made in the present study to evaluate its anti-inflammatory effect.

METHODS

Plant material

The fruits of *C. odorata* were collected from the Dhanwantri garden of University of Horticulture of Bagalkot district, Karnataka, India in the month of November 2014 and it was authenticated by Mr. Harish. B.S. (Asst. Prof, Medicinal and Aromatic Crops) and the specimen (Voucher number: SNMC/Pharma 006), is kept in Department of Herbarium.

Preparation of plant extract

The fruits of the plant were dried under shade for a period of 2 weeks. The dried fruit was milled to a fine powder using a mechanical grinder. The material was extracted with 50% ethanol using soxhlet extraction apparatus and it was evaporated to dry at 60°C. Dried fruits (40 g) of *C. odorata* yielded 8 g of crude extract. The solid residues were stored in the airtight container and preserved in the refrigerator at -20°C.¹⁰ From this stock, fresh preparations were obtained whenever required.

Acute oral toxicity study

It was done according to Organization for Economic Co-operation and Development (OECD) guidelines 425 (up and down procedure). All the five mice were administered 2000 mg/kg of ethanolic extract of *C. odorata* (EECO) orally and observed continuously for a period of 14 days, every hourly for 24 hrs, and every day for 14 days for its movements, grooming activity, exploring activity, writing reflex, and convulsion etc.,¹¹

Experimental animals

All the animals were procured from the Central Animal house, Department of Pharmacology, S. N. Medical College, Bagalkote. Wistar albino rats of either gender weighing 150-200 g were selected for the experiment. Pregnant rats, animals with an infection, animals with injuries, deformities were excluded from the study. Prior to and during study, all the animals were maintained under standard animal house conditions at 12:12 hrs dark: light cycle, 25±2°C, and 35-60% humidity and other micro and macro environment conditions as suggested by Committee for the Purpose of Control and Supervision of Experiment on Animals (CPCSEA). All animals were housed in a polypropylene cage covered with a stainless steel wire mesh and a paddy husk bed, with adequate provision for feed and water. All the animals were maintained on standard laboratory diet (VRK Nutritionals, Pune) and water was provided *ad libitum*. The study was started after getting the Institutional Animal Ethics Committee approval (IAEC/SNMC Reg. No. 829/AC/04/CPCSEA).

Anti-inflammatory activity

Carrageenan induced paw edema

The test was carried out in healthy Wistar albino rats of either sex weighing 150-250 g. After 12 hrs fasting 30 animals were randomly divided into 5 groups of 6 animals each. Group I received 0.5 ml of normal saline (control group), Group II received 300 mg/kg of aspirin (standard group), Group III, IV, and V received EECO (test groups). All the drugs were given orally. After 1 hr, all the animals received 0.05 ml of 1% w/v carrageenan in normal saline solution intradermally in the left hind paw. Right paw served as the control for the same animal. The paw edema volume was measured using the plethysmograph by measuring fluid displacement at 1 hr, 3 hrs, 5 hrs after carrageenan injection.¹²

Phytochemical analysis

The ethanolic extract of *C. odorata* was qualitatively analyzed for alkaloids, flavonoids, tannin, glycosides, carbohydrates saponin, phenol, and steroids.¹¹

Statistical analysis

All the data were analyzed using one-way ANOVA followed by *post-hoc* test. The results were expressed as mean±standard error of mean and $p < 0.05$ was considered as significant.

RESULTS

Acute oral toxicity study

No adverse effect or mortality was detected in Swiss albino mice at 2 g/kg of EECO using five animals. All the animals were alive, healthy, and active during the observational period of 14 days. So the LD 50 was considered as >2000 mg/kg.

Carrageenan-induced paw edema: Table 1 and Figure 1 show the anti-inflammatory activity of EECO in wistar albino rats by Carrageenan-induced paw edema method.

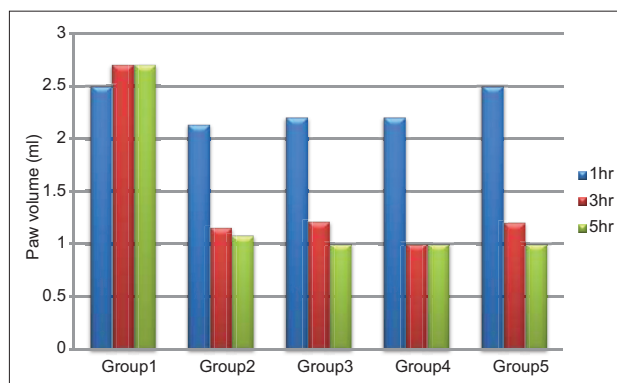


Figure 1: Paw edema - paw volume (ml).

Table 1: Carrageenan-induced paw oedema.

Group	Mean±SEM			Percentage of inhibition at 5 hrs
	1 hr	3 hrs	5 hrs	
Control	2.5±0.44	2.7±0.41	2.7±0.84	-
Standard (300 mg/kg)	2.13±0.17	1.15±0.19***	1.08±0.20***	60.14
Test (100 mg/kg)	2.2±0.26	1.21±0.21***	1.0±0.00***	62.9
Test (200 mg/kg)	2.2±0.22	1.0±0.00***	1.0±0.0***	62.9
Test (400 mg/kg)	2.5±0.52	1.2±0.27***	1.0±0.0***	62.9

*p<0.05, **p<0.01, ***p<0.001. All the values are expressed as mean±SEM (n=10). SEM: Standard error mean

Control group has shown maximum edema around 3 hrs after carrageenan injection. "Mean paw volume" for the control group was 2.7±0.84 ml, which was considered as 100% edema in the present study.

Test drug in the doses of 100 mg/kg, 200 mg/kg and 400 mg/kg body weight per orally produced highly significant inhibition of paw volume compared to control group (mean paw volume is 1.0±0.00 ml, 1.0±0.00 ml, 1.0±0.00 ml; p<0.001, p<0.001, p<0.001) at 5th hr. Test drug showed highly significant reduction in paw volume in the doses of 100 mg/kg, 200 mg/kg, and 400 mg/kg with 62.9%, 62.9%, 62.9% of inhibition of paw volume, which is higher than that of aspirin 300 mg/kg body weight (mean paw volume is 1.08±0.20 ml, p<0.001 and 60.14% of inhibition of paw volume).

DISCUSSION

The extracts derived from fruits of EECO exhibited significant anti-inflammatory activity in Wistar albino rats.

The phytochemical study of EECO possess alkaloids, carbohydrates, glycosides, saponin, tannin, phenol, flavonoids, and steroids.⁹

According to acute oral toxicity study, LD50 was considered as more than 2000 mg/kg body weight. Carrageenan induced hind paw edema is the standard experimental model of acute inflammation. Carrageenan is the phlogistic agent of choice for testing anti-inflammatory drugs as it is not known to be antigenic and is devoid of apparent systemic effects. Moreover, the experimental model exhibits a high degree of reproducibility.^{13,14}

Carrageenan-induced paw edema is a biphasic response. The first phase is mediated by the release of histamine, serotonin, and kinins whereas the second phase is related with the release of prostaglandin and slow reacting substances which peaks at 3rd hr.

In this method, the mean paw volume was found to be 2.7±0.84 ml in the control group. Test drug at the doses of 100 mg/kg, 200 mg/kg, and 400 mg/kg showed 62.9%, 62.9%, and 62.9% of inhibition of paw volume, which is higher than that of aspirin 300 mg/kg body weight (60.14%) of inhibition of paw volume.

The extract of EECO contains flavonoids, glycosides, alkaloids, and tannins. It is suggested that some flavonoids blocks both cyclooxygenase and lipoxygenase pathway is blocked.^{7,15} There are few reports on the role of tannins in anti-inflammatory activity.¹⁶ In the present study, the anti-inflammatory activity of *C. odorata* Lam. might be attributed to the presence of flavonoids and tannins.

CONCLUSION

Here, in this research work, we found that EECO possess significant anti-inflammatory activity in experimental animals. The plant can be recommended for the further studies to isolate the active ingredients.

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Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Animal Ethics Committee.

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