Int J Pharm Bio Sci 2015 April; 6(2): (P) 467 - 474

Research Article **Pharmacology**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

EVALUATION OF ANALGESIC ACTIVITY OF ETHANOLIC EXTRACT OF CANANGA ODORATA LAM IN EXPERIMENTAL ANIMALS

YASMEEN A MANIYAR[1] AND JANAKI DEVI C.H.[*2]

Department of Pharmacology, S Nijilingappa Medical College, Bagalkot, Karnataka, India.

ABSTRACT

The present study evaluates the central and peripheral analgesic activity of ethanolic extract of Cananga Odorata Lam [EECO] in experimental animals.Acute toxicity test was done following OECD guidelines. EECO (100mg/kg, 200mg/kg and 400mg/kg b.w. p.o) was evaluated for central and peripheral analgesic activity by the Tail flick method in wistar rats and 0.7%acetic acid induced Writhing test in Swiss albino mice respectively. Aspirin was used as the standard drug in the dose of 300 mg/kg b.w. in tail flick method and 100 mg/kg b.w. in acetic acid induced writhing test. EECO significantly increased the reaction time in tailflick method($p<0.01$) at all the doses and decreased the number of writings in Writhing test ($p<0.01$) at all the doses.EECO has significant central and peripheral analgesic activity.

KEYWORDS: Ethanolic extract, Cananga Odorata Lam, Analgesic activity, Writhing test, Tail flick method

***Corresponding author**

JANAKI DEVI C.H

Department of Pharmacology, S Nijilingappa Medical College, Bagalkot, Karnataka, India.

INTRODUCTION

Pain is a symptom of many diseases requiring treatment with analgesics. It is the most important symptom that brings the patient to the physician[1,2] . The term pain is derived from Latin “poena” and the Greek “poine” meaning “punishment”[3,4]. It is described as an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage[5,6]. Pain perception involves two components, the nociceptive component and affective component. Based on a clinical diagnosis of pain it is classified as somatic pain, visceral pain, referred pain, psychogenic/functional pain and neuropathic pain. *Canaga Odorata Lam* belongs to Annonaceae (custardapple family) it’s called as Ylang-Ylang in English, Ban champak in hindi. It is a fast growing tree that attains height of 12m. It grows in full or partial sun and prefers acidic soil. It is distributed in both tropical and subtropical regions. The flower, seeds and leaves yield highly fragrant essential oil.[7] The distilled oils are used in perfumes, shampoos, creams and lotions, but also in ice creams, candies and baked goods flavours[7,8]. Ylang-Ylang oil is used in the food industry as a flavouring ingredient. It is approved as a food additive by FDA[9]. It is used for asthma, malaria, fever, cholera, typhoid, dermatitis, ulcers and wounds. Aroma therapist claims that oil is useful for depression, distressed breathing, hypertension, anxiety, as a aphrodisiac[10]. The phytochemical bioactive components are steroids, alkaloids, carbohydrates, glycosides, saponin, tannin, phenol, flavonoids[11]. It is shown to have anti-inflammatory effects which is mediated by inhibition of COX-2 enzyme[9], lipoxygenase inhibitory effect and inhibition of leukotrienes[9].

MATERIALS AND METHODS

Plant material

The fruits of Canaga 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.

Prepration 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 grinder. The material was extracted with 50% ethanol using soxhlet extraction apparatus and it was evaporated to dry at 60˚C. Dried fruits (40gm) of Cananga Odorata yielded 8g of crude extract. The solid residues were stored in the air tight container and preserved in refrigerator at -20˚C.[12] From this stock, fresh preparations were obtained when ever required.

Acute oral toxicity study

It was done according to the Organization for Economic Co-operation and Development (OECD) guidelines 425 (up and down procedure). All the five mice were administered 2000mg/kg of EECO orally and observed continuously for a period of 14 days, every hourly for 24 hours, and every day for 14 days for its movements, grooming activity, exploring activity, writing reflex, and convulsion etc.[13]

Experimental Animals

All the animals were procured from the Central Animal house, Department of Pharmacology, S N Medical College, Bagalkot. Wistar albino rats of either gender weighing 150-200g and Swiss albino mice of either sex weighing 20-30 g were selected for the experiment. Pregnant rats, animals with 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 hr dark: light cycle, 25±2°C, and 35%-60% humidity and other micro and macroenvironment conditions as suggested by CPCSEA (Committee for the purpose of control and supervision of experiment on animals). 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 RegNo 829/AC/04/CPCSEA).

Analgesic activity

1. Acetic acid induced writhing

It tests the peripheral analgesic activity. Following 12 hrs fasting 30 healthy Swiss albino mice of 25-30g were randomly divided into 5 groups of 6 animals each. Group I received 0.5 ml of normal saline (control group), Group II received 100 mg/kg of aspirin (standard group) [14], Group III, IV, V received EECO (100mg/kg, 200 mg/kg, 400 mg/kg) respectively. All the drugs were given orally. After 1 hr all the animals received 10ml/kg of 0.7% v/v acetic acid injection intraperitoneally. Number of writhings were counted between 5 and 20 min after acetic acid injection.[15]

2. Tail flick method

This method is used to screen the central analgesic activity. The test was carried out in healthy Wistar rats. 30 animals weighing 150-250g were randomly divided in to 5 groups of 6 animals each after 12 hrs fasting. Group I received O.5 ml of normal saline (control group), Group II received 300mg/kg of aspirin (standard group) [16], Group III, IV, V received EECO (100mg/kg, 200 mg/kg, 400 mg/kg) respectively. All the drugs were given orally. After ½ hr, lhr, 2hr, 3hr the tail flick response was carried out and the reaction time was measured by placing the distal 1 /3rd of the tail about l cm from the radiant heat source. The time taken by the animal to withdraw the tail was taken as the reaction time. Cutoff time was kept as 20-30s. The animals showing reaction time of >20-30 were excluded from the study.[17]

Phytochemical analysis

The Ethanolic extract of Cananga Odorata was qualitatively analysed for steroids, alkaloids, flavonoids,tannin,glycosides, carbohydrates, saponin and phenol[11].

Stastical analysis

All the data were analysed by using one-way ANOVA followed by Posthoc Test. The results were expressed as Mean $\pm$ SEM and $p<0.05$ was considered as significant.

RESULTS

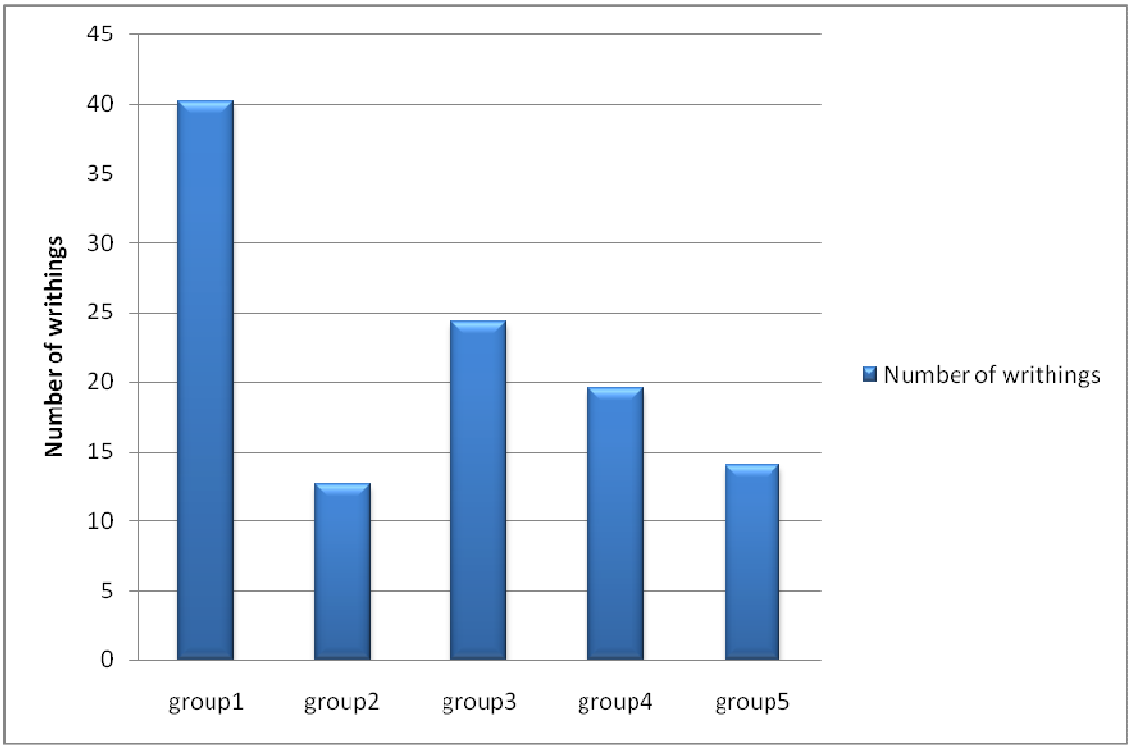
Acute oral toxicity study

No adverse effect or mortality was detected in Swiss albino mice at 2g/kg of EECO by 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 >2000mg/kg.

Peripheral analgesic activity

Acetic acid induced writhing test

Table 1 and Graph 1 shows the analgesic activity of the EECO on acetic acid induced writhing test in albino mice. In the present study the test drug at all the doses of (100mg/kg, 200mg/kg, 400mg/kg) of EECO showed significant reduction in the number of writhings($p<0.001$). The test drug at the dose of, 100mg/kg, 200mg/kg and 400mg/kg produced 24.3 $\pm$ 2.26%,19.6 $\pm$ 1.71% and 14.01 $\pm$ 2.6 % inhibition of writhings movements compared with the control group. The standard drug aspirin showed 68.45% inhibition of writhing movements. The number of writhings was significantly reduced in both the test and standard groups. The peripheral analgesic activity of test drug at 400mg/kg is comparable to that of the standard drug of aspirin 100mg/kg ($p<0.001$).



Graph 1
Number of writhings

Table 1
Number of writhings and percentage of inhibition of acetic acid induced writhing test

Group	Mean±Standard	Percentage of inhibition (%)
Control	40.17 ± 2.40	-
Standard (100mg/kg)	12.67 ± 1.21***	68.45
Test (100mg/kg)	24.3 ± 2.26***	39.5
Test(200 mg/kg)	19.6 ± 1.71***	51.2
Test(400 mg/kg)	14.01 ± 2.6***	65.1

Post-hoc test: when compared with control; *p<0.05, **p<0.01, ***p<0.001. All the values are expressed as mean ±SEM(n=10). SEM=Standard error mean.

Central analgesic activity
Tail flick method

Table 2 and Graph 2 shows the analgesic activity of the EECO on the Tail-flick method in Albino rat. There was no significant difference between mean reaction time of different groups ($p>0.05$) at 0hr. The control group showed the mean reaction time of 10.75 ± 0.79 sec at 3rd hr. Test groups showed increase in the reaction time significantly after 1/2hr in the doses of 100mg/kg, 200mg/kg and 400mg/kg body weight, per orally compared to the control group, (mean reaction time 14.35 ± 2.04 sec, 13.13 ± 1.12 sec and 15.51 ± 0.95 sec, $p<0.01$, $p<0.001$, $p<0.001$). Test drug at the dose of 400mg/kg body weight, per orally showed highly significant increase in reaction time(mean reaction time 41.45 ± 3.15 sec, $p<0.001$) at 3rd hr which is comparable to that of standard drug aspirin 300mg/kg body weight, per orally (mean reaction time 20.56 ± 2.50 sec, $p<0.001$).

Graph 2
Mean reaction time at different hours

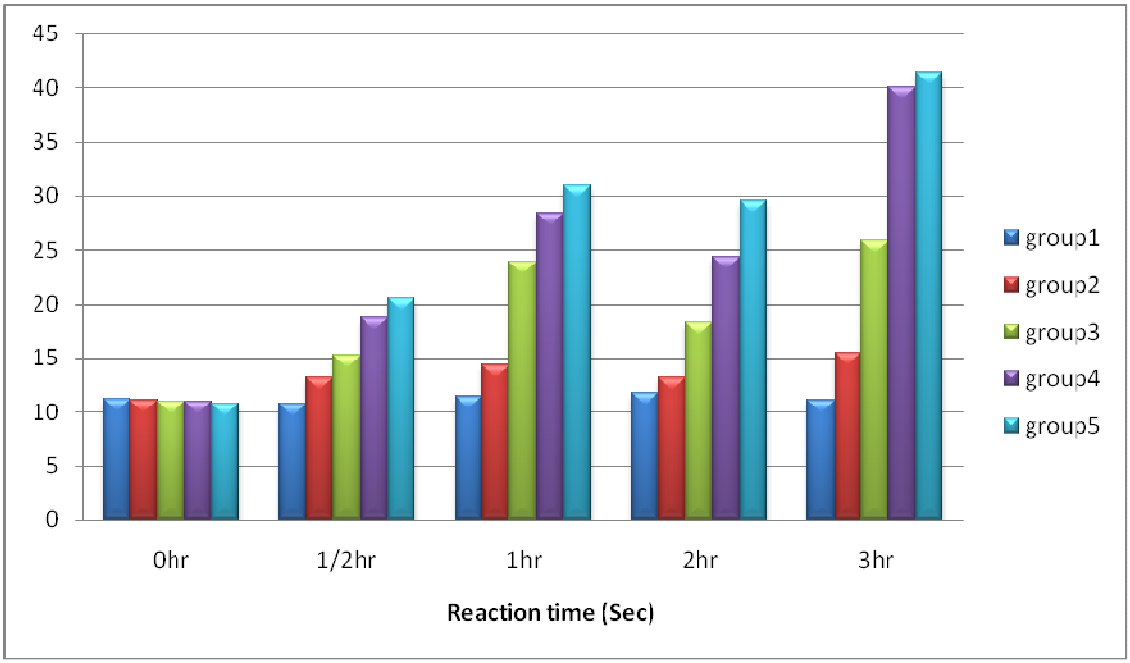


Table 2
Reaction time (seconds) in Tail-flick method

Group	0hr M ± S	½ hr M ± S	1hr M ± S	2hr M ± S	3hr M ± S
Group1 Control	11.2 ± 0.52	11.08±0.64	10.96 ± 0.73	10.99 ± 0.57	10.75 ± 0.76
Group2 Standard Aspirin (300 mg/kg)	10.73 ± 1.86	13.18 ± 1.71**	15.21 ± 1.88***	18.75 ± 1.97***	20.56 ± 2.50***
Group3 Test (100mg/kg)	11.40 ±0.88	14.35 ± 2.04**	23.89 ± 5.86**	28.38 ± 5.93***	30.98 ± 6.61***
Group4Test (200 mg/kg)	11.73 ±1.02	13.13 ±1.12***	18.19 ± 3.78***	24.35 ±4.46	29.5 ± 2.95***
Group5 Test (400 mg/kg)	11.06 ± 1.17	15.51 ± 0.95***	25.87 ± 5.48***	40.06 ± 5.83***	41.45 ± 3.15***

Post-hoc test: when compared with control;*p<0.05, **p<0.01,p<0.001. All the values are expressed as mean ±SEM(n=10). SEM=Standard error mean,***

DISCUSSION

The extracts derived from fruits of EECO exhibited significant analgesic activity in Swiss albino mice and wistar albino rats. The phytochemical study of EECO possess alkaloids, carbohydrates, glycosides, saponin, tannin, phenol, flavonoids and steroids[11]. Peripheral analgesic activity of EECO was evaluated by using Writhing test in mice according to the method of Koster R et al(1959)[18,19]. The extract of EECO exhibited significant analgesic activity in albino rats by inhibiting acetic acid induced writhing, which is a model of visceral pain. Intraperitoneal injection of acetic acid produces pain through activation of chemosensitive nociceptors[18], or irritation of the visceral surface, which lead to the liberation of histamine, bradykinin, prostaglandins and serotonin[19]. The test drug at the dose of 400mg/kg body weight produced 14.01 $\pm$ 2.6 writhing movements in 20 minutes duration. The percentage of protection from writhing test at 400mg/kg was 65.1%. In the present study standard drug aspirin produced 12.67 $\pm$ 1.21 writhings and 68.45% of protection at the dose of 100mg/kg. The results obtained with the test and standard drugs were significant when compared to the control. The test drug, however was found to be equally effective as that of standard drug aspirin (100mg/kg body weight). Although the writhing response test is very sensitive, it has poor specificity of analgesic screening tail flick test was conducted to confirm and study the possible analgesic mechanism of *Cananga Odorata Lam.* Central analgesic activity was evaluated by using the Tail flick test which is considered to be a spinal reflex induced by heat according to Schumacher et al. (1940),Wolff et al. (1940)[20,21],but could also involve higher neural structures (central analgesic activity).[22] In the Tail flick method, a mean of reaction time of control was 10.75 $\pm$ 0.76 sec at 3rd hour. Standard drug aspirin at the dose of 300mg/kg body weight showed the mean reaction time of 20.56 $\pm$ 2.50sec at 3rd hour and test drug in the dose of 400mg/kg showed the mean reaction time of 41.45 $\pm$ 3.15sec at third hour which is comparable and higher to that of the standard drug. EECO at a dose of 100mg/kg, 200 mg/kg, 400 mg/kg showed significant activity from 30 min. The extract of EECO contains steroids, flavonoids, glycosides, alkaloids and tannins. It is suggested that some flavonoids blocks both cyclooxygenase and lipoxygenase pathway is blocked[9, 23]. There are few reports on the role of tannins in analgesic ativity[24]. Previous studies suggest that alkaloids also involve in analgesic action[25]. In the present study the analgesic activity of Cananga Odorata Lam might be attributed to the presence of Steroids, flavonoids, tannins and alkaloids. The significant increase in the threshold of pain by tests and standard in these models suggests involvement of central pain pathways involving dopaminergic descending noradrenergic and serotonergic systems or via peripheral mechanisms involved in the inhibition of prostaglandins, leukotrienes and other endogenous substances that are key players in pain[26].

CONCLUSION

Here in this research work it was found that EECO showed significant analgesia by tail flick method and acetic acid induced writhing test i.e both central and peripheral analgesic activity in experimental animals. The plant can be recommended for the further studies to isolate the active ingredients.

ACKNOWLEDGEMENT

Further, the authors wish to extend their gratitude to the anonymous Reviewers for their valuable comments in improvising the research article.

REFRENCES

1. Tripathi KD, editor. Essentials of Medical Pharmacology. 7th ed. New Delhi: Jaypee Brothers, p.401,2013.
2. Joint Commission on Accreditation of Healthcare Organizations (JCAHO). Pain Assessment and Management an Organizational Approach. OakbrookTerrace: Joint Commission Resources, Inc, p.1,2000
3. Baumann TJ. Pain management. In: Dipiro JT, editor. Pharmacotherapy Apathophysiologic approach. 6th ed. Newyork: McGraw Hill; p.1089–121,2005.
4. Stimmel B, editor. Pain, Analgesia and Addiction: The pharmacology of pain. New York: Raven Press; 1,2,63,241–5,259,266,1983.
5. Merskey H, Bogduk N. Task force on taxonomy of the IASP. Classification of chronic pain - Descriptions of chronic pain syndromes and definitions of pain terms. 2nd ed. Seattle: IASP Press; p. 209-14,1994.
6. Welch SP, Martin BR. Opioid and Nonopioid Analgesics. In: Craig CR, Stitzel RE, editors. Modern Pharmacology with Clinical Applications. 6th ed. Philadelphia, USA: Lippincott William & Wilkins;p.310–29,2004.
7. Hsieh TJ, Chang FR, Chia YC, Chen CY, Chiu HF, Journal of natural products ;64:616-19,2001.
8. Burdock GA, Carabin IG. Safety assesmentof ylang-yalang oil as a food ingredient. Food and Chemical Toxicology, Vol.46,p.433,2008.
9. Fulekar MH, Pathak B, Kale RK. Environment and sustainable Development for anti-inflammatory of cananga.p.69
10. Manner HI, Elevith CR, Cananga odorata(ylang-ylang), Species profile for specific island agroforestry. www.traditionaltree.org.
11. Hook F, Thomson. Phytochemical analysis of leaf extracts of calophyllum inophyllum L. and cananga odorata (Lam). IOSR journal of pharmacy and biological sciences. sep—oct;3(2);35-7,2012.
12. Erturk O. Antibacterial and antifungal activity of ethanolic extract from eleven spice plants. Biologia Bratislava ,61(3).275-8,2006.
13. Manjamalai A, Singh SS, Guruvayoorappa C, Grace VM. Analysis of phytochemical constituents and anti-microbial activity of some medicinal plants in Tamil Nadu, India. Glob J Biotechnol Biochem ,5(2):120-8,2010.
14. Maniyar Y, Bhixavathimath P. Antihyperglycemic and hypoglycemic activities of aqueous extract of Carica papaya Linn. Leaves in alloxan-induced diabetic rats. Journal of Ayurveda and Integrative Medicine, 3(2):70-4,2012.
15. Rajveer CH, Swarnalatha CH et al. Synthesis of 6-Bromooxo quinazoline derivatives and their pharmacological activities. International Journal of Chemistry Research, 1(1): 21-4,2010.
16. Sharma US, Sharma UK, Sutar N et al. Evaluation of analgesic, anti-pyretic and anti-inflammatory activities of Andropogan Muricatus root extract. Journal of Pharmacy Research, 3(7):1652-4,2010.
17. Panthong A et al. Anti-inflammatory, analgesic and antipyretic activities of gamboges from Garcina hanburyi Hook f. Journal of Ethnopharmacology,111(2): 335-40,2007.
18. Indumathy S, Kavimani S. Analgesic activity of angiotensin antagonists. African Journal of Pharmacy and Pharmacology, 5(2):194-8,2011
19. Koster R, Anderson M, de Beer EJ. Acetic acid for analgesic screening. Fed Proc, 18:412.138,1959.
20. Taber RI, Greenhouse DD, Rendell JK, Irwin. Agonist and antagonist interactions of opioids on acetic acid-induced abdominal stretching in mice. J Pharm Exp Ther S,169:29-38,1969.

21. Jayaraman R, Anitha T, Joshi VD. Analgesic and anticonvulsant effect of Acorus calamus roots in mice. International Journal of Phann Tech Research , 2(1):552-5,2010.
22. Schumacher GA, Goodell H, Hardy JD, Wolff HG. Uniformity of the pain threshold in man. Science, 92: 110-2,1940.
23. Wolff HG, Hardy JD, Goodell H. Studies on pain. Measurement of the effect of morphine, codeine, and other opiates on the pain threshold and an analysis of their relation to the pain experience. JClin Invest,19:659-80,1940.
24. Vinegar R, Schreiber W, Hugo R. Biphasic development of carrageenin edema in rats. J Pharmacol Exp Ther,166(1):96-103,1969.
25. Chakraborty A, Devi RK, Rita S, Sharatchandra K, Singh TI. Preliminary studies on anti-inflammatory and analgesic activitieas of Spilanthes acmella in experimental animal models. Indian J pharmacol,36(3):140-50,2004.
26. Mishra D, Ghosh G, Kumar PS, Panda PK. An experimental study of analgesic activity of selective COX-2 inhibitor with conventional NSAIDs. Asian journal of Pharmaceutical and clinical research,4(1):78-81,2011.