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Original Article

COMPARATIVE ANALGESIC STUDIES OF LEAF AND STEM BARK OF *CALOPHYLLUM INOPHYLLUM* IN SWISS ALBINO MICE

MIRZA DANISH BAIG*, SYED BASHEER UDDIN, ASRA BEGUM

Department of Pharmacology, Anwarul Uloom College of Pharmacy, New Mallepally, Hyderabad 500001, Andhra Pradesh, India.
Email: spaciodino@gmail.com

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ABSTRACT

Objectives: The aim of the present study was to perform acute oral toxicity studies and to compare and evaluate the analgesic effect of ethanolic extracts of leaf and stem bark of *Calophyllum inophyllum* on Swiss albino mice.

Methods: Eddy's hotplate method and acetic acid induced writhing were implemented to determine analgesic properties of the extracts. Both the extracts were administered orally. Acute oral toxicity studies were conducted using the OECD guidelines 423 Annexure – 2d.

Results: The results indicate the mortality was not observed during the acute oral toxicity studies and maximum safe does was determined. The analgesic effect of the extracts showed significant dose dependent effect (100 mg/kg b.w and 200 mg/kg b.w) on both models of algesia i.e., Eddy's hotplate method and acetic acid induced writhing.

Conclusion: The comparative studies between the leaf and stem bark of *Calophyllum inophyllum* suggests that *Calophyllum inophyllum* leaf extract showed more activity compared to *Calophyllum inophyllum* stem bark extract.

Keywords: *Calophyllum inophyllum*, *Calophyllum inophyllum* leave extract, *Calophyllum inophyllum* stem bark extract, Analgesic effect, Eddy's hotplate method, Acetic acid induced writhing.

INTRODUCTION

The genus *Calophyllum* belongs to the family Clusiaceae which are native to Tropical Asia and its geographical distribution area also includes Melanesia and Polynesia. It grows near the sea coast throughout India [1]. The Tamanu tree is 2-3 m high, and has a thick trunk covered with a rough, black and cracked bark. It has elliptical, shiny and tough leaves. Its flowers, arranged in axillary cymes, have a sweet, lime-like fragrance. The tree, which flowers twice a year, is said to attain a great age [2]. Pain is a private experience with complex sensory, affective and evaluative qualities that must be measured if peoples in distress are to be helped [3].

Although acute pain has a protective role as a warning system, chronic pain such as neuropathic pain is produced by dysfunction or damage to the peripheral or central nervous system and can persist for days, months or even years after nerve injury [4]. The present study is to evaluate acute oral toxicity and compare the analgesic effects of ethanolic extracts of leaf and stem bark of *Calophyllum inophyllum*.

Flavonoids are known to target prostaglandins which are involved in the late phase of acute inflammation and pain perception; hence its presence in the ethanolic extracts of the plant may be contributory [5]. Presence of xanthones may also be the factors contributing to the analgesic effect [6].

MATERIALS AND METHODS

Plant material and preparation of plant extracts:

The plant *C. inophyllum* was collected from "Sri Kotla Vijaybhaskar Reddy Botanical Garden", Hyderabad, India. The plant was identified by a taxonomist (Annexure – I) and voucher specimens representing *Calophyllum inophyllum* (No. 0555) was deposited at the Department of Biology, Osmania University, Hyderabad, India. The ethanolic leaf and stem bark extracts of *Calophyllum inophyllum* were prepared as per the procedure described earlier [7].

Drugs and chemicals

Indomethacin, Acetic acid and CMC were obtained from S.D. Fines Chemical Limited, Hyderabad. Distilled water was obtained from Stangen Fine Chemicals, Hyderabad.

Phytochemical Screening

Phytochemical screening of *Calophyllum inophyllum* was carried out on the ethanolic extracts of leaf and stem bark using standard methods of analysis [7].

Experimental animals

Healthy Swiss albino mice of either sex, weighing 30-40g, were used for present investigation. Animals were housed under standard environmental conditions of temperature and humidity (25±2°C) and 12h light/dark cycle were utilized for studies. Mice were fed with standard pellet diet and water *ad libitum.* The ethical clearance was obtained from the 'Anwarul Uloom College of Pharmacy Animal Ethical Committee' for using animals in the present study (1534/PO/a/11/CPCSEA, India).

Acute oral toxicity studies

Acute toxicity studies for ethanolic leaf and stem bark extracts of *Calophyllum inophyllum* were conducted as per OECD guidelines 423 Annexure – 2d, using Swiss albino mice. Females were selected as they were considered to be more sensitive [8].

Analgesic Studies

Eddy's hot plate method

The test was carried out using Eddy's hot plate apparatus [9]. The temperature was set to

55±1° C. Mice were placed on the hot plate and the reaction time was recorded in seconds for paw licking or jump response, following the oral administrations at 0, 30, 60, 90 and 120 minutes.

Experimental Design

The mice were divided into 6 groups (n=6) and treated with the respective solutions as given below.

Group I (Control): CMC (1% w/v, 1ml, p.o.).

Group II (Standard): Indomethacin (10mg/kg b.w, p.o.).

Group III (Test-I): CILE (100mg/kg b.w, p.o.).

Group IV (Test-II): CILE (200/kg b.w, p.o.).

Group V (Test-III): CISBE (100/kg b.w, p.o.).

Group VI (Test-IV): CISBE (200/kg b.w, p.o.).

Acetic Acid Induced Writhing

Animals of each group were injected 3% acetic (0.1 ml/10g) intraperitonially after subjecting them to various treatments [10]. The number of writhes (abdominal muscle contractions) and stretching of the hind limbs were counted for 20 min after 5 min of acetic acid injection. Percent inhibition was determined for each experimental group by the following formula:

Percent inhibition = (N-Nt/N) χ 100

Where N is the average number of writhes per control group, and Nt is the average number of writhes per test group.

Experimental Design

The mice were divided into 6 groups (n=6) and treated with the respective solutions as given below.

Group I (Control): CMC (1% w/v, 1ml, p.o.).

Group II (Standard): Indomethacin (10mg/kg b.w, p.o.).

Group III (Test-I): CILE (100mg/kg b.w, p.o.).

Group IV (Test-II): CILE (200/kg b.w, p.o.).

Group V (Test-III): CISBE (100/kg b.w, p.o.).

Group VI (Test-IV): CISBE (200/kg b.w, p.o.).

Statistical Analysis

The values are expressed as mean ± SEM. P<0.05 was considered statistically significant and P<0.01 was considered statistically highly significant. Data obtained was analyzed by one-way ANOVA test (parametric ANOVA) followed by Dunnett's multiple comparisons post-hoc test using Graph pad Instat version 3.05, 32 bit for windows.

RESULTS

Oral acute toxicity studies

Oral acute toxicity studies conducted on Swiss albino mice with ethanolic leaf and stem bark extracts of *Calophyllum inophyllum* had shown no mortality at 2000mg/kg. Therefore, 2000mg/kg dose was considered as maximum safe dose.

Eddy's hot plate method

From Table 1, the dose of 100 mg/Kg doses of the ethanolic CILE and CISBE significantly increased the threshold of pain (p<0.05) after 30 minutes of extract administration. Both doses of 100mg/kg and 200mg/kg of CILE and CISBE were highly significant after 90 minutes of extract administration (100 mg/Kg and 200 mg/Kg, p<0.01). Comparative studies between the leaf and stem bark extracts of *Calophyllum inophyllum* suggests unnoticeable difference in variation while increasing the threshold of pain significantly.

Table 1: Eddy's hot plate method

Groups	Treatment	Dose	Reaction time (in sec) after administration of drug				
			0 Mins	30 Mins	60 Mins	90 Mins	120 Mins
I	Control	1%, 1ML	9 ± 0.577	9.83 ± 0.601	9.3 ± 0.422	10.17 ± 0.601	10 ± 0.730
II	Standard	10mg/kg	11.67 ± 0.426*	15.5 ± 0.428**	20.5 ± 0.671**	22.17 ± 0.477**	23.33 ± 0.558**
III	CILE	100mg/kg	10.33 ± 0.494	12.17 ± 0.477*	15.33 ± 0.558*	17.5 ± 0.5**	18.67 ± 0.667**
IV		200mg/kg	10.5 ± 0.619	13.5 ± 0.567*	17.17 ± 0.401**	19.33 ± 0.843**	20.67 ± 0.882**
V	CISBE	100mg/kg	10 ± 0.577	12 ± 0.516*	15.17 ± 0.601*	17.83 ± 0.703**	19.17 ± 0.601**
VI		200mg/kg	10.33 ± 0.843	13 ± 0.577*	17 ± 0.856**	19.83 ± 1.302**	21 ± 0.817**

** P<0.05, **P<0.01*

Acetic acid induced writhing

From Table 2, the dose of 100 mg/Kg of the ethanolic CILE and CISBE decreased the amount of writhing in mice significantly (P<0.05).

The dose of 200 mg/Kg of the ethanolic CILE and CISBE were highly significant (P<0.01) in decreasing the writhing in mice. The resultant inhibition of ethanol leaf extract v/s ethanol stem bark extract, the ethanol CILE shows high inhibition percentage than CISBE.

Table 2: Acetic Acid Induced Writhing in Mice

Group	Treatment	Dose	Number of Writhing Observed (20mins)	% Inhibition
I	Control (CMC)	1ml	33.17 ± 1.579	-
II	Indomethacin	10mg/kg	9.5 ± 0.7638**	71.36%
III	CILE	100mg/kg	24 ± 0.5774*	27.64%
IV	CILE	200mg/kg	19.33 ± 0.6667**	41.71%
V	CISBE	100mg/kg	25.33 ± 0.7601*	23.62%
VI	CISBE	200mg/kg	21.67 ± 0.8028**	34.67%

** P<0.05, **P<0.01*

DISCUSSION

In Hot plate test, the fraction prolonged the paw withdrawal latencies to thermal stimulation in mice. The ethanol extracts significantly increased paw withdrawal latencies 30, 60, 90, 120 minutes post treatment unlike the writhing test, thermally induced pain responds better to centrally acting agents such as narcotics [11]. The hot plate test involves the transmission of pain from the periphery via C fibers to the spinal cord. The ethanol extracts could therefore be acting by inhibiting the transmission of pain via C fibers to the CNS.

The abdominal injection of acetic acid may have induced the release of mediators of pain such as prostaglandins and other cytokines, meaning that the ethanol extracts may act by inhibiting the actions of cyclooxygenase (COX) which is responsible for producing prostaglandins from arachidonic acids.

Thus possibly it is by this mechanism that the ethanol extracts inhibits the writhing produced by acetic acid [12, 13]. Different flavonoids with analgesic activity have been reported. The three flavonoids; 3-O-methylquercetin, 3, 7-O-dimethylquercetin and 3, 7-O-dimethylkaempferol from the ethanol extract of *Cistus laurifolius* L. [14], nepetin, jaceosidin and hispidulin isolated from dichloromethane extract of *Eupatorium arnottianum* Griseb [15] exhibited analgesic effect. Thus the presence of flavonoids may contribute towards analgesic activity. Presence of xanthones may also be the factors contributing to the analgesic activity [6].

CONCLUSION

The oral acute toxicity studies suggest that ethanolic leaf and stem bark extracts of *Calophyllum inophyllum* doses are safe up to 2000 mg/kg in Swiss albino mice. The experimental data showed that the ethanolic leaf and stem bark extracts of *Calophyllum inophyllum* possess significant analgesic activity at doses 100mg/kg and 200mg/kg. In addition, the comparative studies of ethanolic leaf and stem bark extracts of *Calophyllum inophyllum,* the data suggests that ethanolic leaf extract of *Calophyllum inophyllum* showed more analgesic activity compared to ethanolic stem bark extract of *Calophyllum inophyllum* in acetic acid writhing method.

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CONFLICT OF INTEREST

Conflict of interest declared none.

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