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Analgesic, Anti-Inflammatory and Acute Toxicity Studies on *Cordia macleodii* and *Leucas ciliata* Leaves

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ABSTRACT: Analgesic, anti-inflammatory and acute toxicity studies of ethanolic extract of *Cordia macleodii* and *Leucas ciliata* leaves were carried out in Albino mice and rats. For acute toxicity study stair case method was applied. The extracts were administered intraperitonealy (i.p.) in a dose of 500 mg/kg up to 2000 mg/kg in mice, mortality and toxicity symptoms were observed for 24hrs. For evaluation of Analgesic activity, the extracts were administered intraperitonealy in mice and the latency to lick paw was measured at different time intervals by Hot plate model. For evaluation of anti-inflammatory activity, the extracts were formulated as hydrogel and investigated for anti-inflammatory activity by using carageneeanan induced rat paw edema model in comparison with diclofenac diethylamine. *C. macleodii* and *L. ciliata* extracts showed no mortality in any dose but the animals were well depressed in higher doses. Both the extracts significantly increased the latency to lick paw dose dependently. Both the extracts inhibited increase in paw volume. *C. macleodii* and *L. ciliata* leaf extracts were found to have significant anti-inflammatory activity. Thus *C. macleodii* and *L. ciliata* leaves are therapeutically safe and posses analgesic and anti-inflammatory activity.

KEYWORDS: Analgesic, anti-inflammatory, Carrageenan, *Cordia macleodii*, *Leucas ciliata*.

INTRODUCTION

Pain and inflammation have always been a matter of concern for the physicians. Pain describes the normal process of nociception and the responses to noxious stimuli that are damaging or potentially damaging to normal tissues. The noxious stimuli cause release of chemical mediators including prostaglandin, bradykinin, serotonin, substance p, histamine etc. from the damaged cells [1,2]. Inflammation is a complex pathophysiological response of tissue to injury leading to local accumulation of plasmic fluid and blood cells. Though it is a defense mechanism, it has a tendency to induce, maintain and even aggravate several diseases[3]. Although scores of analgesic and anti-inflammatory agents are available, the search for better ones continues to avert the side effects of the available agents. Many traditionally used plants have been claimed to have analgesic and anti-inflammatory potential [4]. Flavonoids which are ubiquitously present in plants are reported to have analgesic and anti-inflammatory activities[5]. Several plants containing flavonoids have been studied for their analgesic and anti-inflammatory effects. Hence it was considered worthwhile to evaluate analgesic and anti-inflammatory activity of *C. macloedii* and *L. ciliata* leaf extracts.

Cordia macleodii (Boraginaceae) is a tree having broad ovate, glabrous green leaves with a cordate base and crenate dentate margins [4]. Traditionally this plant has been used in the treatment of jaundice [6, 7]. The phytochemical evaluation and constituents of this plant have not been reported anywhere. *Leucas ciliata* is a shrubs with ovate or lanceolate, sparsely hairy green leaves with acute apex and serrate margins [8]. The

phytochemical nature and uses of this plant have not been reported.

MATERIALS AND METHODS

Plant Material: Fresh Leaves of *C. macleodii* and *L. ciliata* were collected in the month of June from Nandurbar district and certified by Botanical Survey of India, Pune. The leaves were shade dried and crushed to make coarse powder and used for ethanol extraction. One Kg each of coarse powder of the *C. macleodii* and *L. ciliata* leaves were extracted with 6 liters of 95%v/v ethanol by continuous extraction method for 20 hours. Solvent was distilled off and the extract was concentrated and dried under reduced pressure. Finally the extracts were kept at 2 to 4°C and used for evaluation of analgesic activity. For administration the extract was dissolved in water for injection and the solutions of different doses were prepared.

Preparation of Hydrogels: For preparation of hydrogels, one gram carbopol was dissolved in 100 ml of 10%v/v aqueous acetic acid solution. To the solution a 10%w/v sodium hydroxide solution was added gradually till a white cloudy precipitate appeared and then the mixture was incubated overnight at 37°C. The gel formed was washed extensively with distilled water and then 50%w/w propylene glycol was added and mixed. The extract was added to this gel base so as to contain 5%w/w of the extract. Finally, 2%w/w ethanol was added as penetration enhancer [9].

Animals: Healthy albino mice (20-22g) either sex and albino Wister rats of either sex (140-170g) were procured from Krishna Institute of Medical Sciences Karad, District Satara. Prior to initialization of experimentation the mice and rats were housed in groups of five for a week for acclimatization to laboratory conditions. They were fed on standard feed and given free access to water. Food was withdrawn 6 hrs before the treatment in mice and the rats were fasted overnight. The experiments were carried out according to guidelines of the 'Committee for Prevention and Control of Scientific Experimentation on Animals' (CPCSEA) and the procedures were approved by Institutional Animal Ethics committee.

Acute Toxicity Study: The acute toxicity study was carried out by 'Staircase method'[10]. Initially the dose of 500mg/kg was administered individually in 2 mice and mortality was observed for next 24 hr. The next dose 1000mg/kg was administered individually in 2 mice and mortality were observed for next 24 hr. Finally the dose was increased by 200mg/kg up to the dose 2000mg/kg and animal were observed for the mortality and toxicity.

Evaluation of Analgesic Activity: The analgesic activity of extracts was evaluated by using hot plate analgesiometer. The mice were divided in eight groups comprising of five animals each. One group received pentazocin injection from Ranbaxy India Ltd. (10mg/kg i.p.) and one group received vehicle. Three groups received three different doses (100, 200 and 400mg/kg) of *C. macleodii* extract while the remaining three groups received three different doses (100, 200 and 400mg/kg) of *L. ciliata*.

A hot plate was maintained at 55±1°C. Extract was administered intraperitonealy. Each mouse was placed on hot plate until the first lick of paw or jump. The test was terminated at 20 sec. [11].

Evaluation of Anti-Inflammatory Activity: The anti-inflammatory activity was evaluated using carrageenan induced rat hind paw edema method [12]. The extracts were used for preparation of carbopol based hydrogels as per the method reported by Salunkhe et al [9] so as to contain 5% w/v of the extract. Albino Wister rats of either sex weighing between 140-170g were selected for the studies. The rats were kept on standard diet and allowed food and water ad libitum. They were divided in four groups comprising of five rats in each group. Group I served as control group and it was applied plain hydrogel. The Group II served as reference standard and it was applied Voveran emulgel (Novartis, 0.5% w/w diaclofenac sodium). To the rats in Group III hydrogel containing *C. macleodii* extract was applied similarly hydrogel containing *L. ciliata* extract was applied to the rats in Group IV.

One hour after the application of 50 mg of respective hydrogels to the paw, 0.1ml of 1%w/v solution of carageenan was injected subcutaneously. At different time intervals the paw volume was noted and accordingly activity of each formulation was evaluated in terms of percent inhibition of edema using following formula;

$$\% \text{ Inhibition of edema} = \frac{V_c - V_t}{V_c} \times 100$$

Where V_t means paw volume of treatment group and V_c means paw volume of control group. The data obtained was statistically analyzed by Students t-test. $P<0.05$ and 0.001 were considered significant.

RESULTS

Acute Toxicity: The results of acute toxicity study indicate that there is no mortality even at a dose of 2000mg/kg. The animals demonstrated signs of depression in higher doses of both the extracts. None of the doses of the extracts showed any mortality, but the animals were depressed.

Analgesic activity: Table 1 shows the effect of pentazocin and extracts of *C. macleodii* and *L. ciliata* Leaves on latency to lick paw in mice at different time intervals in hot plate analgesiometer study. The extract of *C. macleodii* showed significant increase in latency to lick paw dose dependently compared to the vehicle treated group. *C. macleodii* extract at 100 mg/kg significantly increased the latency to lick paw at 30 min. (5.6 ± 0.51*) and the latency to lick paw increased to a maximum (6.8 ± 0.37*) at 60 min. At 200 mg/kg the extract significantly increased the latency to lick paw (7 ± 0.32*) at 30 mint and the latency to lick paw was maximum (10.6±0.57*) at 60 min. At 400 mg/kg, the extract significantly increase latency to lick paw (4.8 ± 0.37*) at 15 min. interval and was maximum (14.6±0.68*) at 90 min. The extract of *L. ciliata* showed significant increase in latency to lick paw dose dependently compared to the vehicle treated group. *L. ciliata* extract at 100 mg/kg significantly increased the latency to lick paw at 30 min. (5.6 ± 0.4*) and the latency to lick paw increased to a maximum (6.4 ± 0.51*) at 60 min. At 200 mg/kg the extract significantly increased the latency to lick paw (4.4 ± 0.24*) at 15 min. and the latency to lick paw was maximum (8.4±0.6*) at 90 min. At 400 mg/kg, the extract significantly increase latency to lick paw (5.2 ± 0.37*) at 15 min. interval and was maximum (14±1.22*) at 90 min. Pentazocin treated group showed significant increase in latency to lick paw as compared to the vehicle treated group. The increase in latency to lick paw was significant (5.8 ± 0.37*) at 15min. and it was highest (15.8±0.58*) at 90min.

Anti inflammatory activity: Table 2 shows the effects of ethanolic extracts of *C. macleodii* and *L. ciliata* leaves on mean increase in paw volume and percent inhibition of edema induced by carageenan in rat paw. At 0.5Hr, the mean increase in paw volume in the group treated with *C. macleodii* extract was 0.252 ± 0.012 which at 5 hrs. got significantly reduced to 0.102 ± 0.004*. The extract of *C. macleodii* leaves showed start in significant reduction in the mean paw volume at 1Hr. At 0.5 Hrs, the mean increase in paw volume in the group treated with *L. ciliata* extract was 0.258 ± 0.012 which at 5 hrs. got significantly reduced to 0.070 ± 0.005*. The extract of *L. ciliata* leaves showed the start in significant reduction in the mean paw volume at 1Hr. The control group showed mean paw volume 0.268 ± 0.006 at 0.5Hr and 0.182 ± 0.006 at 5.0Hrs. While in Voveran emulgel treated group the mean paw volume was 0.170 ± 0.005* at 0.5Hr which got significantly reduces to 0.014 ± 0.005*.

Table 1: Effect of ethanolic extract of *C. macleodii*, *L. ciliata* and pentazocin on latency to lick paw in mice.

Treatment	Latency to lick paw (Sec) at different time intervals				
	0 Min	**15 Min**	**30 Min**	**60 Min**	**90 Min**
Vehicle (5ml/kg)	3.8 ± 0.37	3.4 ± 0.4	2 ± 0.32	2.4 ± 0.24	2.2±0.2
Pentazocin (10 mg/kg)	3 ± 0.55	5.8 ± 0.37*	11 ± 1.22*	15.4 ± 0.81*	15.8±0.58*
C. macleodii (100 mg/kg)	3 ± 0.45	3.6 ± 0.4	5.6 ± 0.51*	6.8 ± 0.37*	6.6±0.4*
C. macleodii (200 mg/kg)	3.4 ± 0.51	4.2 ± 0.37	7 ± 0.32*	10.6 ± 0.57*	9.6±0.51*
C. macleodii (400 mg/kg)	2.6 ± 0.4	4.8 ± 0.37*	10.4±1.03*	14.2 ± 0.97*	14.6±0.68*
L. ciliata (100 mg/kg)	2.8 ± 0.37	3.8 ± 0.37	5.6 ± 0.4*	6.4 ± 0.51*	5.8±0.37*
L. ciliata (200 mg/kg)	3.4 ± 0.24	4.4 ± 0.24*	6.6 ± 0.24*	7.6 ± 0.51*	8.4±0.6*
L. ciliata (400 mg/kg)	3 ± 0.32	5.2 ± 0.37*	8.8 ± 0.37*	13.8 ± 1.02*	14±1.22*

Values represent the mean ± SEM; number of mice used in each group = 5; *P<0.05, compared with the control group.

Table 2: Mean increase in paw volume and inhibition of rat paw edema by ethanolic extracts of *C. macleodii* and *L. ciliata* leaves in comparison with Voveran emulgel

Treatment	Mean Increase in Paw Volume at different time intervals					
	0.5Hr.	1 Hr.	2 Hrs.	3 Hrs.	4 Hrs.	5 Hrs.
Control	0.268 ± 0.006	0.258 ± 0.004	0.236 ± 0.006	0.216 ± 0.007	0.198 ± 0.007	0.182 ± 0.006
Voveran emulgel	0.170 ± 0.005*	0.172 ± 0.005*	0.118 ± 0.004*	0.024 ± 0.008*	0.012 ± 0.004*	0.014 ± 0.005*
L. ciliata	0.258 ± 0.012	0.222 ± 0.006*	0.164 ± 0.005*	0.122 ± 0.006*	0.100 ± 0.003*	0.070 ± 0.005*
C. macleodii	0.252 ± 0.012	0.222 ± 0.007*	0.172 ± 0.004*	0.122 ± 0.010*	0.122 ± 0.010*	0.102 ± 0.004*

Values represent Mean ± SEM; number of rats used in each group = 5; *P<0.001, compared with the control group.

DISCUSSION

Acute toxicity is the adverse effect occurring within a short time of administration of a single dose of substances/ extract or multiple doses given within 24 hours. The acute toxicity of *C. macleodii* and *L. ciliata* leaves extracts were studied at different doses, the last dose was 2000 mg/kg. None of the doses of the extracts showed any mortality, but the animals were depressed, hence it was considered that the LD_{50} was above 2000 mg/kg.

Prostaglandins and bradykinins are suggested to play an important role in the pain process [13]. Anti-inflammatory and analgesic activities of many plants have been attributed to their high sterol/ triterpenoids saponins levels. Preliminary phytochemical analysis of the extracts indicated the presence of sterols, flavonoids and saponins. Flavonoids are also known to inhibit prostaglandin synthesis [14]. Edema represents the early phase of inflammation in carageenan induced paw edema and is the simplest and most widely used acute inflammatory model for studying anti-inflammatory agents. The development of carageenan induced edema is believed to be biphasic of which histamine, serotonin and kinins in the first hour after injection of carageenan and the second phase is related to release of prostaglandin like substances in 2-3 hours [15]. It is during this phase that the extracts have shown maximum anti-inflammatory activity. This suggests that the extracts may be inhibiting inflammation by inhibiting release of prostaglandin like substances from phologenic stimuli.

The present study reveals that the extracts of *C. macleodii* and *L. ciliata* leaves have significant anti-inflammatory and analgesic activities. Attention should be given for further investigation of the constituents of these plants, which are responsible for the activities.

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