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AN ANALGESIC AND ANTI-INFLAMMATORY ACTIVITY OF HYDRO-ALCOHOLIC LEAVES EXTRACTS OF SOME INDIGENOUS HERBS GROWING IN UTTARAKHAND, INDIA

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ABSTRACT

Objective: To study anti-inflammatory and analgesic activities of hydro-alcoholic extracts from leaves of *Callicarpa macrophylla* and *Micromeria biflora* were evaluated for support to their traditional medicinal applications. **Materials and method:** The extracts from the leaves of *C. macrophylla* (CMLE) and *M. biflora* (MBLE) at 50 and 100 mg/kg body weight were evaluated for anti-inflammatory and analgesic activities in mice. Analgesic effect characterized by a reduction in the number of writhes induced by acetic acid and anti-inflammatory activity was determined by carrageenan induced paw oedema method. **Results:** The extracts significantly reduced the formation of oedema induced by carrageenan as well as good analgesic effect. Extracts of *C. macrophylla* produce significant ($p < 0.05$) anti-inflammatory activity with 28.01 % inhibition in paw volume and followed by *M. biflora* (20.26%) at a dose level of 100mg/kg body weight after 24 hr of the administration of drugs (samples) in comparison to the control. CMLE showed significant ($p < 0.05$) analgesic activity with 37.75% inhibition in writhing while 35.34% inhibition was observed with MBLE at 100 mg/kg body weight. The extract of *C. macrophylla* was found to be more potent than *M. biflora* extracts and activities are dose dependent. Both the plants extract strong anti-inflammatory and analgesic effect in comparison to the control but moderate than standards.

INTRODUCTION

The medicinal properties of plants have been investigated throughout the world due to their potent pharmacological activities. In present study *Callicarpa macrophylla* Vahl. (CMLE) and *Micromeria biflora* (Buch.-Ham ex D. Don) Benth.(MBLE), investigated for different anti-inflammatory and analgesic activities. *C. macrophylla* belonging to the family *Verbenaceae* ^[1]. Many scientific reports suggested that leaves of *C. macrophylla* have medicinal properties and play significant role in the treatment of inflammation, pain and fever while its roots have significant role in the treatment of inflammation and pain ^[2, 3 4]. Previously studies suggested that its aqueous extracts from stem contain glycosides, saponins, flavanoids, tannins and carbohydrates while its alcoholic extract have significant glycoside, flavanoid, tannins, carbohydrates and steroid content^[5]. Various species of *Micromeria* (*family lamiceae*) are effective against heart disorders, headache, wounds, skin infections in cattle and treating cold ^[6-9]

The leaf powder mixed with oil is used in ulcer and fungal infection^[10]. About 100 species of this genus are widely distributed throughout the globe. Only three species have been reported from Indian Himalayan region, namely *Micromeria biflora* (Buch.-Ham ex D. Don) Benth., *Micromeria capitellata* Benth. and *Micromeria biflora* var. *hispida* Kitamura ex Murata^[11,12]. This study reports on the anti-inflammatory and analgesic activities of hydro-alcoholic extracts from leaves of *M. biflora* (Buch.-Ham ex D. Don) Benth. and *C. macrophylla* because of its various therapeutic uses in the present study.

MATERIALS AND METHODS

Plant Materials: Fresh leaves of *Callicarpa macrophylla* and *Micromeria biflora* were collected from Kumaun region (Udham Singh Nagar and Almora Districts) of Uttarakhand during August to September, 2012 and were identified by Dr. D. S. Rawat (Assistant Professor and Taxonomist), Department of Biological Science, G.B. Pant University of Agriculture and Technology, Pantnagar. The herbarium specimen has been preserved and stored in the Department for future reference.

Preparation of hydro-alcoholic extracts: For preparation of hydro-alcoholic extract, shade dried and powdered leaves of the plants (*C.macrophylla* and *M. biflora*) (100 g) were macerated separately with 500 ml of EtOH–H₂O (7:3) for 48 h. The extracts were then shake, filtered and evaporated in a rotating evaporator under reduced pressure until dryness^[13].

Evaluation of *in vivo* pharmacological activities: The experiments were carried with the permission of the institutional ethical committee (Registration No. 330/CPCSEA). Swiss albino mice were procured from the Laboratory Animal Division of the Central Drug Research Institute, Lucknow, India. They were randomly divided into six groups, with six mice in each group, and were maintained under standard laboratory conditions (temperature, $25\pm 2^\circ\text{C}$; humidity, $40\pm 5\%$). Two concentrations 50 and 100 mg/kg body weight of the dried extracts from the leaves of *Callicarpa macrophylla* (CMLE) and *Micromeria biflora* (MBLE) were prepared and administered orally at the rate of 10 ml/kg body weight. Ibuprofen was used as standard drugs and saline water was used as the control.

Anti-inflammatory activity:

Carrageenan-induced paw oedema: The method reported earlier ^[14] is used to determine the anti-inflammatory activity of the extracts. Oedema was induced by the injection of carrageenan (0.1 ml, 1% w/v in saline) into the sub plantar tissue of the right hind paw. Four groups were given hydro-alcoholic extract (50 and 100 mg/kg body weight), one group was given Ibuprofen (40 mg/kg body weight), and one group distilled water (10 ml/kg body weight). The paw thickness was measured using a micrometer screw gauge at 1, 3 and 24h after carrageenan injection. Oedema formation in the paw results from a synergy between various inflammatory mediators that increase vascular permeability, and mediators that increase the blood flow ^[15]. The reduction in the volume displacement of hind foot in comparison to control and between 0, 4 and 24 h was taken as an anti-inflammatory effect.

Analgesic activity

Acetic acid-induced abdominal writhing test: Glacial acetic acid was administered intraperitoneally to create pain sensation ^[16]. After 1 h, 0.2 ml of extracts (50 and 100 mg/kg body weight), the standard drug Ibuprofen at 40 mg/kg body weight and saline water were administered orally to their respective groups. The number of writhings was counted for 30 min for each mouse. The inhibition of writhing in mice by the standard analgesic ibuprofen was compared with the groups receiving extracts and the percentage of pain protection was calculated using the following formula:

$$\text{Writhing (\%)} = (T/C) \times 100 \quad \text{Inhibition (\%)} = (C-T/C) \times 100$$

where T= treatments (groups III to VI) and C= control saline group I.

Assessment of toxicity: Lethality was determined by oral administration of 400, 600 and 800 mg/kg of body weight of the extract. Clinically animals were examined for 24 h and the number of deaths, if any, was recorded up to 48 h.

Statistical analysis: The data were expressed as mean \pm standard error (SE) and the results were analysed using one way analysis of variance (ANOVA) and $P \leq 0.05$ which were considered to be statistically significant.

RESULTS

Anti-inflammatory activity: The results of carrageenan induced mice paw oedema test have been summarized in **Table 1**. Both the hydro-alcoholic extracts (CMLE & MBLE) produce significant ($p < 0.05$) anti-inflammatory activity in a dose dependent manner CMLE showed maximum % inhibition (28.01%) and followed by MBLE (20.26%) at 100mg/ kg body weight after 24 hr of the administration of drugs (samples) in comparison to the control (saline water) while standard drug ibuprofen treated group exhibited 36.64% reduction in paw thickness in the same time period.

Acetic acid induced writhing test: Both MBLE and CMLE treated mice showed a significant ($P < 0.05$) reduction in writhing induced by acetic acid after oral administration in a dose dependant manner. The standard drug Ibuprofen was found to be more potent, then MBLE and CMLE at all the dose levels. CMLE showed significant ($p < 0.05$) analgesic activity with 37.75% inhibition while 35.34% inhibition was observed with MBLE at 100 mg/kg body weight **Table 2**.

Table 1 Effect of hydro-alcoholic extracts (MBLE and CMLE) on carrageenan-induced paw oedema (Mean \pm SE, n=6).

Group	Treatment	dose	Paw thickness after different time(in mm)		
			0hr	4hr	24hr
I	Control	0.2ml	2.39 \pm 0.02	2.34 \pm 0.02	2.32 \pm 0.01
II	Ibuprofen	40mg/lg	2.37 \pm 0.03	1.76 \pm 0.04(24.79%) ^a	1.47 \pm 0.02(36.64%) ^a
III	MBLE	50mg/kg	2.41 \pm 0.02	2.25 \pm 0.03(3.855%) ^b	2.06 \pm 0.03(11.21%) ^{ab}
IV	MBLE	100mg/kg	2.42 \pm 0.01	2.14 \pm 0.04(8.55%) ^{ab}	1.85 \pm 0.04(20.26%) ^{ab}
V	CMLE	50mg/kg	2.40 \pm 0.03	2.16 \pm 0.05(7.69%) ^{ab}	1.93 \pm 0.04(16.01%) ^{ab}
VI	CMLE	100mg/kg	2.36 \pm 0.02	1.92 \pm 0.05(17.95%) ^{ab}	1.67 \pm 0.05(28.01%) ^{ab}

There is a statistically significant difference ($P < 0.05$). One way ANOVA followed by Dunnett's multiple comparison test. a = significantly different ($P < 0.05$) as compared to control, b= significantly different ($P < 0.05$) as compared to Ibuprofen. (%) = Percent reduction in paw thickness at different times.

Table 2. Effect of hydro-alcoholic extract (MBLE and CMLE) on acetic acid induced writhing reflex in mice (Mean \pm SE, n=6).

Group	Treatment	Dose	No. of writhings	Writhing %	Inhibition%
I	Control	0.2ml	138.67 \pm 3.41	100	
II	Ibuprofen	40mg/kg	78.67 \pm 3.00 ^a	56.73	43.27
III	MBLE	50mg/kg	106.33 \pm 1.89 ^{ab}	76.68	23.32
IV	MBLE	100mg/kg	89.67 \pm 2.85 ^{ab}	64.66	35.34
V	CMLE	50mg/kg	103.33 \pm 1.69 ^{ab}	74.51	25.49
VI	CMLE	100mg/kg	86.33 \pm 2.80 ^a	62.25	37.75

There is a statistically significant difference ($P < 0.050$). One way ANOVA followed by Dunnett's multiple comparison test. a=significantly different ($P < 0.05$) as compared to control. b=significantly different ($P < 0.05$) as compared to Ibuprofen.

DISCUSSION

The results of the present study indicated that hydroalcoholic extracts of *C. macrophylla* and *M. biflora* at relatively lower doses had moderate anti-inflammatory and analgesic effect in carrageenan induced anti-inflammatory and acetic acid induced writhing test respectively.

It has been reported that the mediators at the injured site is the result of the carrageenan-induced oedema. These mediators include prostaglandins, especially the E series, histamine, bradykinins, leucotrienes and serotonin, all of which also cause pain and fever^[17]. Inhibitions of these mediators from reaching the injured site or from bringing out their pharmacological effects normally ameliorate the inflammation and other symptoms. This study has shown that the aqueous extract of the *C. macrophylla* and *M. biflora* possessed a significant anti-oedematogenic effect on paw oedema induced by carrageenan. Development of oedema induced by carrageenan is commonly correlated with early exudative stage of inflammation^[18-19].

The result has shown that all the doses produced significant analgesic effect. This could be attributed, partly, to its anti inflammatory effect as, in the visceral pain model, the processor releases arachidonic acid via cyclooxygenase and prostaglandin biosynthesis which plays a role in the nociceptive mechanism^[20-21]. Thus the results obtained for the writhing test are similar to those obtained for the oedematogenic test using carrageenan. Therefore, an anti-inflammatory substance may be involved in the peripheral analgesic activity because inhibition of the acute inflammation by these extract led to their inhibitory effect on pain development.

The increase in prostaglandin levels within the peritoneal cavity then enhances inflammatory pain by increasing capillary permeability ^[22].

According to the literature of pervious studies any substance that has got analgesic activity reduce the number of writhing of animals within a given time and with respect to the control group will help analgesic effect preferably by inhibition of prostaglandin synthesis, a peripheral mechanism of pain inhibition ^[23].

The extracts from the leaves of *C. macrophylla* and *M. biflora* exhibited activities in various degrees against inflammation and pain. According to the researcher higher levels of prostaglandin particularly PGE2 produce inflammation, pain and fever which is cause of cyclooxygenase activation ^[24]. As a result, we suppose that some active metabolites of the extracts could inhibit cyclooxygenase activity. Data presented in this work showed the potent effect of the extracts from both the selected plants as anti-inflammatory and analgesic at all tested concentration and its effect found to be concentration dependent.

Acute toxicity: Both MBLE and CMLE did not cause any behavioral changes and no deaths were observed. Thus they were considered to be practically non-toxic substances

CONCLUSION

It can be concluded from results that *C. macrophylla* and *M. biflora* leaves could be beneficial in the management of inflammations and pains and can be used as anti-inflammatory and analgesic besides its other traditional and indigenous applications.

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