

EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF AQUEOUS AND ETHANOLIC EXTRACTS OF *ACAMPE PRAEMORSA* ON CARRAGEENAN-INDUCED PAW OEDEMA IN RATS

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Summary

The anti-inflammatory effect of the aqueous (AEAP) and ethanolic (EEAP) extracts of *Acampe praemorsa* were evaluated using the carrageenan-induced rat paw oedema model. AEAP exhibited significant anti-inflammatory effect at a dose of 300 mg/kg. Maximum inhibition of 48.1 % for AEAP and 11.4 % for EEAP was observed after 3 h of drug treatment, whilst the standard drug-diclofenac, produced 63.0 % of inhibition. The present study reveals that the aqueous extract of *A. praemorsa* is endowed with significant anti-inflammatory activity.

Keywords: *Acampe praemorsa*, inflammation, carrageenan, paw oedema.

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Introduction

Inflammation is a primary physiologic defense mechanism that helps the body to protect itself against infection, burn, toxic chemicals, allergens or other noxious stimuli. Uncontrolled and persistent inflammation may act as an etiologic factor for many chronic illnesses (1). Although it is a defense mechanism, the complex events and mediators involved in the inflammatory reaction can induce, maintain or aggravate several diseases (2). Most of the currently used anti-inflammatory drugs are associated with several side effects. Hence, the development of potent anti-inflammatory drugs with fewer side effects is necessary.

Herbal medicines derived from plant extracts are being increasingly utilized to treat a wide variety of clinical diseases, though relatively little knowledge is available regarding their mode of action. Hence there is a growing interest in the pharmacological evaluation of various plants used in Indian traditional systems of medicine for their anti-inflammatory properties.

Acampe praemorsa, family - Orchidaceae, also known in Kannada as *Marabale* is mainly found in Sri Lanka, Bangladesh and in some regions of India like Karnataka and Kerala. This plant is used in traditional medicine for the treatment of fractures (3, 4) and is applied externally as a poultice in rheumatism and arthritis (5, 6). On the basis of these common uses of this plant in traditional folk medicine and its above reported activities in literature. The present study was an attempt to evaluate the anti-inflammatory effect of the aqueous and ethanolic extracts of *A. praemorsa* in carrageenan induced paw oedema in rats.

Materials and methods

Collection of plant materials

The plant of *A. praemorsa*, family - Orchidaceae (Roxb.) Blatt. and McCann., was collected from the Udipi district of Karnataka state, India in the month of September 2008. The plant was identified and authenticated by Dr. Gopalakrishna Bhat, Professor of Botany, Poorna Prajna College, Udipi, Karnataka.

Extraction

Preparation of the aqueous extract: The whole plant of *A. praemorsa* (500 g) was collected, washed thoroughly and then dried in shade. It was crushed and extracted by cold maceration using chloroform:distilled water (1:99). The marc obtained after filtration with Whatman No. 1 filter paper was removed and the extraction was repeated once again.

The combined filtrate thus obtained was evaporated and concentrated *in vacuo*. The residue (25 g) obtained was designated as the aqueous extract of *A. praemorsa* (AEAP).

Preparation of ethanolic extract: Coarsely powdered dried whole plant of *A. praemorsa* (500 g) was taken and extracted with ethanol (95%) by soxhletation for 72 hrs. After completion of extraction the solvent was removed by distillation and concentrated *in vacuo*. The residue obtained (26.5 g) was designated as the ethanolic extract of *A. praemorsa* (EEAP).

Animals

Male Wistar rats weighing 200-250 g were housed in polypropylene cages at a temperature of 25 ± 2 °C with relative humidity of 40-60 % and 12 hours light/dark cycle. Animals were maintained on a balanced diet and water *ad libitum* during the complete experimental period. All animal experiments were approved by the Institutional Animal Ethical Committee (Registration No. IAEC/KMC/07/2008-2009) at Manipal University.

Drugs and Chemicals

Carrageenan and diclofenac were purchased from Sigma-Aldrich, Germany. Ethanol and Chloroform were obtained from Merck, Germany.

Carrageenan-induced Rat Paw Oedema

The rats were divided into 4 groups (n=6). Acute inflammation was induced by sub-plantar administration of 0.1ml of 1% carrageenan in normal saline in the right paw of the rats. The different groups were orally administered AEAP (300 mg/kg, p.o.), EEAP (300 mg/kg, p.o.), diclofenac (10 mg/kg, p.o.) and control vehicle respectively. The paw volume was measured at 0, 1 and 3 h after carrageenan injection by using a UGO Basile 7141 digital plethysmometer. The animals were pretreated with the extract 1 h before the administration of carrageenan. The anti-inflammatory effect of AEAP and EEAP were calculated by the following equation: anti-inflammatory activity (%) = $(1-D/C) \times 100$, where D represents the percentage difference in paw volume after respective extracts were administered to the rats, and C represents the percentage difference of volume in the control groups.

Statistical analysis

Data were analyzed by one-way ANOVA followed by Tuckey's post-hoc test and P values < 0.05 were considered statistically significant.

Results and discussion

The aqueous and ethanolic extracts of *A. praemorsa* were evaluated for anti-inflammatory activity in acute experimental animal model and the results are tabulated in Table 1. AEAP exhibited significant anti-inflammatory activity at a dose of 300 mg/kg while EEAP exhibited moderate inflammatory inhibition. As shown in Table 1, the aqueous and ethanolic extracts showed maximum inhibition of 48.1 % and 11.4 % respectively at the dose of 300 mg/kg after 3 h of drug treatment in carrageenan-induced paw oedema, whereas the standard drug showed 63.0 % of inhibition.

Table 1. Effect of the *A. praemorsa* Extract on Carrageenan-induced Paw Oedema

Treatment	Dose (mg/kg)	Difference in paw volume between 0 and 3 h (ml)	Percentage of inhibition
Carrageenan control	-	0.8670 ± 0.02	-
Diclofenac	10	0.3206 ± 0.03*	63.0
AEAP	300	0.4504 ± 0.04*	48.1
EEAP	300	0.7681 ± 0.03	11.4

Values are mean ± S.E.M. (n=6). Experimental groups were compared with control *p < 0.05.

Carrageenan-induced oedema is commonly used as an experimental animal model for acute inflammation and is believed to be biphasic, of which the first phase is mediated by the release of histamine and 5-HT followed by kinin release and then prostaglandin in the later phase (7, 8). The AEAP significantly inhibited inflammation in paw edema, thereby indicating that the extract induces its anti-inflammatory action by means of either inhibiting the synthesis, release or action of inflammatory mediators viz. histamine, serotonin and prostaglandins involved in inflammation. *A. praemorsa* has been reported to contain phenanthropyran derivative, praemorsin (1,7-dihydroxy-3-methoxy-9, 10-dihydro phenanthropyran) (9) and flavonoids (10). Hence the activity of this extract may be due to these constituents.

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