

## Anti-inflammatory and Analgesic Activity of Methanol Extracts of *Cyperus tegetum* Roxb. Rhizome

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### Abstract

The anti-inflammatory activity of methanol extract of *Cyperus tegetum* Roxb. was evaluated against carrageenan induced paw edema model and the extract significantly reduced the paw edema swelling. The percentage reduction in the paw edema in animals treated with *C. tegetum* extract was found to be 49.57 and 86.40 at the dose of 250 and 500 mg/kg b.w. respectively. Diclofenac sodium at a dose of 5 mg/kg b.w. was used as reference standard. The extract at a dose of 200 and 400 mg/kg b.w. significantly inhibit acetic acid induced writhing in mice and the percentage inhibition was found to be 68.90 and 80.48 respectively compare to reference standard paracetamol (50 mg/kg b.w.).

**Keywords:** *Cyperus tegetum* Roxb., anti-inflammatory, analgesic, diclofenac sodium

### Introduction

Inflammation is a local response of living mammalian tissues to the injury. It is a body defense reaction in order to eliminate or limit the spread of injurious agents. The relation between inflammation and atherosclerosis, diabetes, cancer, arthritis and Alzheimer's disease is well substantiated.<sup>1,2</sup> There are various components to an inflammatory reaction that can contribute to the associated symptoms and tissue injury. Edema formation, leukocyte infiltration and granuloma formation represent such components of inflammation.<sup>3</sup> Edema formation in the paw is the result of a synergism between various inflammatory mediators that increase vascular permeability and/or the mediators that increase blood flow.<sup>4</sup> Several experimental models of paw edema have been described. Carrageenan-induced paw edema is widely used for determining the acute phase of inflammation. Histamine, 5-hydroxytryptamine and bradykinin are the first detectable mediators in the early phase of carrageenan-induced inflammation<sup>5</sup> whereas prostaglandins are detectable in the late phase of inflammation.<sup>6</sup>

Herbal medicines derived from plant extracts are being increasingly utilized to treat a wide variety of clinical diseases, though relatively little knowledge about their mode of action is available. There is a growing interest in the pharmacological evaluation of various plants used in Indian traditional systems of medicine. The present investigation was carried out to evaluate the anti-inflammatory potential of *C. tegetum* Roxb. in experimental animal models.

### Materials and methods

#### Chemicals

Carrageenan (S. D. Fine Chemicals Ltd, Mumbai), Diclofenac sodium, acetic acid (E. Merck, Mumbai).

#### Test Compounds

The methanol extract of *Cyperus tegetum* Roxb. Rhizome (MECT) at a dose of 250 and 500 mg/kg b.w. were used for the evaluation of anti-inflammatory activity and at a dose of 200 and 400 mg/kg b.w. for analgesic activity.

#### Animals

Healthy adult Wistar strain albino rats (180-200 gm) and Swiss albino mice (25-30 gm) were screened for the study. The animals were housed in standard polypropylene cages maintained under standard laboratory condition at an ambient temperature of 25 ± 2°C and 45 - 55% relative humidity with 12hr light-dark cycle. The animals had free access to standard pellet diet (Hindustan Lever Ltd.) and water ad libitum. Institutional Animal Ethics Committee (No.955/a/06/CPCSEA), constituted under the guidelines of CPCSEA, Ministry of Environment, Govt. of India, New Delhi, approved all the animal experimental protocols.

#### Test for Anti-inflammatory Activity

The methanol extract of the rhizome was tested for anti-inflammatory activity by carrageenan induced rat paw edema.<sup>7</sup> Healthy albino rats of either sex, weighing 100 -160 gm were selected and provided a standard pellet diet for rodents and water ad libitum. Before the experiment, food was withdrawn overnight but adequate water was given to the rats. The animals were divided into 4 groups of 6 animals each. Group-I received gum acacia (5%, 10 ml/kg) and served as control. Group-II, III and IV

received MECT (250 and 500 mg/kg) and diclofenac sodium (5 mg/kg) respectively. All the drugs were given orally half an hour before the administration of carrageenan suspension. Acute inflammation was produced by the sub-planter administration of 0.1 ml of 1% carrageenan in normal saline in the left hind paw of the rats. The paw volume was measured at 0, 1, 3 and 5 with the help of plethysmometer. The average paw swelling in the group of extract treated rat was compared with control group and standard group and percent change in edema was calculated.

The average percent increase in paw volume with time was calculated and compared against the control group. Percentage inhibition was calculated using the formula:

$$\% \text{ Inhibition} = \frac{(V_c - V_t)}{V_c} \times 100$$

Where  $V_t$  represents the percentage difference in increased paw volume after the administration of test drugs to the rats and  $V_c$  represents the percentage difference of increased volume in the control groups.

#### Test for analgesic activity

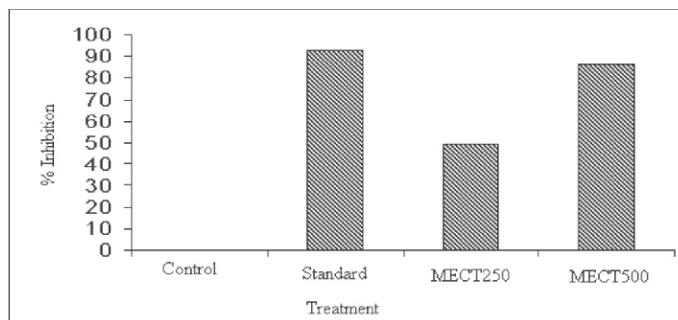
The animals, Swiss albino mice were divided in four groups (n=6). Group-I received vehicle (1% CMC), group-II and III received MECT (200 and 400 mg/kg) and group IV receive reference standard paracetamol (50 mg/kg) orally 30 min before the i.p. administration of 0.2 ml (irrespective of body weight) of 1% acetic acid. The number of writhing during the following 10 min period was counted. The percentage inhibition was calculated using the following

$$\% \text{ Inhibition} = \frac{(N_c - N_t)}{N_c} \times 100$$

Where,  $N_c$  = Average number of writhing of control per group,  $N_t$  = Average number of writhing of test per group.

#### Results and discussion

The anti-inflammatory activity of methanol extract of *C. tegetum* against carrageenan induced paw edema showed that the extracts exhibit significantly reduced the paw edema swelling. The percentage reduction in the paw edema in animals treated with *C. tegetum* extract was found to be 49.57% and 86.40% (Table 1, Fig.1) at the dose of 250 and 500 mg/kg b.w. respectively.



**Fig. 1:** Percentage (%) inhibition of paw volume of rats treated with control (1% CMC), standard (diclofenac sodium, 5 mg/kg) and different test concentration (250 & 500 mg/kg b.w)

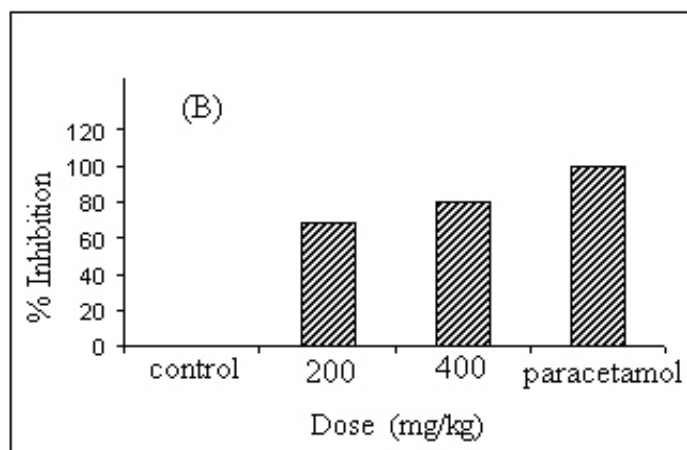
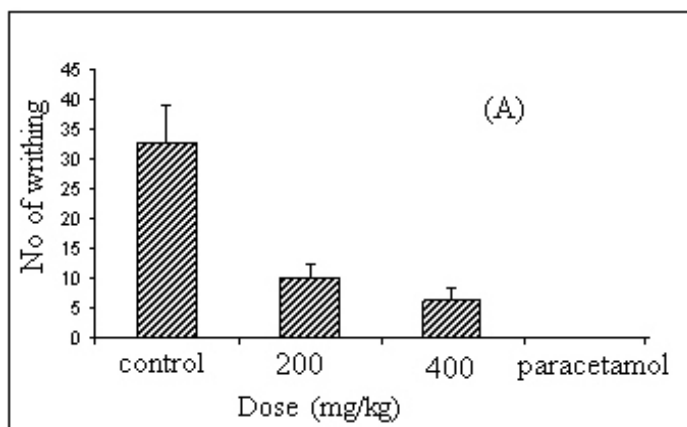
Carrageenan induced edema is commonly used as an experimental animal model for acute inflammation and is believed to be biphasic, of which the first phase is mediated by the release of histamine and 5-HT followed by kinin release and then prostaglandin in the later phase.<sup>8,9</sup> Therefore edema, which develops after carrageenan inflammation, is a biphasic event. The initial phase is attributed to the release of histamine and serotonin. The edema maintained between the first and the second phase is due to kinin like substances. The second phase is said to be promoted by prostaglandin, the cyclooxygenase products and lipoxygenase products like substances.<sup>10</sup> It has been reported that the second phase of edema is sensitive to drugs like hydrocortisone, phenylbutazone and indomethacin. The anti-inflammatory activity of MECT is comparable to reference drug diclofenac sodium, which is a cyclooxygenase and/ or lipoxygenase inhibitor. Therefore it can be concluded that MECT reduces the paw edema may be due to its cyclooxygenase inhibitory property.<sup>11</sup> Therefore inhibition of carrageenan induced paw edema by the crude extract could also be due to its inhibitory activity on the lipoxygenase enzyme. The extract (200 and 400 mg/kg), significantly inhibit acetic acid induced writhing in mice (Table 2, Fig.2).

**Table 2: Acetic acid induced peripheral analgesic activity**

Treatment	Dose (mg/kg b.w)	No of writhing reflex (mean ± sd)	% Inhibition
Control (1%CMC)	0	32.8 ± 6.26	0
MECT	200	10.2 ± 2.16	68.90
MECT	400	6.4 ± 1.94	80.48
Std (Paracetamol)	50	0	100

**Table 1. Effect of MECT on carrageenan induced rat paw edema**

Treatment	Edema volume(ml)				% Inhibition at 5h
	0h	1h	3h	5h	
Control (5% acacia, 10 ml/kg)	0.72 ± 0.05	1.23 ± 0.08	1.82 ± 0.08	1.58 ± 0.05	-----
MECT 250 (250 mg/kg)	0.65 ± 0.08	1.14 ± 0.10	1.13 ± 0.09	1.04 ± 0.11	49.57%
MECT 500 (500 mg/kg)	0.68 ± 0.07	1.15 ± 0.06	0.85 ± 0.11	0.79 ± 0.07	86.4%
Standard (Diclofenac sodium 5 mg/kg)	0.67 ± 0.09	1.08 ± 0.11	0.71 ± 0.05	0.73 ± 0.10	92.5%



**Fig. 2:** Acetic acid induced peripheral analgesic activity of MECT; (A): Dose vs. No of writhing, (B): Dose Vs. % Inhibition

The writhing responses are related to increase in the peritoneal level of prostaglandins and leukotrienes.<sup>12</sup> The result strongly suggests that the mechanism of action of extract may be linked to lipoxygenase and/or cyclooxygenase. Further studies are required to isolate active constituents responsible for the activity.

## Conclusion

The present investigation demonstrated that the methanol extract of rhizomes of *C. tegetum* Roxb. possess anti-inflammatory and analgesic activity. A further study requires involving the purification of the chemical constituents of the plant and the investigations of biochemical pathways for the development of a potent anti-inflammatory agent with low toxicity and better therapeutic index.

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