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Evaluation of Anti-inflammatory Activity of *Ammomum subulatum* Fruit Extract

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ABSTRACT
Ammomum subulatum is a perennial plant cultivated in swampy places in Bengal, Sikkim, Assam and Tamil Nadu. Plant bears fruit having numerous seeds which are traditionally used in spice. Fruits are used as stimulant, aromatic, stomachic, aphrodisiac, in infection of teeth and gums. The present study is an attempt to explore the anti-inflammatory activity of ethanolic and aqueous extract of fruit of *Ammomum subulatum.* Dose of 100 mg/ml and 200 mg/ml of ethanolic and aqueous extract were evaluated for their anti-inflammatory activity against carrageenan induced paw edema in rat. Both the extracts were able to show anti-inflammatory activity in dose dependant manner as compared with standard drug Diclofenac sodium 100 mg/ml. The data were found statistically significant by using one way ANOVA ($P < 0.001$).

Keywords: *Ammomum subulatum; Zingiberaceae;* Anti-inflammatory; Diclofenac sodium.

INTRODUCTION

Ammomum subulatum (*Zingiberaceae*) is a tall, perennial plant, with leafy stems, up to 2.5 m in height, leaves oblong-lanceolate, acuminate, glabrous, spikes globose, very dense, shortly peduncled, [1] cultivated in swampy places in Bengal, Sikkim, Assam and Tamil Nadu, [2] fruit is large, coarsely striated dark brown in colour, measuring 2-3 cm in length and upto 1.5 cm in width. It is a trilocular capsule, anterio-posteriorly flattened, having a number of irregular, dentate-undulate wings which extend from the apex downwards for two third of its length. Internally, the capsule contains several seeds held together by a viscous pulp of dark brown colour. [3] Oil extracted from the seeds is aromatic, stimulant, stomachic, traditionally applied to eyelids to allay inflammation. [4] Decoction of seeds is used as a gargle in infections of the teeth and gums. In combination with the seeds of melon it is used as a diuretic in cases of gravel of the kidneys. [5] Seeds are also used in gonorrhea and as an aphrodisiac. They have been also found useful in neuralgia in large doses. [6]

However so far no systematic study on phytochemical and anti-inflammatory activity has been reported in the literature. In the present context the present study is focused to evaluate the anti-inflammatory activity of *Ammomum subulatum* fruits.

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MATERIALS AND METHODS

Collection of fruits

The fruits of *Ammomum subulatum* were collected from the local market in Delhi and were authenticated by Dr. H. B. Singh Scientist, Incharge, NISCAIR, New Delhi. A voucher specimen NISCAIR/RHMD/Consult/-2007-08/855/39 is preserved for future reference.

Preparation of the extract

In the present study, the ethanolic extract of air dried fruit powdered material (500 g) was prepared using soxhlet apparatus, concentrated and dried using Buchi rotavapour, it gives a brownish mass (56.64 g.) The powdered material (500 g) was percolated with cold water to get the aqueous extract (31.42 g). The dried ethanolic and aqueous extracts were stored in a desiccators to carry out phytochemical and pharmacological studies. Each extract was subjected to qualitative chemical investigation of phytoconstituents such as alkaloids, flavonoids, tannins, carbohydrates, proteins, vitamins, coumarins, etc.

Phytochemical studies

Preliminary phytochemical screening was performed. [7] The presence of phytoconstituents such as glycosides, carbohydrates, flavonoids, steroids and resins were confirmed.

Pharmacological Screening

The animal experiments were performed according to CPCSEA guidelines and after the approval from Institutional Animal Ethics Committee (I.A.E.C.), Rajiv Academy For

Table 1: Anti-inflammatory activity of *Ammomum subulatum* fruit extracts on carrageenan induced paw edema in rats

Treatment	Dose (mg/ml) p.o.	Volume displaced in ml				
		1 h	2 h	3 h	4 h	5 h
Control	-	0.17 ± 0.06	0.24 ± 0.09	0.29 ± 0.07	0.32 ± 0.07	0.35 ± 0.06
Diclofenac sodium	100	0.15 ± 0.03 (11.77)	0.16 ± 0.05* (33.33)	0.12 ± 0.04* (58.63)	0.10 ± 0.04* (68.75)	0.10 ± 0.03* (71.43)
Ethanol Extract	100	0.16 ± 0.04 (5.89)	0.17 ± 0.04 (29.16)	0.19 ± 0.06* (34.49)	0.18 ± 0.06* (43.75)	0.18 ± 0.04* (48.57)
Ethanol Extract	200	0.15 ± 0.04 (11.77)	0.16 ± 0.05* (33.33)	0.17 ± 0.04* (41.39)	0.16 ± 0.03* (50.00)	0.14 ± 0.06* (60.00)
Aqueous Extract	100	0.16 ± 0.09 (5.89)	0.17 ± 0.05* (29.16)	0.20 ± 0.04* (31.03)	0.20 ± 0.03* (37.5)	0.19 ± 0.03* (45.72)
Aqueous Extract	200	0.16 ± 0.04 (5.89)	0.17 ± 0.04* (29.16)	0.19 ±.068* (34.48)	0.18 ± 0.06* (34.75)	0.16 ± 0.03* (54.28)

Results expressed as Mean ± SEM, $n = 6$ animals in each group; Values within parentheses represent the percentage inhibition. Statistical evaluation by one- way ANOVA followed by Dunnett's t – test; Symbols represent statistical significance: * - P< 0.001

Pharmacy, Mathura, experiments were conducted in accordance with the standard guidelines.

Animal used

Albino rats (Wistar strain) of either sex (150- 180 g) were obtained from the animal house of Rajiv Academy For Pharmacy, Mathura. Animals were kept in animal caging system (four rats per cage on beds of sawdust) under the laboratory conditions (25 ± 2°C, 12 h light). They were provided with animal feed pellets manufactured by Hindustan Lever (India) Ltd. Mumbai. Food was withdrawn 12 h before the experimental work and water was provided *ad libitum*. After a 7 days of acclimatization period, animal were randomly selected for different experimental groups (6 animal/ group) and used for the *in vivo* determination of anti-inflammatory activity. During the course of the experiment the animal behavior was normal.

Drugs

Ethanolic, aqueous extracts and diclofenac sodium were prepared as suspension using 0.6% w/v sodium carboxymethyl cellulose as suspending agent.

Experimental Method

Anti-inflammatory activity was evaluated using carrageenan – induced hind paw edema method. [8-9] Carrageenan (0.1 ml of 1% w/v suspension) was injected into the sub plantar region of the right hind paw of each rat. The extracts (100 and 200 mg/ml) and diclofenac sodium (100 mg/ml) were administered orally to rats one hour before carrageenan injection.

Control group received an equal volume of vehicle (0.6% w/v Sod. CMC). The dosage details are given in Table 1. The volume of the paw was measured with a volume differential meter (Model 7140 UGO Basile) after 1, 2, 3, 4 and 5 h of carrageenan injection. Results were determined as the percentage inhibition of edema compared to the control.

Percent inhibition of edema volume between treated and control group was calculated as follows:

$$\text{Percent inhibition} = \frac{Vc - Vt}{Vc} \times 100$$

Where, Vc and Vt represent mean increase in paw volume in control and treated groups respectively.

Statistical analysis

All data are expressed as mean ± SEM. Statistical significance was calculated using one - way ANOVA with Dunnett's t test. [10] P valves < 0.001 were considered significant.

RESULT

There was significant and dose dependent anti-inflammatory activity of both the ethanolic and aqueous extracts in the acute carrageenan induced rat paw edema model.

Orally administered doses of 100 and 200 mg/ml of ethanolic extract of the fruit of *Ammomum subulatum* produced 34.49% and 41.39% inhibition respectively after 3 h. The remaining aqueous extract of fruit of *A. subulatum* produced 27.59% and 34.48% inhibition respectively after 3 h as compared to diclofenac sodium (Standard) (100 mg/ml) which showed 58.63% inhibition after 3 h.

Orally administered doses of 100 and 200 mg/ml of ethanolic extract of the fruit of *Ammomum subulatum* produced 48.57% and 60% inhibition respectively after 5 h. The remaining aqueous extract of fruit of *A. subulatum* produced 45.72% and 54.28% inhibition respectively after 5 h as compared to Diclofenac sodium (Standard) (100 mg/ml) which showed 71.43% inhibition after 5 h (P< 0.001). Results of anti-inflammatory activity are presented in Table 1. Carrageenan induced rat paw edema throughout the observation period.

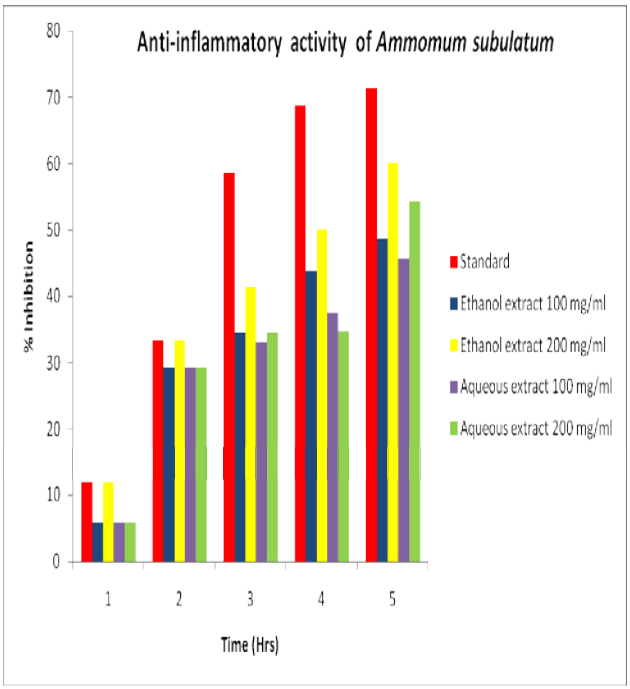

Fig. 1: Anti-inflammatory activity of ethanolic and aqueous extract of the fruit of *Ammomum subulatum* compared with standard drug diclofenac sodium

DISCUSSION

Percentage inhibition of edema volume of ethanolic, aqueous and standard drugs were calculated after every hour upto 5 h duration. There is dose dependent inhibition of paw edema in rats as shown in Fig. 1. Prostaglandins and bradykinins were suggested to play important role in carrageenan induced edema. [11-12] Both steroidal and non steroidal anti-inflammatory drugs can be tested by the carrageenan-induced paw inflammation test. The edema induced in the rat paw by the injection of 1% carrageenan is brought about by

autocoids, histamine and 5-hydroxy tryptamine (5-HT) during the first one hour, after which kinins act, to increase the vascular permeability upto two and a half hours. The maximum inflammation is seen approximately three hours post the carrageenan injection, after which it begins to decline. Following that the prostaglandins act from two and a half hours to six hours, which results in the migration of leucocytes into the inflamed site. [13-14] The carrageenan induced paw edema model in rats is known to be sensitive to cyclo-oxygenase (COX) inhibitors and has been used to evaluate the effect of non-steroidal anti-inflammatory agents. [15-16] *Ammomum subulatum* shows a significant inhibition of inflammation, which is comparable to the standard drug diclofenac sodium. As Phytochemical tests showed the presence of glycosides, carbohydrates, flavonoids, steroids and resin in both the ethanolic and aqueous extract, they might suppress the formation of prostaglandins and bradykinins or antagonize their action and exert its activity. Further studies are required to identify the actual chemical constituents that are present in the crude extracts of this plant which are responsible for anti-inflammatory activity.

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