EVALUATION OF ACUTE ANTI-INFLAMMATORY EFFECT OF *ANANAS COMOSUS* LEAF EXTRACTS IN RATS

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Summary

Ananas comosus (L.) Merr. (Bromiliaceae), commonly known as Pineapple, is native to Central and South America and grown in several tropical and subtropical countries including the Indian subcontinent. The present study assessed the chloroform and methanol extracts of *A. comosus* leaf for their acute anti-inflammatory potential by carrageenan induced paw oedema in Wistar albino rats. All of the test extracts exhibited significant anti-inflammatory activity. The methanol extract was found to be the most potent followed by the chloroform extract. The present preliminary study demonstrated marked acute anti-inflammatory activity of *A. comosus* leaf in Wistar rats.

Key words: *Ananas comosus,* anti-inflammatory, oedema, leaf.

Introduction

Ananas comosus (L.) Merr. (Bromiliaceae), commonly known as Pineapple, is native to Central and South America and grown in several tropical and subtropical countries including Hawaii, India, China, Kenya, South Africa, Malaysia, the Philippines and Thailand. It has been used as edible fruit and medicinal plant in several native cultures and its active extract bromelain has been chemically known since 1876 (1). Botanicals such as *A. comosus* (Pineapple) and their extracts (bromelain) have been used medicinally as anti-inflammatory agents in rheumatoid arthritis, soft tissue injuries, colonic inflammation, chronic pain and asthma (2-4). Bromelain have been reported to have antidiarrhoeal activity (5). It is also useful in the prevention and treatment of thrombosis and thrombophlebitis, breaks down cholesterol plaques and exerts a potent fibrinolytic activity (3). However, the anti-inflammatory assessment of chloroform and methanol extracts from *A. comosus* leaf is still not reported. Therefore, in the present investigation we attempted these studies on the leaf extracts of *A. comosus* grown in India.

Materials and methods

Plant material: The mature leaves of *Ananas comosus* (L.) Merr. (Bromiliaceae), were collected during August 2011 from Burdwan region of Bardhhaman district of West Bengal, India. The plant material was taxonomically identified at the Central National Herbarium, Botanical Survey of India, Howrah, West Bengal, India. The voucher specimen [CNH/99/2011/Tech II/595] was maintained in our research laboratory for future reference. The plant material was shade-dried with occasional shifting and then powdered with mechanical grinder, passing through sieve no. 40, and stored in an air-tight container.

Preparation of plant extracts: Preparation of plant extracts: The dried powdered material was defatted with petroleum ether (60-80°C), the percentage extractive value was 0.048 % w/w. The defatted powdered material thus obtained was further extracted successively with chloroform and methanol for 72 h. The solvent was distilled off in reduced pressure and resulting semisolid mass was vacuum dried to yield the dry extracts and the percentage extractive values were accordingly 2.056 % w/w and 4.112 % w/w respectively. The preliminary phytochemical analysis was performed for all three extracts to identify the phytoconstituents present in the extracts (6). However, for anti-inflammatory evaluation only the chloroform and methanol extracts were used.

Drugs and chemicals: λ-Carrageenan (type IV) was obtained from S. D. Fine Chemicals Ltd., Bombay; indomethacin was from Recon, Bangalore, India. All other chemicals and reagents were of analytical grade obtained commercially.

Experimental animals: Studies were carried out using adult male Wistar albino rats of weighing 150-180 g. The animals were grouped in polyacrylic cages (38 cm × 23 cm × 10 cm) with not more than four animals per cage and maintained under standard laboratory conditions (temperature 25 ± 2 °C, dark and light cycle 14/10 h). They were allowed free access to standard dry pellet diet (Hindustan Lever, Kolkata, India) and water *ad libitum*. The rats were acclimatized to laboratory condition for 10 days before commencement of experiment. All experimental methods were reviewed and approved by the Institutional Animal Ethical Committee.

Evaluation of anti-inflammatory activity

***Carrageenan-induced rat paw oedema*:** The rats were divided into four groups (n = 6). The first group (which served as control) received normal saline (3 ml/kg body wt., p.o.). The second and third group received the chloroform and methanol extracts (200 mg/kg body wt., p.o., each). The fourth group (which served as reference) received indomethacin (10 mg/kg body wt., p.o.). After 30 mins, acute inflammation was produced by the sub-plantar administration of 0.1 ml of 1 % (w/v) of freshly prepared suspension of carrageenan in the right hind paw of each rat. The paw volume was measured at 0 hour and at each hour up to 4 hours after carrageenan injection by using plethysmometer (Ugo Basile, Italy). The difference between the two readings was taken as the volume of oedema and the percentage of inhibition was calculated by using the following formula (7, 8).

(Control mean− Treated mean/ Control mean) × 100 %.

Statistical analysis: The values were expressed as mean ± standard error of mean (SEM). Statistical significance was analyzed by Student's *t* test. Values of $p < 0.001$ were considered as statistically significant.

Results and discussion

Preliminary phytochemical studies on *A. comosus* leaf extracts showed the presence of triterpenoids and steroids in the petroleum ether extract; alkaloids and steroids in the chloroform extract; and alkaloids, steroids, saponins, glycosides and carbohydrates in the methanol extract.

Anti-inflammatory activity of *A. comosus* leaf extract was evaluated against carrageenan induced acute paw oedema in rats and the results are summarized in Table 1. The methanol extract was found to be the most potent followed by the chloroform extract after 4 hours of treatment, whereas the reference drug indomethacin was found to be the most potent when compared with the saline control group.

The present study establishes the significant anti-inflammatory activity of *A. comosus* leaf against the experimentally induced acute inflammation in rodents. Carrageenan-induced paw oedema has been commonly used as an experimental animal model for acute inflammation and it is believed to be a biphasic response. The early phase (1 - 2 h) of the carrageenan model is mainly mediated by histamine and serotonin (5-HT). The late phase (2 - 4 h) is mediated by bradykinin, leukotrienes, polymorphonuclear cells and prostaglandins produced by tissue macrophages (10). All the test extracts produced significant ($p < 0.001$) inhibition of carrageenan-induced rat paw oedema after a period of 4 h.

Based on the results obtained from the present preliminary investigation, it can be inferred that all the test extracts from *A. comosus* leaf possessed remarkable acute anti-inflammatory property in the Wistar rats. The present preliminary study confirms marked anti-inflammatory activity of *A. comosus* leaf which may be due to presence of multitude of constituents as revealed by its phytochemical profile.

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Table 1. Effect of *A. comosus* leaf extracts on carrageenan induced rat paw oedema.

Treatments	1 h	2 h	3 h	4 h	% Inhibition
Normal control	0.73±0.08	1.40±0.57	1.80±0.57	1.66±0.08	-
Indomethacin (10 mg/kg)	0.20±0.05*	0.50±0.05*	0.36±0.03*	0.23±0.03*	86.14
$CHCl_3$ extract (200 mg/kg)	0.31±0.06*	0.55±0.07*	0.42±0.04*	0.29±0.06*	82.53
MeOH extract (200 mg/kg)	0.26±0.04*	0.43±0.06*	0.31±0.04*	0.25±0.05*	84.93

Values are mean ± SEM (n = 6). *$p < 0.001$ when compared with normal control.

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