

Anti-inflammatory activity of *Annona squamosa* Linn.

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ABSTRACT

An attempt was made to prepare the alcoholic and aqueous extracts of root of *Annona squamosa* were subjected for chemical analysis and anti-inflammatory (carrageenan induced rat paw edema model method) activity. Phytochemical screening was done performed for both the extracts. Both the extracts were subjected to preliminary pharmacological studies at the doses 200 and 400 mg/kg b. w. and the results were compared with standard drugs. The results of anti-inflammatory studies at the doses 200 and 400 mg/kg b. w with reference to standard drug diclofenac sodium (100 mg/kg) showed better inhibition ($P < 0.01$) for alcoholic extract.

Key words: *Annona squamosa*; Ethanolic extract; Aqueous extract; anti-inflammatory.

INTRODUCTION

The World Health Organization (WHO) estimated that 80 % of population developing countries still relies on traditional medicines, mostly plant drugs, for their primary health care¹. Plant products also play an important role in the health care systems of the remaining 20 % population, mainly residents of developed countries. The scientific data generated by research on the plants serves as a valuable tool for identifying plant species and for characterization of the pharmacological active constituents for their biological activities. In the search for new plant it is always important to screen for its activity as first step. Once the plant is identified for beneficial biological activity it is imperative to collect supporting scientific data generated through pharmacognostic and phytochemical properties of plant under investigation.

In the present study we have carried out work on the root of *Annona squamosa* Linn (Annonaceae) to find out anti-inflammatory activity. The plant *Annona squamosa* is commonly called

Custard apple in English and Sharifa in Hindi². The plants reputed to possess varied medicinal properties like, mosquitocidal³, cytotoxic⁴ and anti-oxidant activities⁵.

Both extracts were taken and analyzed for anti-inflammatory activity. This study was carried to give vital information regarding pharmacological activities.

MATERIALS AND METHODS

Plant collection and authentication

Roots of *Annona squamosa* were collected from Medicinal garden of SDM College of Ayurveda, Udupi and authenticated by Dr. T. Shirdhar Bairy by comparison with the standard specimens deposited at the department of Drava Guna, SDM College of Ayurveda. Udupi. Voucher specimen is kept at the NGSM Institute of Pharmaceutical Sciences, Deralakatte, Mangalore. Karnataka. India.

Preparation of extracts

In the present study, the alcoholic extract of air dried root *A. squamosa* powdered material

(500 g) was prepared using Soxhlet apparatus, concentrated and dried using Buchi rotavapor to give a solid reddish brown mass (42.65 g). The powdered root material (500 g) was percolated with cold water to get the aqueous extract (34.55 g). The dried alcoholic and aqueous extracts were stored in desiccators to further carry out phytochemical and pharmacological studies.

The extracts so obtained were further dried in vacuum desiccators and the residue obtained from various extracts was used for further studies by preserving it in refrigerator.

Phytochemical studies

Preliminary phytochemical screening was carried out for both the extracts. These studies were performed according to described standard methods⁶.

Experimental protocol

The animal experiments were carried according to CPCSEA guidelines and after the approval from Institutional Animal Ethics Committee (I.A.E.C). Experiments were conducted in accordance with the standard guidelines. Swiss albino mice and albino rats of either sex (25 g), (180 g) respectively, were obtained from animal colony of NIGM Institute of Pharmaceutical Science, Deralakatte, Mangalore. Animals were kept in animal caging system (four rats per cage on beds of sawdust) under the laboratory conditions (25 ± 2 °C, 12 hour light and dark). They were provided with animal feed pellets manufactured by Hindustan lever (Indian) Ltd., Mumbai. Food was withdrawn 12 hour before the experimental work and water was provided ad libitum. During the course of the experiment the animal behavior was normal. After a 7-day acclimatization period, animals were randomly selected for different experimental groups (six animal /group) and were used for the in vivo determination of anti-inflammatory activity

Dose Preparation

Both extracts and diclofenac sodium were prepared as suspension using 0.6% w/v carboxy methyl cellulose as suspending agent (Sod. CMC) (vehicle).

Anti-inflammatory activity

Anti-inflammatory activity was evaluated using carrageenan-induced hind paw edema method⁷. Carrageenan (0.1 ml of 1% suspension) was injected sub plantar tissue of the right hind paw of each rat. Both extracts (200 and 400 mg/kg b.w) or diclofenac sodium (100 mg/kg) was administered orally to rats 1 hour before carrageenan administration. 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 3 hour and 24 hour of carrageenan injection. Results were determined as percentage inhibition of edema was compared to the control.

Data analyses

All the data are expressed as mean ± SEM. Statistical analyses was performed by one-way ANOVA with Dunnett's-test⁸. P values < 0.01 were considered significant.

RESULT AND DISCUSSION

Phytochemical analysis

The results of phytochemical screening indicates the presence of alkaloids flavonoids, steroids, volatile oil, triterpenoids, glycosides, saponins, proteins, resins, glycosides, tannins and lipids in case of alcoholic extract, where as aqueous extract contains triterpenoids, saponins, alkaloids, flavonoids, tannins, resins.

Anti-inflammatory activity

There was significant dose-dependent in anti-inflammatory activity of both the extracts in which is induced with acute carrageenan-induced rat paw edema model. Orally administered doses of 200 and 400 mg/kg of alcoholic extract of root of *A. squamosa* produced 40 % and 54 % inhibition respectively after 24 hours. Aqueous extract of root of *A. squamosa* produced 24% and 47% inhibition respectively after 24 hour as compared to diclofenac sodium (standard, 100 mg/kg) which showed 72% inhibition after 24 hour (P < 0.01). However, after 3 hour both alcoholic and aqueous extracts did not show any significant anti-inflammatory activity (Table 1).

Table 1: Anti-inflammatory activity of alcoholic and aqueous extracts from *Annona squamosa* root on carrageenan induced paw edema in rats

Treatment (p.o)	Dose (mg/kg) p.o.	Edema volume (ml) (mean \pm SEM)		Percentage inhibition (%)	
		3 hr	24 hr	3hr	24 hr
Control	-	0.37 \pm 0.01	0.32 \pm 0.01	-	-
Diclofenac sodium	100 mg/kg	0.27 \pm 0.01*	0.09 \pm 0.01*	27%	72%
Alcoholic extract	200 mg/kg	0.33 \pm 0.01	0.19 \pm 0.01*	11%	40%
Alcoholic extract	400 mg/kg	0.25 \pm 0.01*	0.15 \pm 0.01*	33%	54%
Aqueous extract	200 mg/kg	0.34 \pm 0.01	0.24 \pm 0.01*	8%	24%
Aqueous extract	400 mg/kg	0.30 \pm 0.01*	0.21 \pm 0.01*	27%	47%

n = 6 per group. Values are mean \pm SEM.

*P<0.01 significant compared to control

Carrageenan induced hind paw edema is the standard model of acute anti-inflammation. It is widely known that carrageenan is the phlogistic agent of choice for testing anti-inflammatory drugs as it is not known to be antigenic and is devoid of apparent systemic effects. Moreover, the experiment model exhibits a high degree of reproducibility. Acute inflammation in rats was induced by sub-plantar injection of carrageenan (phlogistic agent). Various mediators are released like histamine and serotonin (initial phase), kinins (middle phase) and prostaglandins (final phase after 3 to 5 hour of carrageenan injection) play an important role in the development of inflammation⁹.

CONCLUSION

The present study indicates that, the plant contains potential anti-inflammatory components such as sterols, triterpenoids, flavonoids and tannins that may be of use for development of phytomedicine for the therapy of inflammations. Further research work is to needed to establish the exact anti-inflammatory mechanism of action of alcoholic extract of *Annona squamosa*.

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