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Evaluation of anti-inflammatory activity of leaf extract of *Annona muricata*

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ABSTRACT

Annona muricata Linn. (Fam: Annonaceae) is a widely claimed medicinal plant with wide ranging biological activity. In the present study, Petroleum ether and ethanolic extracts of *Annona muricata* which is traditionally used in the treatment of inflammation and fracture was screened for its anti-inflammatory activity by carragenan induced rat paw oedema method. Petroleum ether extract showed significant ($P < 0.01$) anti-inflammatory activity as compared to ethanolic extract. Results were comparable with standard drug (200 mg/kg.p.o). © 2009 Trade Science Inc. - INDIA

KEYWORDS

Annona muricata;
Ibuprofen;
Anti inflammatory activity.

INTRODUCTION

Annona muricata is a small evergreen tree. Leaves leatherly, ill-smelling, obovate or lanceolate, acute or abruptly acuminate, glossy above and rusty beneath, but at length glabrous, with the minute pockets in the axile of the lateral veins scarcely perceptible without a lens. Flowers large, the interior petals lapping. Fruits very large, fleshy, ovoid or heart-shaped, dark green, the glabrous ill-smelling skin bearing numerous recurved. Fleshy spines, pulp white and juicy, pleasant with a slight mango like flavour. The Plant is native of tropical America, India, Karnataka, Maharashtra and Tamilnadu.

Traditionally the seeds are used as emetic and astringent. In Brazil the leaves are steeped in hot water or ground with oil, and used as a maturant. The root is considered as antispasmodic and parasitocidal, the leaves are given in fevers, neuralgia, inflammation, rheumatism and also used in the form of a poultice to produce suppuration^[1]. The flower buds and the flowers are con-

sidered an excellent remedy for cough, the unripe fruits when dried and powdered are given in chronic dysentery, and they are used for apthae in the form of a decoction, the seeds are valued for their astringent and emetic properties^[2,3].

The leaves of the plant contains steroids^[4], flavonoids^[5] and tannins^[6]. Till now no work has been reported regarding antiinflammatory activity of leaves of *Annona muricata*. Hence, the present investigation was designed to evaluate for anti-inflammatory activity and to justify its use in traditional system of medicine.

MATERIALS AND METHODS

Plant material and extraction

The leaves of *Annona muricata* were collected from farms of Bellary districts in Karnataka and authenticated by Dr.G.R. Hegde, Professor and Head, PG department of Botany, Karnataka University, Dharawad, India.

The dried leaves of *Annona muricata* was finely powdered, extracted with Pet-ether(60-80^oc) and with 90% alcohol in a Soxhlet apparatus and extracts were concentrated using rotary flash evaporator. The residue (Yield: 4.5% and 20% W/W) was dried in a desiccator. The extract was formulated into 12.5% W/V suspension using 1% tween 80 as a suspending agent in distilled water and used for the study^[7,8].

Phytochemical analysis

Preliminary phytochemical tests and thin layer chromatographic studies of the leaves of Petroleum ether and ethanolic extract of *A.muricata* indicated the presence of steroids, flavonoids, tannins and terpenoids as a major phytoconstituents.

Animals

The study was carried out using Wistar albino rats of either sex weighing between 150-200 g and mice weighing between 25-30 g. Which were randomly distributed in control, standard and test groups of six animals each. They were kept on a standard diet and water ad libitum. .

Acute toxicity studies

Albino mice of either sex weighing between 20-25 g were used to determine LD 50 of the petroleum ether and ethanolic extracts. 2% Gum acacia was used as suspending agent and extract was administered orally. Study was carried out as per "Up and Down or Staircase" method^[9]. Petroleum ether and ethanolic extracts of *A.muricata* did not show any toxicity and behavioral changes in mice up to 5000 mg/kg, Hence 1/10th of the dose i.e 500 mg/kg, were selected to screen for antiinflammatory activity.

EXPERIMENTAL

Screening of anti-inflammatory activity

Edema represents the early phase of inflammation in carrageenan induced rat paw edema and is the simplest and most widely used model for studying the anti-inflammatory activity of new compounds^[10,11]. Rats were divided into four groups of six animals each. The first group served as the control and received vehicle only (1% tween 80 solution in distilled water), second group

of animals were administered with standard drug, Ibuprofen (200mg/kg, orally). Third group was treated with petroleum ether extract (500 mg/kg, orally) and the fourth group was treated with ethanolic extract (500 mg/kg, orally). The dose of extracts were selected on the basis of acute toxicity test (LD₅₀ dose of extracts). A mark was made on both the hind paws just below the tibio-tarsal junction so that every time the paw could be dipped in the mercury column of plythysmograph up to the mark to ensure constant paw volume. Thirty minutes after treatment, an inflammatory edema was induced in the left hind paw by injection of 0.1ml of carrageenan (1% w/v) in the plane tissue of the paw of all the animals. The right paw served as a reference to non-inflamed paw for comparison. The relative increase in the paw volume was measured in control, standard and sample treated groups in the time duration of 1, 2, 3, 4 and 5 hr after carrageenan injection.

The degree of edema formation was assayed by the percentage increase in paw treated with standard drug and extracts of *Annona muricata*. These were compared with the increased paw volume of control animals. Thus, percentage inhibition of paw volume in treated animals i.e. edema rate (E) % = $(Vt/Vc) \times 100$ which was used for calculating the percentage inhibition rate $(1)\% = 1 - (vt/vc) \times 100$. Where vt and vc are the mean relative changes in the paw volume of the test and control respectively.

Statistical analysis

The experimental results were expressed as the mean \pm standard error of mean (SEM). The statistical significance was evaluated by using the Student's t test. Values of $P < 0.001$ were considered statistically significant^[12].

RESULTS AND DISCUSSION

The Petroleum ether and ethanolic extracts of leaves of *Annona muricata* was subjected for qualitative analysis to identify the possible chemical composition. It showed the positive test for the presence of steroids, flavonoids, carbohydrates, tannins and terpenoids.

The petroleum ether and ethanolic extracts was found to be non-toxic up to the dose of 5g/kg body wt. hence 1/10th of the lethal dose i.e 500 mg/kg body

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TABLE 1: Anti-inflammatory activity of leaf extracts of *Annona muricata* in carrageenan Induced rat hind paw edema model

Groups	Dose (mg/kg, p.o)	Paw volume (mean ± SE)					% Inhibition of edema
		1 hr	2hr	3hr	4hr	5hr	
Control	--	1.367±0.009	1.398±0.008	1.421±0.006	1.476 ±0.010	1.543±0.01	-
Standard(Ibuprofen)	200	0.893±0.006	0.674±0.005	0.612±0.010	0.592±0.010***	0.698±0.009	78.45%
Pet.etherextract	500	0.986±0.006	0.845±0.0003	0.826±0.0009	0.798±0.0006**	0.806±0.0009	66.37%
Ethanolic extract	500	1.381±0.007	1.28±0.004	1.071±0.006	1.043±0.008*	1.006±0.004	40.75%

Each value represents the mean ± SE from 6 animals in each group ** P< 0.01, ***P< 0.001 as compared with control group (student-t-test)

wt. was taken as therapeutic dose for the subsequent study. In acute toxicity study, no toxic symptoms were observed for the drug up to 5000 mg/kg body weight. So the 1/10th of the above dose i.e. 500 mg/kg was selected to study the antiinflammatory activity.

Results of antiinflammatory activities are presented in TABLE 1. Carrageenan induced rat paw exhibited mean increase in edema volume, throughout the observation period. Ibuprofen significantly reduced (P<0.001) the paw volume after 1 hour with an inhibition of 78.45%. Petroleum ether extract showed significant anti-inflammatory activity (P<0.01) as compared to ethanolic extract and it was comparable with the standard drug Ibuprofen with an inhibition of 66.37% at the end of 4hr. The carrageenan model for inflammation was chosen for this study, as it is the widely used noxious stimulus for inducing edema. The development of carrageenan induced edema is biphasic, the first phase is attributed to the release of histamine 5-HT and kinins, while the second phase is related to the release of prostaglandins^[13-15]. The anti-inflammatory activity of leaf extract of *Annona muricata* may be due to presence of steroids, flavonoids, tannins and terpenoids which play vital role in inhibition of inflammation.

CONCLUSION

The petroleum ether extract of leaves of *A.muricata* exhibited significant anti-inflammatory activity (P<0.01) as compared to ethanolic extract. From the results we can conclude that leaves of *A.muricata* are beneficial in the treatment of inflammation and pain threshold. However, Further isolation and characterization is necessary to identify the phytoconstituents responsible for the above activity.

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