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Analgesic and antipyretic activity of leaves of *Annona muricata*

R.A.Shastry*[1], P.V.Kulkarni[1], V.H.Kulkarni[1], B.S.Patil[1], P.V.Habbu[1]

[1]Department of Pharmacognosy and Pharmacology, Post graduate Studies and Research Center, S.E.T'sCollege of Pharmacy, S.R.Nagar, Dharwad, Karnataka-580002, (INDIA)

Tel.: 0836-2448540; Fax: 0836-246719

E-mail: ra_shastri@rediffmail.com

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ABSTRACT

Annona muricata Linn. (Fam: Annonaceae) is a widely claimed medicinal plant with wide ranging biological activities. In the present study, ethanolic and aqueous extracts of leaves of *Annona muricata* was screened for analgesic and antipyretic activity in albino rats using various experimental models viz acetic acid induced writhing method, hot plate method and yeast induced pyrexia. The ethanolic extract (500 mg/kg, p.o) exhibited significant analgesic and antipyretic activity as compared to aqueous extract.

KEYWORDS

Annona muricata;
Aspirin;
Pentazocin;
Analgesic;
Antipyretic 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, flesy, 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, Maharastra 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 parasiticidal, the leaves are given in fevers, neuralgia, rheumatism and also used in the form of a poultice to produce suppuration[1]. The flower buds and the flowers are considered an excellent remedly for cough, the unripe fruits when dried and powered are given in chronic dysentry, 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 analgesic and antipyretic activity of leaves of *Annona muricata*. Hence, the present investigation was designed to evaluate for analgesic and antipyretic activity and to justify it's 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.

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The dried leaves of Annona muricata was finely powdered, extracted with 95% alcohol and distilled water in a Soxhlet extract and concentrated using rotary flash evaporator. The residue (Yield: 25% and 12% W/W) was dried in a desicator. 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 ethanolic and aqueous 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. The rectal temperature was recorded using digital theromometer and rats having temperature between 36°C and 37°C were selected for the study.

Acute toxicity studies

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

EXPERIMENTAL

Analgesic activity

a. Acetic acid induced writhing method

Anagesic activity was evaluated by acetic acid induced writing method using Wister albino mice (25-30 g). The alcoholic and aqueous extract was administered orally (500 mg/kg body wt.) to the test group animals 30 minutes prior to the administration of writhing agent (0.6% v/v aqueous acetic acid, 1ml/100 g by i.p route). Aspirine (100 mg/kg body wt.) served as standard. Writhings produced in the mice were observed for 30 minutes and compared with the standard[10].

b. Hot plate method

Glassman's method was used. The extract was administerd orally to a group of six mice after overnight fast. The time of reaction to the pain stimulus of the mice placed on the heated plate at 55+0.5 °C was recorded at different time intervals. The increase in reaction time against control group was calculated. Another group of animals recived Pentazocin (10mg/kg) by i.p. route[11].

c. Antipyretic activity

Antipyretic activity was evaluated by Brewer's yeast induced pyrexia method. Fever was induced by injecting 20 ml/kg body wt. of 15% aqueous suspension of yeast in sterile water by subcutaneous route. Eighteen hours after the induction of pyrexia, first group of animals received 500 mg/kg of ethanolic and aqueous extract and the third group received 100 mg /kg of aspirin which served as standard for comparison. Rectal temperature was recorded before as well as after 18 hours of yeast administration at different time intervals[12].

Statistical analysis

The data were expressed as Mean ±SEM (n=6). The data were analysed using Student's 't'-test. $P<0.01$ were considerd statistically significant.

RESULTS AND DISCUSSION

The ethanolic and aqueous 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 ethanolic and aqueous extracts was found to be non- toxic up to the dose of 5g/kg body wt. and hence $1/10^{th}$ of the lethal dose i.e 500 mg/kg body wt. was taken as therapeutic dose for the subsequent study.

In acetic acid induced writhing test, ethanolic extract (500mg/kg, p.o.) reduced writhing counts, pro-

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ducing 69.64% analgesia (TABLE 1) Aspirin significantly increased the pain threshold and produced 81.3% analgesic effect.

Analgesic activity by hot plate method was carried out further to confirm the efficiency of the extract in reducing pain. The results indicated that extract has comparable analgesic effects. Pentazocin was used as a standard and exhibited significant($P<0.001$) analgesic activity. Results are depited in the TABLE 2.

The results of antipyretic activity are given in TABLE 3. After 18 hours of Brewer's yeast injection, the experimental rats showed a mean increase of about 1°C in rectal temperature. Ethanolic extract produced significant antipyretic activity ($p<0.01$) from third hour onwards till the end of the study as compared to aqueous extract. Aspirin showed significant ($p<0.001$) antipyretic activity throughout the observation period up to 5 hours.

It is well known that most of the anti-inflammatory analgesic drugs possess antipyretic activity. Ethanolic extract of leaves produced marked analgesic and antipyretic activity in rats. In general, NSAIDs produce their antipyretic action through the inhibition of PG synthetase within the hypothalamus[13]. Although there is evidence of the extract to interfere with PG synthesis in hypothalamus , it appears that antrpyretic action of the extract may be related to the inhibition of PG synthesis[14,15]. The present study demonstrates that the ethanolic extract of leaves of *Annona muricata* exhibited significant antipyretic and analgesic activities. The presence of steroids, flavonoids, tannins and terpenoids in the ethanolic extract may be responsible for the above activity. Aqueous extract showed moderate activity as compared to ethanolic extract.

Results were comparable with standard drug aspirin and pentazocin at a dose of 100 mg/kg and 10 mg/kg respectively.

TABLE 1: Effect of ethanolic and aqueous extract on acetic acid induced writhings

Treatment	Dose	Number of writhings	%Anagesia
Control	-	41.7 ± 0.79	
Ethanolic extract	500mg/kg	10.83 ± 0.47	69.64%**
Aqueous extract	500mg/kg	16.28 ± 0.74	55.48%*
Aspirin	100mg\kg	7.8 ± 0.252	81.33%**

Values are mean±SE from 6 animals in each group; *p<0.01; **p<0.001 as compared with control group

TABLE 2: Analgesic activity of ethanolic and aqueous extract by hot plate method

Treatment	Dose	1 hour	2 hour	3 hour
Control	-	1.50±0.52	1.50±0.58	2.00±0.00
Ethanolic extract	500mg/kg	2.80±0.25	4.50±0.12	5.40±0.25**
Aqueous extract	500mg/kg	1.98±0.24	3.46±0.41	4.89±0.23*
pentazocin	10mg/kg	4.30±0.121	6.40±0.48	6.80±0.21**

Values are mean ± SE from 6 animals in each group; *p<0.01; **p<0.001 as compared with control group

CONCLUSION

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

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TABLE 3 : Effect of ethanolic and aqueous extract on yeast induced pyrexia in rats

Treatment	Dose	Predrug rectal Temperature temperature	after18 hrs of yeast injection	Mean change in temperature 1hour	3hour	5hour
Vehicle	-	36.66±0.07	37.43±0.07	37.80±0.22	38.01±0.21	37.88±0.17
Ethanolic extract	500mg/kg	36.63±0.10	37.73±0.03	37.43±0.16	37.08±0.04*	36.48±0.15**
Aqueous extract	500mg/kg	37.35±0.12	38.85±0.05	38.54 ± 0.17	37.99±0.05	37.95±0.12*
Aspirin	100mg/kg	36.20±0.05	37.33±0.11	36.83 ± 0.20	36.50±0.07**	36.33±0.03**

Values are mean + SE from 6 animals in each group; *p<0.01; **p<0.001 as compared with control group

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