

ANTIPYRETIC ACTIVITY OF ANNONA PLANTS LEAVES ON BREWER'S YEAST INDUCED FEBRILE RATS

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ABSTRACT

Objective: To evaluate and compare the in vivo antipyretic activity of the methanolic extracts of selected three Annona plant leaves.

Methods: The acute oral toxicity determined by OECD class method and the in vivo antipyretic activity was determined by brewer's yeast induced pyrexia method.

Results: The results showed that the methanolic extract of leaves of Annona plants are non-toxic and possessed significant antipyretic effect which may be attributed to the presence of flavonoids and saponins in the extracts.

Conclusion: This study provides evidences for the antipyretic activity of Annona squamosa, Annona reticulata and Annona muricata possess antipyretic activity at the tested doses 100 and 200 mg/kg body weight without any side effects, which could partly contribute to its ethno medical use.

Keywords: *Annona squamosa* L, *Annona reticulata* L, *Annona muricata* L, Antipyretic activity, Brewer's yeast.

INTRODUCTION

Fever or pyrexia is an elevated body temperature above the normal level characterized by an increase in thermoregulatory set-point, which results from the interaction of the central nervous and immune system. Fever is body's natural defense mechanism against infectious agents which can damage the tissue. This in turn triggers the enhanced formation of pro-inflammatory cytokines like tumor necrosis factor- α (TNF- α) and interleukin 1 β , α and β , these pro-inflammatory mediators increase the synthesis of prostaglandin E₂ (PGE₂) near hypothalamus area and thereby trigger the hypothalamus to elevate the body temperature. The thermoregulatory system governed by nervous feedback mechanism alters the fever by vasodilation and vasoconstriction of blood vessels. Although fever is body's defensive mechanism, some studies have suggested that raising temperature may be harmful. Therefore, in clinical practices in which fever-associated risks offset benefits, antipyretic treatment is necessary [1].

Most of the marketed anti-inflammatory drugs possess antipyretic activity like paracetamol, aspirin, nimesulide, etc. These non-steroidal anti-inflammatory drugs inhibit the synthesis of PG to reduce the inflammation, as well as fever. Greater of these drugs have toxic effect to the various organs of the body [2]. Therefore, the development of novel compounds having antipyretic and anti-inflammatory activities with improved safety profiles remains a clinical need [3]. Therefore, the present study aimed to evaluate the antipyretic effect of methanolic leaf fractions of three Annona plants.

METHODS

Plant material

The leaves of three plants were collected from Kondapalli forest range, Krishna District of Andhra Pradesh during January and authenticated by Dr. Kasim, Department of Botany, Acharya Nagarjuna University.

Preparation of plant extract

The collected leaves of three plants were shade dried and pulverized to a coarse powder by the mixer and sieved through the mesh. Each powder was subjected to extraction individually with methanol using

soxhlet apparatus at 64.7°C. The extraction was carried out until the plant material become colorless. The extract is then concentrated and dried under reduced pressure by using rotary evaporator. The solvent free semisolid mass thus obtained respectively. The methanol fractions of three plants (coded as ASME [*Annona squamosa*], ARME [*Annona reticulata*] and AMME [*Annona muricata*]) were considered for the screening of antipyretic activity.

Experimental animals

The female Wistar rats (180-200 g, 8-12 weeks) were procured from Venkateswara Enterprises, Bengaluru and housed (3 animals/cage) in polypropylene cages with stainless steel with paddy husk bedding floor and fed with Nutrilab standard rodent diet manufactured (Provimi. Pvt. Ltd.) and filtered water was supplied *ad libitum* for all the animals. The animal room was maintained at a controlled temperature (22 \pm 3°C) and light (12 hrs' light and dark cycles). All experiments were carried out according to the guidelines for care and use of experimental animals and approved by Committee for Control and Supervision of Experiments on Animals. The study was approved by Institutional Animal Ethics Committee (IAEC) and the approval number is 001/IAEC/NCPA/Ph.D/2012-2013.

Acute toxicity study

The acute toxicity of methanolic fractions of three Annona plants were determined as per the OECD guideline no. 423 (acute toxic class method). The 18 female Wistar rats (6 animals per group) were used for the screening of acute toxicity. After a single dose administration of different test item at 2000 mg/kg, observed over a period of 14 days and based on the results the median lethal dose of ASME, ARME, and AMME was found that LD₅₀ is >2000 mg/kg. Hence, 1/20th (100 mg/kg) and 1/10th (200 mg/kg) of this dose were selected for further study [4].

Antipyretic activity study

Brewer's yeast induced pyrexia in rats

This antipyretic activity animal model was slightly modified method described by Adams *et al.* [5]. Antipyretic activity on Wistar rats was screened with Brewer's yeast induced pyrexia. The rats were divided into eight groups of six each. The basal rectal temperature of the rats

was measured by introducing 1-2 cm of digital thermometer in rectum. After measuring the basal rectal temperature, the pyrexia was induced by intraperitoneal injection, 20% suspension of brewer's yeast in normal saline at a dose of 10 ml/kg of body weight. After 18 hrs of yeast injection, rats which showed a raise in temperature of at least 1°C were taken for the study. Immediately after 18 hrs of yeast injection, animals in the various groups were treated as follows:

Group I: 0.5 % w/v carboxymethylcellulose sodium solutions (CMC-Na) (10 ml/kg body wt., p.o) (Control Group)

Group II: ASME (100 mg/kg body wt., p.o) suspended in 0.5% CMC-Na

Group III: ASME (200 mg/kg body wt., p.o) suspended in 0.5% CMC-Na

Group IV: ARME (100 mg/kg body wt., p.o) suspended in 0.5% CMC-Na

Group V: ARME (200 mg/kg body wt., p.o) suspended in 0.5% CMC-Na

Group VI: AMME (100 mg/kg body wt., p.o) suspended in 0.5% CMC-Na

Group VII: AMME (200 mg/kg body wt., p.o) suspended in 0.5% CMC-Na

Group VIII: Paracetamol (100 mg/kg body wt., p.o) (standard group).

The rectal temperature was measured at 1-4 hrs after treatment.

Statistical analysis

The data were expressed as a mean \pm standard deviation for 8 groups of six rats each. The data were subjected to statistical analysis using ANOVA followed by Dunnett's test to draw a comparison between control and treatment groups. $p \leq 0.05$ was considered as statistically significant.

RESULTS

Effect of methanolic leaf extract of *A. squamosa*, *A. reticulata* and *A. muricata* on brewer's yeast induced rectal temperature in rats is presented in Table 1. The intraperitoneal injection of brewer's yeast suspension markedly elevated the rectal temperature after 18 hrs of administration. Treatment with ASME, ARME, and AMME at a dose of 100, 200 mg/kg decreased the rectal temperature of the rats in dose-dependent manner. It was found that all the three extract at a dose of 100 and 200 mg/kg caused significant ($p < 0.001$) lowering of rectal temperature at 4 hrs following its administration. The ASME, ARME, and AMME at 4 hrs rectal temperature measurement point showed reduction of 47.06%, 40.44%, and 59.56% at 100 mg/kg and 66.91%, 55.88%, 69.85% at 200 mg/kg, respectively, and the standard drug paracetamol at 100 mg/kg showed 90.44% (Fig. 1). Whereas, all the three methanolic fractions maximum effect disclosed at dose of 200 mg/kg. The antipyretic effect started as early as 1st hr and the effect was maintained for 4 hrs, after its administration. The standard drug paracetamol 100 mg/kg and tested drug *A. squamosa* L, *A. reticulata* L and *A. muricata* L methanolic fractions were significantly reduced the yeast-elevated rectal temperature, at 2nd, 3rd and 4th hrs compared to control group. However, the *A. muricata* fraction showed better rectal temperature reduction when compared to other two plants.

DISCUSSION

The results of the above experiment suggested that the leaf methanolic fractions of *A. squamosa*, *A. reticulata* and *A. muricata* possess

antipyretic activity at the tested doses. These plant leaves have phytochemicals such as β -sitosterol, triterpenes, flavonoids, saponins, glycosides, tannins, alkaloids, proteins, lipids, and carbohydrates. The β -sitosterol reduces PG and leukotrienes synthesis and in turn shows anti-inflammatory and antipyretic activity by inhibiting the pro-inflammatory cytokines and TNF- α [6,7]. Adaptive immunity can be increased by these phytosterols by the stimulation of innate immunity system called adaptogen which helps for health without any side effects [8]. The steroids, tannins, and flavonoids are predominant inhibitors of PG synthetase and cyclooxygenase or lipoxygenase, this mechanism helps in inhibition pyrexia [9]. The antipyretic activity observed can be attributed to the presence of flavonoids, saponins, glycosides, tannins [10].

The infection, tissue damage, graft rejection, inflammation or disease states may lead to pyrexia. Antipyretic are the agents, which cause the hypothalamus to supersede an interleukin induced fever to normal levels. Brewer's yeast induced fever is pathogenic fever, in which the production of PGs involves [11]. The present results shows the leaves of three *Annona* plants has significant antipyretic activity in yeast induced pyrexia in rats, and this effect is comparable to standard drug paracetamol. Hence, there may be a possible mechanism of antipyretic action by inhibiting the synthesis of PGs like paracetamol [12]. Furthermore, there are several multiprocesses or mediators emphasizing the pathogenesis of fever. Inhibition of any of these mediators may bring about antipyresis [13].

CONCLUSION

The outcome of this study indicates that the leaf methanolic fractions of *A. squamosa*, *A. reticulata* and *A. muricata* possess antipyretic property at the tested doses. However, *A. muricata* leaf methanolic fraction showed better action when compared to others. This could provide a rationale for the use of these plants in fever as an herbal medicine without any side effects.

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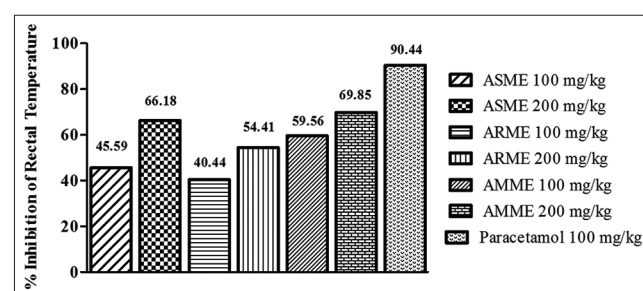


Fig. 1: Percentage inhibition of methanolic fractions of three *Annona* plants at 4 hrs after treatment

Table 1: Effect of plant extracts against Brewer's yeast induced Pyrexia in Wistar rats

Test item	Dose (mg/kg)	Body weight (g)	Initial rectal temperature (°C)	Rectal temperature (°C) 18 hrs after Brewer's yeast induction	Rectal temperature (°C) after treatment with extract			
					1 hr	2 hr	3 hr	4 hr
Control	-	188.33 \pm 1.63	37.07 \pm 0.25	39.35 \pm 0.22	39.62 \pm 0.38	39.57 \pm 0.36	39.45 \pm 0.36 [#]	39.33 \pm 0.37 [#]
ASME	100	188.33 \pm 4.63	37.10 \pm 0.26	39.25 \pm 0.25	39.12 \pm 0.22*	38.88 \pm 0.08 [#]	38.63 \pm 0.14 [#]	38.30 \pm 0.23 [#]
	200	191.67 \pm 6.22	37.10 \pm 0.29	39.20 \pm 0.14	39.02 \pm 0.15**	38.70 \pm 0.11 [#]	38.18 \pm 0.13 [#]	37.85 \pm 0.16 [#]
ARME	100	188.17 \pm 6.08	37.10 \pm 0.26	39.28 \pm 0.13	39.07 \pm 0.18*	38.85 \pm 0.14 [#]	38.62 \pm 0.16 [#]	38.45 \pm 0.23 [#]
	200	192.17 \pm 7.19	37.08 \pm 0.25	39.30 \pm 0.28	39.12 \pm 0.35*	38.90 \pm 0.39 [#]	38.50 \pm 0.32 [#]	38.08 \pm 0.21 [#]
AMME	100	190.33 \pm 4.76	37.07 \pm 0.18	39.32 \pm 0.29	38.93 \pm 0.39**	38.62 \pm 0.40 [#]	38.30 \pm 0.29 [#]	37.98 \pm 0.37 [#]
	200	190.33 \pm 5.28	37.08 \pm 0.17	39.35 \pm 0.33	38.97 \pm 0.37**	38.37 \pm 0.21 [#]	38.05 \pm 0.23 [#]	37.77 \pm 0.16 [#]
Paracetamol	100	193.33 \pm 6.12	37.10 \pm 0.18	39.27 \pm 0.18	38.58 \pm 0.26 [#]	38.22 \pm 0.19 [#]	37.50 \pm 0.29 [#]	37.32 \pm 0.19 [#]

Values are expressed as mean \pm SD, n=6 and * $p \leq 0.05$, ** $p \leq 0.01$ and, [#] $p \leq 0.001$ versus control, SD: Standard deviation

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