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Comparative antipyretic activity of methanolic extracts of some species of *Amaranthus*

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ABSTRACT

Objective: To provide scientific validation for the antipyretic activities of *Amaranthus viridis* (Linn.), *Amaranthus caudatus* (Linn.) and *Amaranthus spinosus* (Linn.). **Methods:** The antipyretic activity of methanol extracts of all three plants at doses of 200 and 400 mg/kg was investigated by yeast induced pyrexia in rats. Paracetamol (150 mg/kg, p.o.) was used as reference drug and control group received distilled water. Rectal temperatures of all the rats were recorded and compared at 19 h, immediately before extract or vehicle or paracetamol administration, and again at 1 h interval up to 24 h by thermal probe Eliab themistor thermometer. **Results:** At 400 mg/kg dose all the three methanolic extracts showed significant ($P<0.01$) reduction in yeast provoked elevated temperature as compared with that of standard drug paracetamol, whereas 200 mg/kg dose is less effective when compared with higher dose ($P<0.05$). **Conclusions:** The results show that methanol extract of three plants of *Amaranthus* possesses a significant antipyretic effect in maintaining reducing yeast-induced elevated body temperature in rats and their effects were comparable to that of the standard antipyretic drug paracetamol.

1. Introduction

Amaranthus viridis L (Amaranthaceae) (*A. viridis*) (commonly called as 'Chilaka Thota-Kura' in Telugu) has been used in Indian traditional medical system and in Nepal to lessen labour pain and as antipyretic[1,2]. A. viridis contains amino acids lysine, arginine, histidine, cystine, phenylalanine, leucine, isoleucine, valine, threonine, methionine, tyrosine and tryptophan[3]. The Negritos of the Philippines apply the bruised leaves directly to eczema, psoriasis and rashes, *etc*[4]. Other traditional uses are anti-inflammatory of the urinary tract, in venereal diseases, respiratory problems, eye treatment and asthma[1,3,5-11]. Furthermore, the plant possesses antiproliferative and antifungal lactin properties as well as ribosome inactivating protein, beta-carotene[12-14] and antiviral activities[15]. In addition the whole plant possesses analgesic and antipyretic properties and is used for the treatment of pain and fever respectively in traditional systems of medicine[16].

Amaranthus caudatus Linn, (Amarnathaceae), (*A. caudatus*) commonly known as "Peddathotakura" in Telugu, was once nearly as important a food as maize and beans in central and South America. The *Amaranthus* plants are spread throughout the world, growing under a wide range of climatic conditions and they are able to produce grains and leafy edible vegetables. In India *A. caudatus* was traditionally used to cure kidney stones, stomach pain, leprosy, fever, piles[17], as blood purifier, diuretic, vermifuge, astringent[18]. In Southeastern Ethiopia seeds of *A. caudatus* were used in amoebiasis, jaundice and kidney diseases[19]. The leaf has also been used as tea for relieving pulmonary conditions. In South Africa leaf is used as an abortifacient[20].

Antimicrobial peptides, triterpenoid saponins, agglutinin, vitamin E isomers and amaranthin were isolated from *A. caudatus*, which showed antiatherosclerotic[21], anthelmintic activities[22]. *A. caudatus* seeds showed cholesterol lowering, *in vitro* antioxidant and alpha amylase inhibition activities[23,24]. The *Amaranthus* seed oil is used as

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nutraceutical resource from Ecuadorian flora.

Amaranthus spinosus Linn., (Amaranthaceae) (*A. spinosus*), commonly known as "Mulluharivesoppu" in Kannada, is an annual or perennial herb, native to tropical America and found throughout India as a weed in cultivated as well as fallow lands. In Indian traditional system of medicine (Ayurveda) the plant is used as analgesic, antipyretic, laxative, diuretic, digestible, antidiabetic, anti-snake venum, antileprotic for blood diseases, bronchitis, piles and anti-gonorrheal [3]. Some tribes in India apply *A. spinosus* to induce abortion. The juice of *A. spinosus* was used by tribe of Kerala, India to prevent swelling around stomach while the leaves were boiled without salt and consumed for 2-3 days to cure jaundice[25]. *A. spinosus* is also used as reported for its anti-inflammatory[26], antimalarial[27], immunomodulatory[28], anti-diabetic, anti-hyperlipidemic and spermatogenic activities[29]. The betalains in stem bark of *A. spinosus* were identified as amaranthin, isoamaranthine, hydroxycinnamates, rutin, quercetin and kaempferol glycosides[30,31]. It also contains amaranthoside, a lignan glycoside, amaricin, a coumaroyl adenosine along with stigmasterol glycoside, betaine such as glycinebetaine and trigonelline. Betalains are well known for their antioxidant, anticancer, antiviral and antiparasitosis properties[32].

A. viridis, *A. caudatus* and *A. spinosus* have been used for the treatment of pain in Indian traditional system of medicine. However, there is lack of scientific report regarding to its analgesic activity, so our aim is to provide scientific validation for traditional claims.

2. Materials and methods

2.1. Collection of plant material and extraction

The fresh plant of *A. viridis*, *A. caudatus* and *A. spinosus* was collected from Chickballapur, and was authenticated by Dr. Rajan from Department of Botany, Government Arts College, Ootcamund, Tamilnadu. A voucher specimen (SKVCP 11, 12, 13) was deposited in college herbarium. Whole plant of *A. viridis* and *A. caudatus* and leaves of *A. spinosus* were shade dried and coarsely powdered. The coarse powder was subjected to extraction with methanol by Soxhlet apparatus and extracts were concentrated to dryness in vacuum. Methanolic extract of all the three plants were screened for the presence of various phytoconstituents[33].

2.2. Animals

Male Swiss albino mice (20-25 g) of either sex were acclimatized to the experimental room at temperature of (23±2) ℃, controlled humidity conditions (50%-55%) and 12 h light and dark cycle. They were caged with a maximum of two animals in polypropylene cage and were fed with standard food pellets (Kamadenu Enterprises, Bangalore) and water *ad libitum*.

2.2. Acute toxicity studies

Methanol extracts of *A. viridis*, *A. caudatus* and *A. spinosus* were studied for acute oral toxicity as per revised OECD (Organization for Economic Cooperation and Development) guidelines No. 423[34]. The extract was devoid of any toxicity in mice when given in dose up to 2 000 mg/kg by oral route. Hence, for further studies 200-400 mg/kg doses of extract were used.

2.3. Induction of fever by yeast-induced pyrexia

The antipyretic activity of methanolic extracts of *A. viridis*, *A. caudatus* and *A. spinosus* was evaluated using Brewer's yeast-induced pyrexia in rats as described by Loux *et al*[35]. Fever was induced by administration of 20 mL/kg of 20% aqueous suspension of Brewer´s yeast (*Saccharomyces cerevisiae*) in normal saline subcutaneously. Methanolic extracts of *A. viridis*, *A. caudatus* and *A. spinosus* at doses of 200 and 400 mg/kg were administered orally. Paracetamol (150 mg/kg, p.o.) was used as reference drug and control group received distilled water. Rectal temperatures of all the rats were recorded and compared at 19 h, immediately before extract or vehicle or paracetamol administration, and again at 1 h interval up to 24 h by thermal probe Eliab themistor thermometer.

2.4. Statistical analysis

Data were expressed as mean±SEM. The statistical significance of differences between groups was determined by analysis of variance (ANOVA), followed by Dunnett's test. Differences of $P<0.05$ were considered statistically significant.

3. Results

3.1. Preliminary phytochemical screening

Preliminary phytochemical screening of methanol extracts of all the three plants revealed the presence of steroids, flavonoids, glycosides, carbohydrates, terpenoids and aminoacids, respectively.

3.2. Acute toxicity studies

The preliminary acute oral toxicity test of methanol extract of all the three plants at the highest dose of 2 000 mg/kg did not show any mortality or behavioral changes in the animals.

Table 1
Effect of methanolic extracts of three plants on yeast induced pyrexia in rats (mean±SEM) (n=6).

Treatment	Dose (mg/kg)	Rectal temperature (℃) after yeast injection						
		0 h	19 h	20 h	21 h	22 h	23 h	24 h
Control		37.39±0.03	39.16±0.02	39.20±0.15	39.20±0.15	39.05±0.18	39.20±0.15	38.58±0.21
Paracetamol	150	36.93±0.41	39.20±0.56	37.90±0.21**	37.70±0.31**	37.52±0.20**	37.40±0.17**	37.32±0.16**
A. viridis	200	37.26±0.17	38.78±0.45	37.86±0.42*	38.71±0.34*	38.12±0.21**	38.40±0.17	38.10±0.10
	400	37.40±0.17	39.10±0.18	38.90±0.34	37.63±0.22**	37.57±0.20**	37.45±0.19*	37.40±0.17**
A. spinosus	200	37.30±0.11	39.00±0.27	38.25±0.37	38.11±0.30	38.32±0.33*	38.45±0.18	38.04±0.22
	400	37.28±0.09	39.26±0.39	38.90±0.30*	37.75±0.27**	37.43±0.20**	37.65±0.20*	37.30±0.18**
A. caudatus	200	37.26±0.08	38.70±0.42	38.53 ±0.37	38.20±0.31	38.13±0.17**	38.40±0.31*	38.08±0.20
	400	37.25±0.16	39.50±0.20	38.80±0.28*	37.83±0.29**	37.58±0.20**	37.70±0.19**	37.61±0.18**

*P<0.05, ** P<0.01 *vs* control.

3.3. Antipyretic activity

The effect of three plant extracts on yeast-induced pyrexia was presented in Table 1. The data revealed that the rectal temperature of 37.2 ℃ at 0 h was markedly elevated to 39.2 ℃ for vehicle control and paracetamol group 19 h after the subcutaneous injection of yeast suspension. The animals treated with methanolic extracts of three plants at 200 and 400 mg/kg showed a decrease in the rectal temperature by 0.20, 0.92, 0.36, 0.75, 0.17, 0.70 ℃, respectively within 1 h. On the otherhand, from 21 h to the end of the experiment 400 mg/kg dose of methanol extract of three plants showed significant antipyretic activity (P<0.01). At 200 mg/kg dose, *A. viridis* showed significant activities at 21 and 22 h, *A. spinosus* (P<0.05) at 22 h and *A. caudatus* (P<0.01 and P<0.05) at 22 h and 23 h. However, paracetamol (150 mg/kg) treated group which showed rectal temperature of 39.2 ℃ was reduced by 1.3 at 20 h, 0.19 at 21 h, 0.18 at 22 h, 0.11 at 23 h and 0.08 at 24 h, respectively. The antipyretic effect of methanolic extract of three plants at 400 mg/kg was similar to the paracetamol group.

4. Discussion

Pyrexia or fever is caused as a secondary impact of infection, tissue damage, inflammation, graft rejection, malignancy or other diseased state. It is the body's natural defence to create an environment where infectious agent or damaged tissue cannot survive. Normally the infected or damaged tissue initiates the enhanced formation of pro-inflammatory mediators (cytokines like interleukin 1β, α, β and TNF-α), which increase the synthesis of prostaglandin E2 (PEG2) near preoptic hypothalamus area and thereby triggering the hypothalamus to elevate the body temperature[35]. As the temperature regulatory system is governed by a nervous feedback mechanism, so when body temperature becomes very high it will dilate the blood vessels and increase sweating to reduce the temperature; but when the body temperature becomes very low, hypothalamus will protect the internal temperature by vasoconstriction. High fever often increases faster disease progression by increasing tissue catabolism, dehydration, and existing complaints, as found in HIV, when fever during seroconversion results in disease progression[36]. Most of the antipyretic drugs inhibit COX-2 expression to reduce the elevated body temperature by inhibiting PGE2 biosynthesis[35]. Moreover, these synthetic agents irreversibly inhibit COX-2 with high selectivity but are toxic to the hepatic cells, glomeruli, cortex of brain and heart muscles, whereas natural COX-2 inhibitors have lower selectivity with fewer side effects[36]. Search for herbal remedies with potent antipyretic activity received momentum recently as the available antipyretics, such as paracetamol, nimusulide *etc*, have toxic effect on the various organs of the body[36]. The results showed that methanol extract of three plants of *Amaranthus* possesses a significant antipyretic effect in maintaining reducing yeast-induced elevated body temperature in rats and their effects were comparable to that of the standard antipyretic drug paracetamol. Preliminary phytochemical study indicated the presence of alkaloids, steroids, glycosides, flavonoids, phenolic compounds, terpenoids, proteins and carbohydrates which may be responsible for the antipyretic activity.

Conflict of interest statement

We declare that we have no conflict of interest.

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