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Pain management in mice using methanol extracts of three plants belongs to family *Amaranthaceae*

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ABSTRACT

Objective: To investigate the analgesic activity of methanolic extract of *Amaranthus viridis(A. viridis)*, *Amaranthus caudatus (A. caudatus)* and *Amaranthus spinosus (A. spinosus)*. **Methods:** In this study, the analgesic activity of methanol extracts of all three plants at doses of 200 and 400 mg/kg were investigated by acetic acid-induced writhings test, hot plate test and tail immersion test for mice. **Results:** It was found that all the three plants showed significant pain management effect($P<0.01$) at a dose of 400 mg/kg, but showed a less significant effect at a dose of 20 mg/kg in the entire tests used for evaluation of analgesic activities ($P<0.05$). **Conclusions:** Methanol extracts of *A. viridis*, *A. caudatus* and *A. spinosus* show potent analgesic activities, and this study provides the scientific proof for their traditional claims.

1. Introduction

Amaranthus viridis (*A. viridis*)L (Amaranthaceae) commonly called as "Chilaka Thota-Kura" in Telugu. *A. viridis* has been used in Indian traditionally system and in Nepal to relief labour pain and as antipyretic[1,2]. The Negritos of the Philippines apply the bruised leaves directly to cure eczema, psoriasis and rashes[3]. Other traditional uses are antiinflammatory of the urinary tract, vermifuge for venereal diseases, diuretic, antirheumatic, antiulcer, analgesic, antiemetic, laxative, antileprotic. It can improve appetite, and treat respiratory problems, eye diseases and asthma[1,4-6]. A novel antiproliferative and antifungal lactin and ribosome inactivating protein, β-carotene isisolated from *A. viridis*[7] and it also possesses antiviral activity[8].

Amaranthus caudatus (A. caudatus) Linn, (Amarnathaceae), is commonly known as "Peddathotakura" in Telugu. It was once a food nearly as important 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* is traditionally used to cure kidney stones, stomach pain, leprosy, fever, piles[9], as blood purifier, diuretic, vermifuge, astringent[10]. In Southeastern Ethiopia seeds of *A. caudatus* has been used in amoebiasis, jaundice and kidney diseases[11]. The leaf has also been used as tea for relieving pulmonary conditions. In South Africa leaf is used as an abortifacient[12].

Antimicrobial peptides, triterpenoid saponins, agglutinin, vitamin E isomers and amaranthin were isolated from *A. caudatus*. *A. caudatus* showed antiatherosclerotic[13], and anthelmentic[14]. *A. caudatus* seeds showed cholesterol lowering, *in vitro* antioxidant and alpha amylase inhibition activities[15,16]. The amaranth seed oil is used as nutraceutical resource from Ecuadorian flora.

Amaranthus spinosus (A. spinosus) Linn., (Amaranthaceae), commonly known as "Mulluharivesoppu" in Kannada, is an annual or perennial herb, native to tropical America and found as a weed in cultivated as well as fallow lands throughout India. In Indian traditional system of medicine (Ayurveda) the plant is used as analgesic, antipyretic, laxative, diuretic, digestible, antidiabetic, anti-snake venum, antileprotic, anti-gonorrheal, and in treatement for blood diseases, bronchitis, and piles[1]. Some tribes

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in India apply *A. spinosus* to induce abortion. The juice of *A. spinosus* used by tribal of Kerala, India to prevent swelling around stomach while the leaves are boiled without salt and consumed for 2-3 days to cure jaundice[17]. *A. spinosus* is also reported to be used as anti-inflammatory[18], antimalarial[19], immunomodulatory[20], anti-diabetic, anti-hyperlipidemic and spermatogenic activating agent[21]. With effect on hematology, antiandrogenic biochemical agent changes in epididymis. The betalains in stem bark of *A. spinosus* were identified as amaranthin, isoamaranthine, hydroxycinnamates, rutin, quercetin and kaempferol glycosides[22,23]. 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[24].

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 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 plants of *A. viridis*, *A. caudatus* and *A. spinosus* were collected from Chickballapur, and was authenticated by Dr. Rajan, Department of Botany, Government Arts College, Ootcamund, Tamilnadu. A voucher specimen (SKVCP 11, 12, 13) was deposited in college herbarium. Whole plants 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 extracts of all the three plants were screened for the presence of various phytoconstituents[25].

2.2. Animals

Male Swiss albino mice (20-25 g) of either sex were acclimatized to the experimental room at temperature (23 ±2) ℃, controlled humidity conditions (50-55%) and 12 h light and 12 h 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*.

2.3. Acute toxicity studies

Methanol extracts of *A. viridis*, *A. caudatus* and *A. spinosus* were studied for acute oral toxicity as per revised Organization for Economic Cooperation and development (OECD) guidelines No. 423[26]. 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 extracts were used.

2.4. Acetic acid-induced writhing test

This test was done using the method described by Collier *et al*[27]. Muscle contractions were induced in rats by intra peritoneal injection of 0.6% solution of acetic acid (10 mL/kg). Immediately after administration of acetic acid, the animals were placed in glass cages, and the number of 'stretching' per animal was recorded during the following 15 min. Methanol extracts of three plants were administrated orally at doses of 200 mg/kg and 400 mg/kg and diclofenac sodium at 50 mg/kg was administered 30 min before the acetic acid injection.

2.5. Hot plate method

The hot plate test described by Eddy *et al*[28] was used. The mice were firstly treated with different doses of methanol extracts of three plants at 200 mg/kg and 400 mg/kg p.o. After 1 h of extracts administration they were placed on a hot plate maintained at (55± 1) ℃. A cut-off period of 15 sec was considered as maximal latency to avoid injury to the paws. The time when the animals licked the hind paw or jump out of the place was taken as the reaction time and was measured at 0, 30, 60, and 120 min. morphine at 5 mg/kg was used as a reference drug.

2.6. Tail immersion

Tail immersion was conducted as described by Aydin *et al*[29]. This involved immersing extreme 3 cm of the rat's tail in a water bath containing water at a temperature of (55.0±0.5) ℃. Within a few minutes, the rats reacted by withdrawing the tail. The reaction time was measured at 0, 30, 60, 120, 180, 240 and 300 min. The test groups were given methanol extracts of three plants at 200 mg/kg and 400 mg/kg, morphine at 5 mg/kg and distilled water.

2.7. Statistical analysis

Data were recorded as Mean±SEM. The statistical significance of differences between groups was determined by analysis of variance (ANOVA), followed by Dunnett's test for multiple comparisons among groups. 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 showed no death occurred, even at the highest doses of methanol extracts of three plants at 2 000 mg/kg, which indicated it may have a reasonable safety margin with regards to acute toxicity.

3.3. Acetic acid-induced writhing test

Methanol extracts of three plants were significantly reduced writhing and stretching induced by 0.6% acetic acid at dose of 10 mL/kg. Dose dependent antinociceptive effect was noted with the extract at the tested dose levels (Table 1). Maximum percentage of inhibition of writhing responses exhibited by the Methanol extracts of *A. viridis*, *A. caudatus* and *A. spinosus* at 400 mg/kg was 64.01%, 60.60% and 66.70% while at 200 mg/kg it showed 51.01%, 52.07% and 45.46% reduction in acetic acid induced writhing responses respectively, and diclofenac sodium at 50 mg/kg group had

72.88% pain reduction.

Table 1
Effect of methanolic extract of *A. viridis*, *A. caudatus* and *A. spinosus* on acetic acid induced writhing test in mice (n=6).

Treatment	Dose (mg/kg)	Number of writhes	Inhibition (%)
Control	--	57.17±1.66	--
Diclofenac sodium	50	15.50±0.18**	72.88
AV	200	28.00±0.89**	51.01
	400	20.16±0.16**	64.01
AC	200	31.16±1.04**	45.46
	400	22.50±0.17**	60.60
AS	200	27.00±1.10**	52.07
	400	19.00±0.89**	66.70

**: P<0.01 VS control; AV: *A. viridis*, AC: *A. caudatus*, AS: *A. spinosus*.

3.4. Hot plate test

Figure 1 showed the analgesic profile of methanol extracts of three plants in hot plate test of mice. Methanol extract of *A. spinosus* showed maximum analgesic effect compared to other plant extracts. It also exhibited a dose-dependent analgesic activity with doses of 200 and 400 mg/kg extracts (P<0.01), comparing to the effect of standard morphine at 5 mg/kg.

3.5. Tail immersion test

Figure 2 showed analgesic activity of methanol extracts of three plants were significantly (P<0.01) and dose dependent. It reduced the painful sensation in mice due to tail immersion in warm water when compared to morphine. The inhibitory effect was prominent between 30 and 180 mim after administration of extracts at dose of 400 mg/kg.

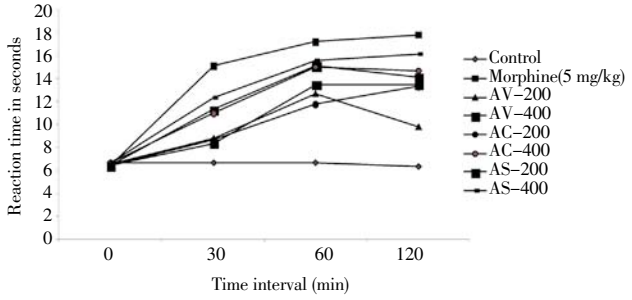

Figure 1. The analgesic profile of methanol extract of three plants in hot plate test of mice.
AV: *A. viridis*, AC: *A. caudatus*, AS: *A. spinosus*.

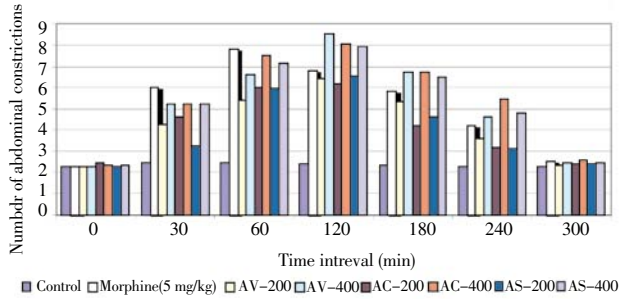

Figure 2. The analgesic profile of methanol extract of three plants in tail immersion test of mice.
AV: *A. viridis*, AC: *A. caudatus*, AS: *A. spinosus*.

4. Discussion

Pain and inflammation are associated with many pathophysiology of various diseases like arthritis, cancer and vascular diseases. A number of natural products are used in various traditional medicinal systems to relief of symptoms such as pain and inflammation. The methanol extracts of three plants demonstrated significant analgesic activity at two different dose levels. The results from the present study show that all three plants exhibited activities in various degrees against pain. By activating the cyclooxygenase, the levels of prostaglandin, especially prostaglandin E2 (PGE2), increases markedly and its production provokes pain and fever. Therefore, we assume that some active metabolites of the extract in this study could inhibit cyclooxygenase activity.

The study indicated that all these three plant extracts have both peripheral and central analgesic properties. Its peripheral analgesic activity was deduced from its inhibitory effects on chemical induced nociceptive stimuli. In acetic acid-induced abdominal writhing, the hot plate and tail immersion methods elucidated peripheral central activity. The intraperitoneal administration of agent that irritates serous membranes provokes a stereotypical behaviour in mice which is characterized by abdominal contractions, movements of the body as a whole, twisting of dorsoabdominal muscles and a reduction in motor activity and coordination[30].

The hot plate test and tail immersion test have been found to be suitable for evaluation of centrally acting analgesics. The tail immersion test indicated that the pharmacological actions were mediated by mu (μ) opioid receptors rather than kappa (K) and delta (δ) receptors. The tail flick or tail immersion model is an index to evaluate acute pains in animals. The processor releases arachidonic acid via cyclooxygenase, and prostaglandin biosynthesis plays a role in the nociceptive mechanism. Results of the present study show that all the doses of methanolic extracts of three plants produced significant analgesic effect and this effect may be due to inhibition of the synthesis of the arachidonic acid metabolite[31].

Preliminary phytochemical study indicated the presence of alkaloids, steroids, glycosides, flavonoids, phenolic compounds, terpenoids, proteins and carbohydrates which may be responsible for the antinociceptive effect of methanolic extracts of three plants. Flavonoids and phenolic compounds have been reported to have multiple biological effects such as antioxidant activity, antinociceptive activity *in vivo*, anti-inflammatory action, inhibition of platelet aggregation, inhibition of mast cell histamine release and inhibitory action on arachidonic acid metabolism as demonstrated by *in vitro* and *in vivo* tests[32].

Methanol extracts of *A. viridis*, *A. caudatus* and *A. spinosus* showed potent analgesic activity and this study provides the scientific proof for their traditional claims.

Conflict of interest statement

We declare that we have no conflict of interest.

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