Original Article

Antinociceptive and Antipyretic Activities of Amaranthus Viridis Linn in Different Experimental Models

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Received: 28 Oct 2009
Accepted: 1 Dec 2009

Abstract

Methanolic extract of whole plant of *Amaranthus viridis* L (MEAV), was screened for antinociceptive activity using acetic acid induced writhing test, hot plate test and tail immersion test in mice. In a similar way a screening exercise was carried out to determine the antipyretic potential of the extract using yeast induced pyrexia method in rats. Administration of the extracts was applied to both laboratory animals at the doses of 200 and 400 *mg/kg* body weight, respectively. The results of the statistical analysis showed that MEAV had significant ($p<0.01$) dose dependent antinociceptive and antipyretic properties at 200 and 400 *mg/kg*. Hence present investigation reveals the antinociceptive and antipyretic activities of methanolic extract of *Amaranthus viridis.*

Avicenna J Med Biotech 2009; 1(3): 167-171

Keywords: Analgesics, Animals, Dose response relationship, Methanol, Plant extracts

Introduction

Amaranthus viridis L (A. viridis Amaranthaceae), commonly called 'Chilaka Thota-Kura' in Telugu, has been used in Indian and Nepalese traditional system to reduce labour pain and act an antipyretic [1,2]. The Negritos of the Philippines apply the bruised leaves directly to eczema, psoriasis and rashes etc [3]. Other traditional uses range from an anti-inflammatory agent of the urinary tract, venereal diseases vermifuge, diuretic, anti-rheumatic, antiulcer, analgesic, antiemetic, laxative, improvement of appetite, antileprotic, treatment of respiratory and eye problems, to treatment of asthma [1,4-11].

Furthermore, the plant possesses antiproliferative and antifungal lactin properties as well as ribosome inactivating protein, β-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]. However, there is not enough scientific reports to support these supposed analgesic and antipyretic activities. This has prompted us to conduct the studies to ascertain the authenticity of these important claims of traditional potency.

Materials and Methods

Collection and extraction of plant material

Fresh plant material of *A. viridis* was collected from surroundings of Chickballapur,

Karnataka in the month of May 2009. The plant material was identified and authenticated by Dr. Rajan, Department of Botany, Government Arts College, Ootcamund, Tamilnadu.

A voucher specimen (SKVCP 13) was deposited in college herbarium. Plant material was washed with water to remove dirt and shade dried for one week. The dried material was powdered by using grinder and passed through 10-mesh sieve. The coarsely powdered material was extracted with methanol by using soxhlet apparatus. The extract was later evaporated to dryness under reduced pressure and the residue was preserved for future use.

Preliminary phytochemical screening

The methanol extract of *A. viridis* was screened for the presence of various phytoconstituents like steroids, alkaloids, glycolsides, flavonoids, carbohydrates, proteins and phenolic compounds [17].

Animal models

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

Acute toxicity studies

Methanol extracts of *A. viridis* was studied for acute oral toxicity as per revised OECD (Organization for Economic Cooperation and Development) guidelines No. 423. The extract was devoid of any toxicity in rats when given in doses up to 2000 *mg/kg* by oral route. Hence, in our study 200 and 400 *mg/kg* doses of extract were dissolved in 0.1% Carboxy Methyl Cellulose (CMC) and used for the study [18].

Antinociceptive activity

Acetic acid-induced writhing test: This test was done using the method described by Collier et al [19]. Muscle contractions were induced in mice by intra peritoneal injection of 0.6 % solution of acetic acid (10 *ml/kg*). Thirty *minutes* before this administration the animals were treated with diclofenac sodium (50 *mg/kg*), MEAV orally at doses of (200 and 400 *mg/kg*) and 0.1 % CMC (5 *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*.

Writhing movement was accepted as contraction of the abdominal muscles accompanied by stretching of hind limbs. There was significant reduction in the number of writhes by drug treatments as compared to vehicle treated animals. This was considered a positive analgesic response and the percentage inhibition of writhing was calculated [19].

Hot plate method: The hot plate test described by Eddy and Leimback (1953) was used. The mice were first treated with different doses of MEAV (200 and 400 *mg/kg* orally). One *hour* after this administration the animals were placed on a hot plate maintained at 55±1.0 °*C*. A cut-off period of 15 *sec* was considered as maximal latency to avoid injury to the paws. The time taken by the animals to lick the hind paw or jump out of the place was taken as the reaction time and was measured at 0,30,60 and 120 *mins*. Morphine (5 *mg/kg*) was used as a reference drug [20].

Tail immersion: Tail immersion was conducted as described by Aydin et al [21]. This involved immersing extreme 3*cm* of the rat's tail in a water bath containing water maintained at a temperature of 55±0.5°*C*. 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 *mins*.

The test groups were given MEAV (200 and 400 *mg/kg*), morphine (5*mg/kg*) and 0.1% CMC in water were administered orally [21].

Screening for antipyretic activity

The antipyretic activity of MEAV was evaluated using Brewer's yeast-induced pyrexia in rats as described by Loux et al [22]. Fever was induced by administering 20 *ml/kg* of 20% aqueous suspension of Brewer's yeast in normal saline subcutaneously. The MEAV

Table 1. Effect of methanolic extract of *Amaranthus viridis* (MEAV) on acetic acid induced writhing test in mice

Treatment	Dose (*mg/kg*)	Number of writhes	% inhibition
Control	--	57.166±1.66	--
Diclofenac sodium	50	15.5±0.18	72.88
MEAV			
	200	28±0.89	51.07
	400	20.16±0.10	64.73

Values are in mean ±SEM; (n=6)

(200 and 400 *mg/kg*, orally) was administered orally, paracetamol (150 *mg/kg*, orally) was used as reference drug and control group received distilled water. Rectal temperature was determined by thermal probe Eliab themistor thermometer at 1,2,3,4,5 and 6 *hrs* after test extract/reference drug administration [22].

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.

Results

The present study was conducted to assess the antinociceptive and antipyretic properties of methanolic extract of *A. viridis*. The methods selected were chemical nociception in the test model of acetic acid-induced writhing and thermal nociception hot plate and tail immersion test. These methods were selected to evaluate both centrally and peripherally mediated effects of MEAV. The acetic acid induced abdominal constriction is believed to show the involvement of peripheral mechanisms, whereas the hot plate and tail immersion tests are believed to do same by central mechanisms [23].

The results of the present study demonstrated that MEAV-possessed antinociceptive activity is evident in all the nociceptive models, suggesting the involvement of both central and peripherally mediated activities.

In acetic acid-induced abdominal constriction test, the results showed that the MEAV (200 and 400 *mg/kg*) potently and significantly reduced the number of abdominal writhing in a dose dependent manner with 51.01 % and 64.73% of inhibition, respectively as compared to control animals (Table 1). The positive control group treated with diclofenac sodium (50 *mg/kg*) also showed significant reduction in the number of writhes (72.66%).

It has been postulated that acetic acid acts indirectly by inducing the release of endogenous mediators, such as PGE_2 (prostaglandin E_2) and $PGE_2\alpha$ in peritoneal fluids as well as lipooxygenase products, which stimulate the nociceptive neurons sensitive to NSAIDs [18,24]. Therefore, the results of the acetic acid-induced writhing strongly suggests that the mechanism of this extract may be linked partly to inhibition of lipooxygenase and/or cyclooxygenase in peripheral tissues, reducing in PGE_2 synthesis and interfering with the mechanism of transduction in primary afferent nociceptor (Table 1).

The central analgesic effect of the MEAV may be supported by the results recorded in the hot plate and tail immersion tests, a selective method used to screen centrally acting opiate analgesic drugs [23]. It was demonstrated that oral administration of MEAV (200 and 400 *mg/kg*) significantly prolonged the latency time to the heat stimulus (Table 2, Figure 1). This effect began early at 30 *mins* after administration of MEAV and persisted until the following 120 *mins*. As expected, morphine (5 *mg/kg*) significantly increased the latency time to the nociceptive response compared with control group.

Table 2. Effect of methanolic extract of *Amaranthus viridis* (MEAV) on tail immersion test in mice

Treatment	Dose (*mg/kg*)	Post treatment reaction time (seconds)						
		0 *min*	**30 *min***	**60 *min***	**120 *min***	**180 *min***	**240 *min***	**300 *min***
Control		2.31±0.08	2.48±0.07	2.43±0.09	2.31±0.056	2.34±0.06	2.3±0.03	2.3±0.06
Morphine	5	2.45±0.095	6.0±0.15**	7.8±0.13**	9.8±0.19**	8.2±0.15**	3.8±0.06**	3±0.014
MEAV								
	200	2.45±0.12	4.62±0.17*	6.0±0.07**	6.2±0.07**	5.6±.0.148**	2.8±0.12*	2.4±0.15
	400	2.33±0.06	5.23±0.12**	7.48±0.12**	8.05±0.14**	5.7±0.15**	3.14±0.08**	2.67±0.08

Values are in mean ±SEM; (n=6) *p<0.05, ** p<0.01 vs control

Table 3. Effect of methanolic extract of *Amaranthus viridis* (MEAV) on yeast induced pyrexia

Treatment	Dose (*mg/kg*)	Rectal temperature (^{0}C) after yeast injection						
		0*hr*	19*hr*	20*hr*	21*hr*	22*hr*	23*hr*	24*hr*
Control		37.39±0.03	39.16±0.02	39.2±0.15	39.2±0.15	39.2±0.15	39.05±0.18	38.58±0.21
Paracetamol	150	36.93±0.41	38.6±0.56	37.33±0.21**	37.33±0.31**	37.52±0.17**	37.41±0.2**	37.26±0.16**
MEAV								
	200	37.26±0.17	39.1±0.18	38.5±0.34	38.35±0.335*	38.28±0.17*	38.12±0.21*	38.1±0.106
	400	37.4±0.17	38.78±0.45	37.86±0.42*	37.63±0.22**	37.45±0.19**	37.57±0.20**	37.4±0.17**

Values are in mean ±SEM; (n=6) *p<0.05, ** p<0.01 vs control

Fever may be a result of infection or one of the sequelae of tissue damage, inflammation, graft rejection, or other disease states. Antipyretics are drugs, which reduce the elevated body temperature. Regulation of body temperature requires a delicate balance between production and loss of heat, and the hypothalamus regulates the set point at which body temperature is maintained. In fever this set point elevates and a drug like paracetamol does not influence body temperature when it is elevated by the factors such as exercise or increase in ambient temperature [25].

The results obtained (Table 3) revealed that MEAV showed significant (p<0.05) antipyretic activity at all doses tested. The MEAV at 400 *mg/kg* showed antipyretic activity after 19 *hrs* of administration of Brewer's yeast and continued until the end of the experiment, while 200 *mg/kg* dose showed reduction in temperature after 22 *hrs* of administration of Brewer's yeast extending up to 23 *hrs*. Therefore, the MEAV possesses a significant antipyretic effect in yeast-induced elevation of body temperature in rats and this may be attributed to the anti-inflammatory properties of the plant.

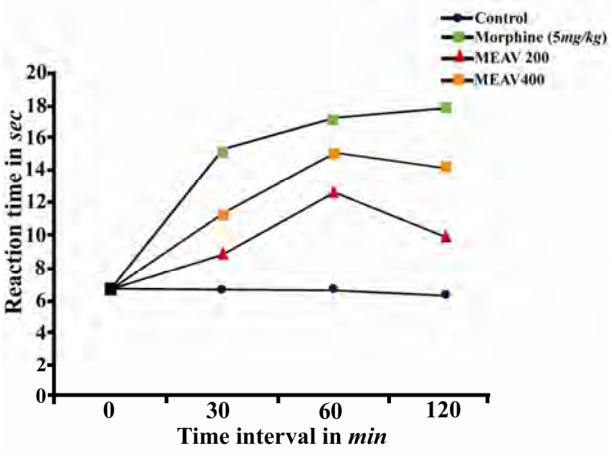

Figure 1. Effect of methanolic extract of *Amaranthus viridis* (MEAV) on hot plate test in mice

Discussion

Preliminary phytochemical study indicated the presence of alkaloids, steroids, glycosides, flavonoids, phenolic compounds, terpenoids, proteins and carbohydrates which might be responsible for the antinociceptive and antipyretic effects of the MEAV.

Flavonoids and phenolic compounds have been reported to have multiple biological effects such as anti-oxidant activity [26], antinociceptive activity *in vivo* [27,28], anti inflammatory action [29,30], inhibition of platelet aggregation [31], inhibition of mast cell histamine release [32] and inhibitory action on arachidonic acid metabolism as demonstrated by *in vitro* and *in vivo* tests [33].

Acknowledgement

The authors wish to thank Sri K.V. Naveen Kiran, Chairman, Sri K.V. College of Pharmacy, Chickballapur, Karnataka (India), for providing the research facilities to carry out this work successfully.

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