ANTINOCICEPTIVE AND ANTIPYRETIC ACTIVITIES OF
AMARANTHUS VIRIDIS LINN. IN DIFFERENT EXPERIMENTAL MODELS

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Abstract - The methanolic extract of the whole plant extract of *Amaranthus viridis* L (MEAV) was screened for antinociceptive activity using the acetic acid writhing test, hot plate test and tail immersion test in mice and for antipyretic activity using the yeast-induced pyrexia method in rats, at doses of 200 and 400 mg/kg body weight. Significant ($p<0.01$) dose-dependent antinociceptive and antipyretic properties were observed with 200 and 400 mg/kg.

Keywords: *Amaranthus viridis,* antinociceptive activity, acetic acid writhing test, hot plate test, antipyretic activity.

INTRODUCTION

Amaranthus viridis L (Amaranthaceae) is commonly called 'Chilaka Thota-Kura' in Telugu. *A. viridis* has been traditionally used in India and Nepal to lessen labor pains and as an antipyretic (Kirithikar and Basu, 1986; Mark Turin, 2003). The Negritos of the Philippines apply the bruised leaves directly to eczema, psoriasis and rashes (Eduardo and Quisumbing, 1951). Other traditional uses are as an anti-inflammatory agent of the urinary tract, in venereal diseases, as a vermifuge, diuretic, antirheumatic, antiulcer, analgesic, antiemetic; laxative, for improving appetite, as an antileprotic, for the treatment of respiratory problems, eye treatment and for asthma (Anonymous, 1988; Agra et al., 2007; 2008; Kirithikar and Basu, 1986; Hassan Sher and Khan, 2006; Quershi et al., 2008; Muhammad Ejaz Ul Islam Dar, 2003; Muhammad Arshad, 2000; Muhammad and Amusa, 2005)

A novel antiproliferative and antifungal lactin and a ribosome inactivating protein, β-carotene, were isolated from *A. viridis* (Kaur et al., 2006; Kwon et al., 1997; Sena et al., 1998) and it also possesses antiviral activity (Obi et al., 2006). The whole of the *A. viridis* plant is used for the treatment of pain and fever in traditional medicine. However, there is insufficient scientific proof regarding the analgesic and antipyretic activity of *A. viridis,* so our aim is to provide scientific validation for traditional uses.

MATERIALS AND METHODS

Collection of Plant Material and Extraction

The fresh plant of *A. viridis* was collected from Chickballapur, and was authenticated by Dr. Rajan, Department of Botany, Government Arts College, Ootcamund, Tamilnadu. A voucher specimen (SKVCP 12) was deposited in the college herbarium. The whole plant was shade-dried and coarsely powdered. The coarse powder was subjected to extraction with methanol by Soxhlet extractor and the extract was concentrated to dryness in a vacuum.

Animals

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

Acute toxicity studies

The methanol extract of *A. viridis* was studied for acute oral toxicity as per the revised OECD (Organization for Economic Cooperation and development) guidelines No. 423 (2000). The extract was devoid of any toxicity in rats given a dose of up to 2000 mg/kg orally. For further studies 200-400 mg/kg doses of extract were used.

Antinociceptive activity

Acetic acid-induced writhing test: This test was performed using the method described by Collier et al., (1968). Muscle contractions were induced in the rats by an intra-peritoneal injection of a 0.6% solution of acetic acid (10 ml/kg). Immediately after the 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. MEAV was administrated orally at doses of 200 and 400 mg/kg and diclofenac sodium (5 mg/kg) was administered 30 min before the acetic acid injection.

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 p.o). One h after extract administration they 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 away was taken as the reaction time and was measured at 0, 30, 60, 120 min. Morphine (5 mg/kg) was used as a reference drug.

Tail immersion

Tail immersion was conducted as described by Aydin et al., (1999). This involved immersing 3 cm of the rat's tail in a water bath containing water 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 min. The test groups were given MEAV (200 and 400mg/kg), morphine (5 mg/kg) and distilled water.

Screening of Antipyretic Activity

The antipyretic activity of MEAV was evaluated using Brewer's yeast-induced pyrexia in the rats as described by Loux et al. (1972). Fever was induced by the subcutaneous administration of 20 ml/kg of a 20% aqueous suspension of Brewer's yeast in normal saline. The MEAV (200 and 400 mg/kg) was administered orally, paracetamol (150 mg/kg, p.o.) was used as a reference drug and the control group received distilled water. Rectal temperature was determined by a thermal probe Eliab themistor thermometer at 1, 2, 3, 4, 5 and 6 h after the test extract/reference drug administration.

Statistical analysis

Data were recorded as mean ± S.D. The statistical significance of differences between the groups was determined by analysis of variance (ANOVA), followed by Dunnett's test for multiple comparisons among the groups. Differences of $P < 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

The present study was conducted to assess the antinociceptive effect of the MEAV, a medicinal substance claimed in Indian traditional medicine to have analgesic and antipyretic properties. Its chemical antinociceptive effect was examined in the test model of acetic acid-induced writhing while ther-

Table 1. The effect of methanolic extract of *Amaranthus viridis* (MEAV) assessed by the 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) *p<0.05, ** p<0.01 Vs control

mal nociception was assessed by the hot plate and tail immersion test. These methods were selected so that both the centrally and peripherally mediated effects could be investigated. 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 show that of central mechanisms (Abbott and Melzack, 1982).

The results of this study demonstrated that the MEAV possessed antinociceptive activity which was evident in all the nociceptive models, which suggests the presence of both central and peripherally mediated activities. In the acetic acid-induced abdominal constriction test the results showed that the MEAV (200 and 400 mg/kg) potently and significantly reduced the amount of abdominal writhing in a dose-dependent manner with 51.01% and 64.73% inhibition, respectively, as compared to the control animals (Table 1). The positive control group treated with diclofenac sodium (50 mg/kg) also manifested a 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 (Derardt et al., 1980; Collier et al., 1968). Therefore, the results of the acetic acid-induced writhing strongly suggests that the mechanism of this extract may be linked partly to the inhibition of lipooxygenase and/or cyclooxygenase in the peripheral tissues, thereby reducing PGE_2 synthesis and interfering with the mechanism of transduction in the primary afferent nociceptor.

The central analgesic effect of the MEAV could be supported by the results recorded in the hot plate and tail immersion tests, which is a selective method able to screen centrally acting opiate analgesic drugs (Abbott and Melzack, 1982). It was demonstrated that oral administration of MEAV
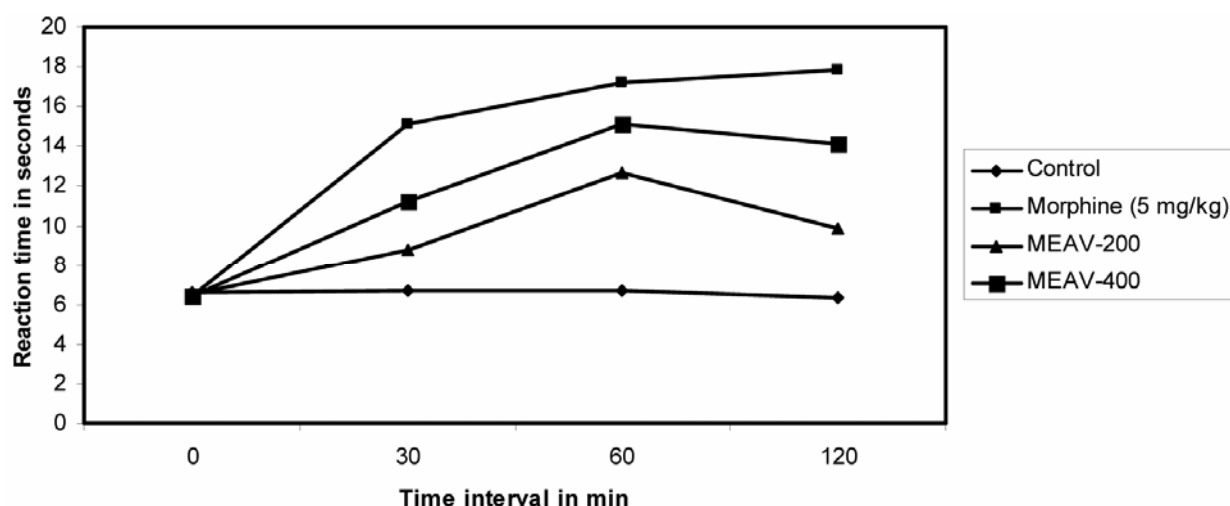
Fig. 1. The effect of methanolic extract of *Amaranthus viridis* (MEAV) examined by the hot plate test in mice.

Table 2. The effect of methanolic extract of *Amaranthus viridis* (MEAV) assessed by the 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. The effect of methanolic extract of *Amaranthus viridis* (MEAV) on yeast-induced pyrexia

Treatment	Dose (mg/kg)	Rectal temperature (^{0}C) after yeast injection						
		0h	19h	20h	21h	22h	23h	24h
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

(200 and 400 mg/kg) exerts significant prolongation in the response latency time to the heat stimulus (Table 2 and Fig. 1). This effect began 30 min after the administration of MEAV and persisted for the following 120 min. As expected, morphine (5 mg/kg) significantly increased the latency time to the nociceptive response compared with control group.

Fever may be a result of infection or one of sequelae of tissue damage, inflammation, graft rejection, or other disease states. Antipyretics are drugs which reduce an elevated body temperature. The regulation of body temperature requires a delicate balance between the production and loss of heat. The hypothalamus regulates the set point at which body temperature is maintained. In fever this set point is elevated and a drug like paracetamol does not influence body temperature when it is elevated by factors such as exercise or an increase in ambient temperature (Goodman and Gilman, 1996). Our results (Table. 3) revealed that MEAV shows a significant ($p<0.05$) antipyretic activity at all doses. MEAV at 400 mg/kg shows an antipyretic activity 19 h after the administration of Brewer's yeast up until the end of the experiment, while a 200 mg/kg dose showed a reduction in temperature 22 h after the administration of Brewer's yeast and it lasts up to 23 h. The MEAV possesses a significant antipyretic effect in a yeast-induced elevation of body temperature in rats and this may be due to an anti-inflammatory effect.

The preliminary phytochemical study indicated the presence of alkaloids, steroids, glycosides, flavonoids, phenolic compounds, terpenoids, pro-

teins and carbohydrates which may be responsible for the antinociceptive and antipyretic effects of MEAV. Flavonoids and phenolic compounds have been reported to have multiple biological effects such as antioxidant activity (Bors and Saran, 1987), antinociceptive activity *in vivo* (Delorme et al., 1977; Mills and Bone, 2000), anti-inflammatory action (Moreira et al., 2000; Rao et al., 2003), inhibition of platelet aggregation (Van Wauve and Goosens, 1989), inhibition of mast cell histamine release (Amresh et al., 2007a) and inhibitory action on arachidonic acid metabolism as demonstrated by *in vitro* and *in vivo* tests (Amresh et al., 2007c).

In addition, the preliminary acute oral toxicity test obtained with this plant showed that no death occurred, even at the highest dose of MEAV (2000 mg/kg), indicating it may have a reasonably safe margin with regards to acute toxicity. This further justifies its application in the treatment of pain and fever.

REFERENCES

Abbott, F.V. and R. Melzack, (1982). Brainstem lesions dissociate neural mechanisms of morphine analgesia in different kinds of pain. *Brain Res.* **251** (1), 149-155.

Agra, M.R., Baracho, G.S., Nurit, K., Basilio, I.J.L.D. and VPM. Coelho (2007) Medicinal and Poisonous diversity of the flora of "Cariri Paraibano" Brazil. *J Ethnopharmacol.* **111**, 283-395.

Anonymous.(1988) The Wealth of India. Vol. 1. New Delhi, India, Publications and Information directorate. CSIR. Council of Scientific and Industrial Research. 221.

Amresh, G., Reddy, G.D., Rao, Ch.V. and P.N. Singh (2007a) Evaluation of antiinflammatory activity of *Cissampelos pareira* root in rats. *J. Ethnopharmacol.* **110**, 526–531.

Amresh, G., Zeashan, H., and Ch.V. Rao, (2007c) Prostaglandin mediated antiinflammatory and analgesic activity of *Cissampelos pareira*. *Acta Pharmaceutica Science.* **49**, 153-160.

Aydin, S., Demir, T., Ozturk, Y., and K.H.C. Baser, (1999) Analgesic activity of *Nepeta italica* L. *Phytotherapy Research.* **13**, 20-23.

Bittar, M., De Souza, M.M., Yunes, R.A., Lento, R., Delle Monache, F. and V. Cechinel Filho, (2000) Antinociceptive activity of I3, II8-Binaringenin, a bioflavonoid present in plants of the guttiferae. **Planta Medica. 66**, 84-86.

Bors, W. and M. Saran, (1987) Radical scavenging by flavonoid antioxidant. *Free Radical Research Communication.* **2,** 131.

Calixto JB, Beirith A, Ferreir J, Santos AR, Cechinel Filho V, and RA. Yunes (2000) Naturally occurring antinociceptive substances from plants. *Phytotherapy Research.* **14**, 401-418.

Collier HO, Dinneen LC, Johnson CA, and C. Schneider (1968) The abdominal constriction response and its suppression by analgesic drugs in the mouse. *British Journal of Pharmacology and Chemotherapy.* **32**, 295-310.

Delorme P, Jay M, and S. Ferry (1977) Antiinflammatory and analgesic activity from roots of *Angelica pubescens*. *Planta Medica.* **61**, 2-8.

Deraedt R., Jougne S., Delevalcee F., and M. Falhout (1980) Release of prostaglandin E and F in an algogenic reaction and its inhibition. *Eur.J. Pharmacol.* **51**, 17-24.

Eddy, N.B. and D. Leimback, (1953). Synthetic analgesic. II. Dithienyl-butenyl and dithienybutylamines. *J Pharmacol. Exp.Ther.* **107**, 385-402.

Eduardo Quisumbing (1951) Medicinal plants of the Philippines, Manila, Department of Agriculture and Natural Resources. Bureau of Printing.

Galati EM, Monforte MT, Kirjavainen S, Forestieri AM, Trovato A, and MM. Tripodo (1994) Biological effects of hesperidins, a citrus flavonoid (Note I); antiinflammatory and analgesic activity. *Farmaco.* **40**, 709-712.

Goodman L.S. and A.G. Gilman (1996) The pharmacological Basis of Therapeutics nineteenth ed. Mc Graw-Hill, New York. 959-975.

Hassan Sher and ZD. Khan (2006) Resource utilization for economic development and folk medicine among the tribal people. Observation from Northern part of Pakistan. *Pak. J. Plant Science.* **12**(2), 149-162.

Kaur N., Dhuna V., Kamboja S.S., Agrewala J.N., and J. Singh (2006) A novel antiproliferative and antifungal lactin from *Amaranthus viridis* Linn, seeds. *Protein Pept. Letter.* **13**(9), 897-905.

Kirtikar K.R. and *B.D. Basu* (1987) Vol.3. Indian Medicinal Plants. International book distributors, Dehra Dun, India. 2061-62.

Kwon S.Y., An C.S., Liu J.R. and *K.H. Pack* (1997) A Ribosome Inactivating Protein from *Amaranthus viridis. Biosci. Biotechnology Biochem.* **61**(9), 1613-14.

Loux J.J., De Palma P,D, and *SL. Yankell* (1972) Antipyretic testing of aspirin in rats. *Toxicol. Appl. Pharmacol.* **22**, 672-675.

Maria de Fatima Agra, Kiriaki Nurisilva, Ionaldo Jose Lima Diniz Basilio, Patricia Franca De Freitas and *Jose Maria Barbosa Filho.* (2008) Survey of medicinal plants used in the region northeast of brazil. *Brazilian journal of pharmacognosy.* **18**(3), 472-508.

Mark Turin. (2003) Ethnobotanical notes on Thangmi plant names and their medicinal and ritual uses. *CNAS Journal.* **30**, 1.

Mills S. and *K. Bone* (2000) Principles and practice of Phytotherapy. Churchill living stone. Edinburgh. pp. 23-24, 31-34, 229-231.

Moreira A.S., Spitzer V., Schapoval E.E. and *E.P. Schenkel* (2000) Antiinflammatory and antinociceptive activity of extracts and fractions from the leaves of *Gochnatia polymorpha, Phytotherapy Research.* **14**, 638-640.

Muhammad Ejaz-Ut Islam Dar. (2003) Ethnobotonica l uses of plants of Lawat district Muzaffarabad Azad Jammu and Kashmir. *Asian Journal of Plant Sciences.* **2** (9), 680-682.

Muhammad Arshad and *Qurrat Ul Ain Khan* (2000) Ethnobotonical study of some medicinal plants of Rawal Town. *Pak. J. Biological Sciences.* **3**(8), 1245-1246.

Muhammad S. and *N.A. Amusa* (2005) The important food crops and medicinal plants of north-western Nigeria. *Research Journal of Agriculture of Biological Sciences.* **1** (3), 254-260.

Obi R.K., Iroagba I.I. and *OA. Ojiako* (2006) Virucidal Potential of Some Edible Nigerian Vegetable, *African Journal of Biotechnology.* **5** (19), 1785-1788.

Patkai G., Barta J. and *I. Varsanyi* (1997) Decomposition of anticarcinogen factors of the beet root during juice and nectar production. *Cancer Letters.* **114**, 105-106.

Quershi S.J., Khan M.A. and *M. Ahmed* (2008) A survey of useful medicinal plants of Abbottabad, in Northern Pakistan. *Trakia Journal of Science.* **6** (4), 39-51.

Rao ChV, Ojha S.K., Amresh G., S. Mehrotra and *Pushpangadan P.* (2003) Analgesic, antiinflammatory and antiulcerogenic activities of unripe fruite of *Aegle marmelos. Acta Pharmaceutica Turcica.* **45**, 85-91.

Sena L.P., Vanderjagt D.J., Rivera C., Tsin A.T.C., Muhamadu I., Mahamadou O., Millson M., Pastuszyn A. and *RH. Glew* (1998) Analysis of Nutritional Components of Eight Famine Foods of the Republic of Nigeria. *Plant Foods and Human Nutrition.* **52** (1), 17-30.

Schmauss C. and *T.L. Yaksh* (1984) *In Vivo* studies on spinal receptor systems mediating antinociceptive. II. Pharmacological profiles suggesting a differential association of mu. Delta and kappa receptor with visceral chemical and cutaneous thermal stimuli in the rat. *Journal of Pharmacology and Experimental Therapeutics.* **228**, 1-12.

Van Wauve J.P. and *J.G. Goosens* (1989) Arabinolactan and dextran induced ear inflammation in mice: differential inhibition of H1-antihistamines, 5HT-serotonin antagonist and lipoxygenase blockers. *Agents Action.* **28**, 78-82.