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Analgesic activity of extracts of the whole plant of *Amaranthus spinosus* Linn.

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Abstract

Successive petroleum ether, ethyl acetate and methanol extracts of the whole plant of *Amaranthus spinosus* Linn. were investigated for the analgesic activity. Experiments were carried out with these extracts for their peripheral and central antinociceptive potentials on acetic acid induced writhing and radiant heat tail-flick models in mice, respectively. In both the models, methanolic extract showed significant writhing inhibition as well as the elongation of tail-flick time at a dose of 500 mg/kg body weight. A linear dose response relationship was also observed.

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Introduction

Amaranthus spinosus Linn. (Bengali name: Kantanotey; Family: Amaranthaceae) is an erect, glabrous, herbaceous weed with dense or interrupted spikes growing wild in all parts of Bangladesh [1]. The plant is commonly found throughout tropical, subtropical and Himalayan regions [2]. The plant is

traditionally used as febrifuge, antipyretic, laxative and diuretic. It is also used to treat bronchitis, leprosy and piles [1]. Previous chemical investigations have revealed that the leaves and stems contain hentriacontane, octacosanoid, α-spinasterol, saponin and fatty acids. The whole plant contains amaranthine and isoamaranthine. As a part of our continuing studies on the medicinal plants of Bangladesh we investigated the analgesic activity of different fractions of *Amaranthus spinosus* and herein, report the results of our examinations.

Materials and Methods

Collection of plant material: The whole plant of *Amaranthus spinosus* (AS) was collected from Jessore in October 2003. The plant was identified and a voucher specimen (Accession Number DACB 33536) representing this collection has been deposited in the Bangladesh National Herbarium, Dhaka, for further reference.

Extraction of the plant material: The dried, coarsely powdered plant material (1.5 kg) was successively extracted by maceration (5 L) over 72 hour period with petroleum ether, ethyl acetate and finally with methanol at room temperature. Then the extracts were filtered and concentrated with a rotary evaporator at low temperature (40-50°C) and reduced pressure and were subsequently defatted [3] to get the dried petroleum ether (ASPE), ethyl acetate (ASEA) and methanol (ASME) extracts. The final amounts of the extracts were 5.6 g, 9.4 g and 13.3 g for petroleum ether, ethyl acetate and methanolic fractions respectively.

Experimental animal: The investigations of analgesic activity of the extracts were done on Swiss albino mice. The animals were purchased from the Animal Research Branch of the International Centre for Diarrhoeal Diseases and Research, Bangladesh (ICDDR, B). The weight of the mice used ranged from 20-24 g. The animals were kept in polyvinyl cages (BIK industries, India) at room temperature under condition of natural light and dark schedule and were supplied with ICDDR, B formulated food pellets and water *ad libitum*. To keep the hydration rate constant, the food and water were withdrawn 12 hours before the experiments. The research protocol was approved by the Bangladesh Medical Research Council (BMRC) Dhaka, Bangladesh.

Preparation of the test material and standard: For the preparation of the test material at a dose of 500, 250 and 125 mg/kg body weight 125, 62.5 and 31.25 mg of all the extracts were triturated by the addition of small amount of DMSO. After proper mixing of the extracts and DMSO, distilled water was slowly added and the final volume of the suspension of each extract was adjusted to 2.5 ml. For the preparation of standard, 12.5 mg of acetyl salicylic acid (ASA) was taken and suspension of 2.5 ml was made with DMSO and distilled water.

Acetic acid induced writhing test: The peripheral analgesic activity of different crude extracts of *Amaranthus spinosus* (AS) was studied by the acetic acid induced writhing method [4]. The inhibition of writhing in mice by the plant extracts were compared against inhibition of writhing by a standard analgesic, acetyl salicylic acid (ASA) given orally at a dose of 50 mg/kg. Acetic acid (0.7%) at a dose of 0.1 ml/10g was administered intraperitoneally to create pain. The number of writhing was calculated for 10 min, 10 min after the acetic acid injection. The percentage of pain protection was calculated.

Radiant heat tail-flick method: The analgesic activity was determined by measuring drug-induced changes in the sensitivity of the mice to heat stress applied to their tails [5]. A Medicraft Analgesiometer Mask-N was employed for this experiment. Intensity of the current passing through the naked nicrome wire was 6 ampere. The distance between the heat source and the tail skin was 1.5 cm and cut-off reaction time was fixed at 10 second to avoid tissue

damage. Morphine (Jayson Pharmaceuticals Ltd., Bangladesh) was used as the standard analgesic for comparing the tail-flick latencies of crude extracts. Tail-flick latency after 60 minutes of the drug administration was considered to be the optimum.

Statistical analysis: The results were analyzed for statistical significance using one-way ANOVA followed by Dunnett's test. P <0.05 was considered significant.

Results and Discussion

In acetic acid induced writhing model methanol extracts at a dose of 250 and 500 mg/kg body weight produced 41.16 and 60.92% (Table 1 and Figure 1) reduction of writhing response. The results were found to be highly significant (P<0.05) in comparison to the control. The pet ether and ethyl acetate extracts also produced 16.09 and 22.22% reduction of writhing response at a dose of 500 mg/kg body weight respectively. In the radiant heat tail-flick model, (Table 2 and Figure 2) the methanolic extract showed 36.18 and 42.28 % increase in the tail flick latency both at a doses of 250 and 500 mg/kg body weight respectively 60 min after the administration of the test materials.

The results of the present study show that the methanol extracts of AS administered orally to mice, produces significant antinociceptive action against chemical (acetic acid-induced visceral pain) and thermal (radiant heat tail-flick test) models of nociception in mice. The abdominal constriction response induced by acetic acid is a sensitive procedure to evaluate the potential analgesic activity of drugs. It has been suggested that acetic acid acts by releasing endogenous mediators that stimulate the nociceptive neurons [6]. It is sensitive to non-steroidal anti-inflammatory drugs (NSAIDs) and to narcotics and other centrally acting drugs [6,7,8]. Recently, it was found that the nociceptive activity of acetic acid may be due to the release of cytokines, such as TNF-α, interleukin-1β and interleukin-8, by resident peritoneal macrophages and mast cells [9]. Thus, the present study presented here might indicate that the antinociceptive action of methanolic fractions in the acetic acid-induced writhing test could be due to inhibition of the release of TNF-α, interleukin-1β and interleukin-8 by resident peritoneal cells. However, this possibility remains to be tested in future studies. In the tail-flick method methanolic fraction increased the stress tolerance capacity of the animals and hence also indicates the possible involvement of a higher center [10]. From the study it may also be said that traditional uses of *Amaranthus spinosus* for the treatment of various types of pain conditions has got definite basis. However further investigations are required to identify the active constituent(s) and to verify the therapeutic merits of the active constituent(s).

Table 1: Effects of crude extracts[a] on acetic acid induced writhing response in mice.

Treatment	Dose (mg /kg, p.o.)	Writhings[b]	% Inhibition
Control (vehicle, 10ml/kg)	-	21.75 ± 0.67	-
ASPE	250 500	20.58 ± 2.39 18.25 ± 0.70	5.36 16.09
ASEA	250 500	18.83± 3.46 16.92± 1.04	13.41 22.22
ASME	250 500	12.67 ±2.59* 8.50 ± 1.71*	41.16 60.92
ASA	50	5 ± 0.62**	77.01
One-way ANOVA	F	3.559	
	df	9, 50	
	P	<0.05	

[a]1 hr after treatment, mice were injected i.p. with 0.7%(v/v) acetic acid (0.1ml/10g); 10 minutes after the injection, the number writhing was counted for 10 min.

[b] Values are mean ± SEM (n = 6); One-way ANOVA; **P<0.01, *P<0.05 compared to control.

Table 2: Effects of crude extracts[a] on radiant heat tail-flick response in mice.

Treatment	Dose (mg/kg)	Reaction time (sec)[c]		
		30 min	60 min	120 min
Control (vehicle, 10ml/kg)	-	4.3± 0.86	4.1 ± 0.8	3.93 ±0.07
ASPE	250	4.85± 0.23	4.62 ± 0.36	4.57 ± 0.38
	500	5.02± 0.16	4.85 ± 0.22	4.62 ± 0.16
ASEA	250	5.02± 0.57	4.97 ± 0.55	4.67 ± 0.40
	500	5.35± 0.35	5.4 ± 0.38	4.93 ± 0.40
ASME	250	5.33± 0.60	5.58 ± 0.48*	5.1± 0.51
	500	5.45± 0.48	5.83 ± 0.51*	5.48 ± 0.48
Morphine	2[b]	6.35 ± 0.21	6.67 ± 0.20	6.23 ± 0.29*
One-way ANOVA	F	2.067	4.311	3.312
	P	> 0.05	< 0.05	< 0.05

[a] per oral administration of vehicle and crude extracts, radiant heat intensity was 6 amp.
[b] Morphine was administered sub-cutaneously.
[c] Values are mean ± SEM (n = 6); One-way ANOVA; df = 10, 55; **P*<0.05 compared to control.

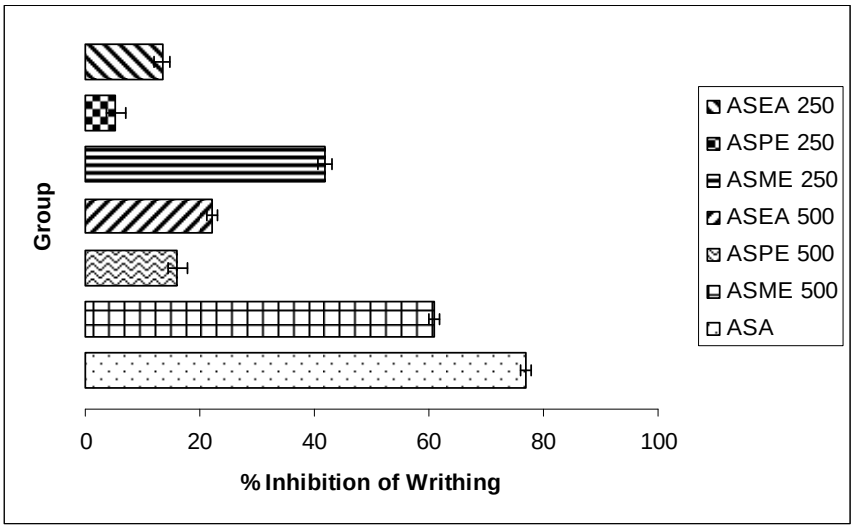

Figure 1: Percentage inhibition of writhing of different extracts of *Amaranthus spinosus*.

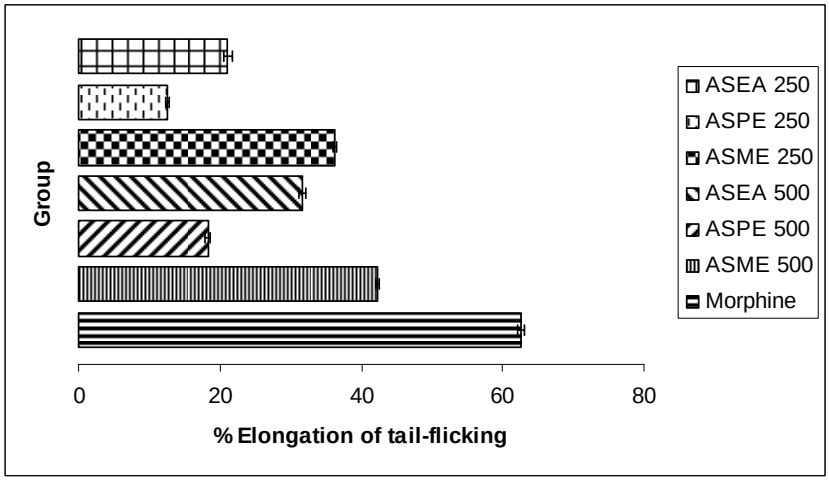

Figure 2: Percentage of elongation of tail-flick after 60 minutes of different extracts administration.

Conclusion

The activity of whole plant of *Amaranthus spinosus* Linn, found in this study could be of particular interest in relation to find out its unexplored efficacy and can be a potential source of chemically interesting and biologically important molecules.

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