Department of Pharmacy[1], University of Dhaka, Department of Pharmacy[2], University of Development Alternative, Dhaka, Bangladesh

Evaluation of neuropharmacological effects of *Rumex maritimus* Linn. (Polygonaceae) root extracts

M. S. Islam[1], M. T. Rahman[2], A. S. S. Rouf[1], F. Rahman[1]

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Md. Taufiq-Ur-Rahman, Department of Pharmacy, Faculty of Life Sciences, University of Development Alternative, Road No. 25 (old), House No. 315/A, Dhanmondi, Dhaka – 1205, Bangladesh
mtrahmanbd@yahoo.com

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Hexane, ethyl acetate and methanol extracts of the root of *Rumex maritimus* Linn. (Polygonaceae) were evaluated for neuropharmacological activities in different models. All the extracts significantly and dose dependently inhibited acetic acid induced abdominal constrictions in mice. The hexane and methanol extract exhibited significant central analgesic activity in the radiant heat method. Only the methanol extract showed statistically significant mild to moderate central nervous system depressant activity assessed by hole cross, open field and hole board test in the mice model.

1. Introduction

Rumex maritimus Linn. (Polygonaceae) is an annual herb widely distributed throughout Bangladesh, India, North Africa and America. The Plant is a good refringent. Leaves are applied to burns; seeds are tonic, remove pain from the back and the lumber region and aphrodisiac [1]. Roots of the plant are traditionally used in diarrhoea and painful ailments. A chemical investigation of *Rumex maritimus* resulted in the isolation of anthraquinone, chromone and flavone derivatives [2].

In continuation of our pharmacological evaluations of important medicinal plants of Bangladesh, we investigated analgesic and neuropharmacological activities of the root of the plant by established experimental models.

2. Investigations, results and discussion

The hexane (HE), ethyl acetate (EA) and methanol (ME) extract of *Rumex maritimus* root showed significant and dose dependent inhibition of acetic acid induced writhing in mice. At an-oral dose of 100 mg/kg body weight, the HE, EA and ME produced 63.38, 42.25 and 57.18% inhibition of acetic acid induced writhing, respectively. On the other hand, at 200 mg/kg body weight, the extracts showed 81.13, 70.14 and 74.65% ($p < 0.001$) inhibition of acetic acid induced writhing in mice (Table 1). Among all the extracts, the anti-writhing activity of HE at 200 mg/kg body weight dose was the maximum and comparable to that of aminopyrine, which offered 87.60% ($p < 0.001$) inhibition of writhing at a dose of 50 mg/kg body weight.

The writhing response in rats or mice induced by intraperitoneal administration of dilute acetic acid, phenylquinone, benzoquionone or bradykinin is prevented by salicylates and similarly acting drugs. The test is not entirely specific as several compounds without analgesic action in humans can also prevent the writhing response. Nevertheless, when taken in conjunction with other tests, including the ability to inhibit prostaglandin (PG) synthetase, especially that from nervous tissue, the anti-writhing test can provide useful information [3].

To evaluate central analgesic activity, all the extracts were subjected to the tail flick test at oral doses of 100 and 200 mg/kg body weight in mice. At the dose of 100 mg/kg body weight and 30 min after oral administration, only ME significantly exhibited ($p < 0.05$) prolongation of tail flicking time. At 200 mg/kg body weight dose, significant prolongation of tail flicking time was observed with HE ($p < 0.05$) and ME ($p < 0.02$) 30 min after oral administra-

Table 1: Evaluation of analgesic activity of *Rumex maritimus* root extracts by acetic acid induced writhing method

Treatment	Dose (mg/kg, p. o.)	Writhings Mean ± SEM (t value)	Inhibition (%)
Control (vehicle, 10 ml/kg)	–	35.5 ± 2.24	–
AP	50	4.4 ± 1.10 (12.462)*	87.60
HX	100	13.0 ± 2.16 (7.230)*	63.38
	200	6.7 ± 2.10 (9.379)*	81.13
EA	100	20.5 ± 1.68 (5.357)*	42.25
	200	10.6 ± 2.44 (7.517)*	70.14
ME	100	15.2 ± 1.78 (7.095)*	57.18
	200	9.0 ± 2.17 (8.497)*	74.65

* $p < 0.001$ vs. control; Student's t-test ($n = 6$)

Table 2: Evaluation of analgesic activity of *Rumex maritimus* root extracts the by tail flick test

Treatment	Dose (mg/kg body wt.)	Tail flick latency (s) Mean ± SEM (t value)		
		30 min	60 min	120 min
Control	–	3.8 ± 1.11	4.1 ± 0.90	4.2 ± 2.20
Morphine	2	7.6 ± 0.80 (2.777)**	7.2 ± 1.08 (2.205)*	5.8 ± 1.80 (0.562)
HX	100	5.9 ± 0.44 (1.758)	5.5 ± 0.88 (1.112)	4.9 ± 2.10 (0.230)
	200	6.7 ± 0.58 (2.315)*	6.1 ± 1.44 (1.177)*	5.4 ± 1.88 (0.414)
EA	100	4.8 ± 1.04 (0.657)	4.2 ± 1.48 (0.057)	4.2 ± 0.88 (0.000)
	200	6.1 ± 1.42 (1.276)	5.5 ± 1.78 (0.701)	4.9 ± 1.08 (0.285)
ME	100	6.6 ± 0.44 (2.345)*	6.1 ± 1.14 (1.376)	5.4 ± 1.46 (0.517)
	200	7.4 ± 0.62 (2.831)**	6.9 ± 0.44 (2.794)**	5.8 ± 1.88 (0.552)

* $p < 0.05$, ** $p < 0.01$ vs. Control; Student's t-test (n = 6)

tion. However, EA did not show any central analgesic activity in either of the doses (Table 2).

In the hole cross test at 100 mg/kg body weight (Table 3), only ME was found to possess mild to moderate depressant activity on the CNS and the peak effect was observed 30 min after administration of extract. The data for ME at 30, 60, 120 and 240 min were highly significant.

Results of the open field test at 100 mg/kg oral dose (Table 4) demonstrated that HE and ME have mild to moderate but significant ($p < 0.05$) depressant activity on the CNS. The peak depressant activity was observed with ME at 30 ($p < 0.01$) and 60 min ($p < 0.01$). Although EA showed mild activity which, however, was statistically insignificant.

The results of the hole board test on mice at an oral dose of 100 mg/kg (Table 5) showed that ME has moderate depressant activity on ambulation. The peak depressant activity was observed after 60 min of extract administration and the data from the experiment at 60 and 120 min were significant ($p < 0.05$). But the data obtained for HE and EA were statistically insignificant and indicative of almost no depressant activity on ambulation.

For head-dripping in the hole board test on mice, HE and EA were found to possess very mild depressant activity and the data obtained were significant ($p < 0.01$) only at 60 min after the administration of extracts. However ME exhibited moderate depressant activity on the head drippings of mice; the peak depressant activity was observed 30 min after administration of the extract and the data from the experiment at 60 min was significant ($p < 0.001$).

In case of defecation (number of stool pellets) in the hole board test of mice, HE and EA exhibited very mild and statistically insignificant depressant activity on immotional defecation of the animals. But ME moderately lowered the frequency of defecation of mice with a peak depressant activity at 120 min of the experiment and the data obtained from the experiment at 30 min was statistically significant.

In summary, ME was found to have the most active regarding CNS depressant activity as indicated by reduced explorating activity in the open field test, reduced movements in the hole cross test, reduced ambulation, reduced defecation and head-dipping frequency in the hole board

Table 3: Evaluation of the neuropharmacological activity *Rumex maritimus* root extracts by the hole cross test

Groups	Dose (mg/kg)	Number of movements (= number of hole crossed) Mean ± SEM (t values)				
		0 min	30 min	60 min	120 min	240 min
Control	100	3.40 ± 0.81	2.0 ± 0.71	3.60 ± 1.03	3.0 ± 1.05	3.0 ± 0.63
HX	100	4.33 ± 1.09 (0.684)	0.17 ± 0.17 (2.506)*	1.0 ± 0.63 (2.329)*	0.67 ± 0.33 (2.116)*	1.67 ± 0.62 (1.504)
EA	100	2.83 ± 1.2 (0.393)	1.83 ± 1.01 (0.137)	1.83 ± 0.65 (1.453)	1.33 ± 0.56 (1.403)	2.0 ± 1.03 (0.828)
ME	100	1.33 ± 1.15 (1.472)	0.0 ± 0.0 (2.816)**	0.0 ± 0.0 (3.495)**	0.17 ± 0.17 (2.660)*	0.5 ± 0.5 (3.108)**

* $p < 0.05$, ** $p < 01$ vs. Control; Student's t-test (n = 6)

Table 4: Evaluation of the neuropharmacological activity *Rumex maritimus* root extracts by the open field test

Groups	Dose (mg/kg)	Number of movements Mean ± SEM (*t* values)				
		0 min	30 min	60 min	120 min	240 min
Control	100	110.66 ± 5.7	56.26 ± 5.09	55.86 ± 5.7	51.29 ± 4.61	45.03 ± 4.77
HX	100	89.17 ± 12.03 (1.614)	16.50 ± 8.0 (4.193)**	9.50 ± 5.45 (5.878)***	9.17 ± 3.34 (7.398)***	22.0 ± 6.91 (2.742)*
EA	100	118.67 ± 25.93 (0.301)	49.50 ± 32.94 (0.202)	62.0 ± 26.11 (0.229)	19.83 ± 10.13 (2.826)*	29.67± 14.25 (1.022)
ME	100	100.0 ± 13.66 (0.720)	9.0 ± 3.67 (7.531)***	13.17 ± 7.44 (4.554)**	8.83 ± 7.83 (4.672)***	4.5 ± 3.5 (6.850)***

* $p < 0.05$, ** $p < 01$, *** $p < 001$ vs. Control; Student's t-test (n = 6)

Table 5: Evaluation of the neuropharmacological activity *Rumex maritimus* root extracts by the hole board test at a dose of 100 mg/kg body weight

Groups	Hole board test parameters	Observations Mean ± SEM (t value)				
		0 min	30 min	60 min	120 min	240 min
Control	Ambulation	16.5 ± 2.49	10.83 ± 4.25	7.0 ± 2.30	11.5 ± 4.12	13.17 ± 3.81
	Defecation	1.33 ± 0.72	1.67 ± 0.40	1.33 ± 0.62	0.67 ± 0.33	0.67 ± 0.49
	Head-dipping	14.33 ± 1.54	4.83 ± 2.1	5.67 ± 1.02	2.0 ± 0.63	5.17 ± 2.1
HX	Ambulation	17.8 ± 4.27 (0.262)	6.2 ± 1.69 (1.012)	5.0 ± 1.82 (0.681)	9.4 ± 3.04 (0.410)	11.4 ± 3.57 (0.339)
	Defecation	1.2 ± 0.74 (0.125)	1.4 ± 0.25 (0.572)	3.2 ± 2.17 (0.828)	1.0 ± 0.63 (0.464)	0.4 ± 0.25 (0.490)
	Head-dipping	5.8 ± 1.46 (4.019)**	1.4 ± 0.87 (1.508)	0.8 ± 0.58 (4.150)**	1.8 ± 1.11 (0.156)	2.2 ± 1.2 (1.227)
EA	Ambulation	18.4 ± 7.44 (0.242)	7.2 ± 3.01 (0.697)	3.6 ± 1.25 (1.298)	5.6 ± 2.82 (1.181)	6.0 ± 2.86 (1.505)
	Defecation	1.0 ± 0.63 (0.344)	0.2 ± 0.2 (3.287)*	0.2 ± 0.2 (1.734)	0.8 ± 0.45 (0.232)	0.6 ± 0.4 (0.110)
	Head-dipping	2.2 ± 1.11 (6.389)***	0.2 ± 0.2 (2.194)	0.6 ± 0.4 (4.627)**	1.6 ± 1.12 (0.311)	1.2 ± 0.2 (1.881)
ME	Ambulation	14.2 ± 1.74 (0.757)	1.4 ± 0.25 (2.214)	1.2 ± 0.2 (2.512)*	1.4 ± 0.25 (2.446)*	9.8 ± 5.19 (0.523)
	Defecation	1.0 ± 0.32 (0.418)	0.4 ± 0.25 (2.692)*	0.2 ± 0.2 (1.734)	0.0 ± 0.0 (2.030)	0.0 ± 0.00 (1.367)
	Head-dipping	1.2 ± 0.8 (7.565)***	0.0 ± 0.0 (2.300)	0.2 ± 0.2 (5.262)***	0.0 ± 0.0 (3.174)*	1.2 ± 0.49 (1.841)

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. Control; Student's t-test ($n = 6$)

test. Data from the HE and EA extracts were partly indicative of mild to moderate CNS depressant activity but lacked statistical significance. Although anthraquinone, chromone and flavone derivatives were isolated previously from the aerial parts of the plant, a bioassay guided phytochemical investigation of the root extracts is necessary to find out the active principle(s) to which the pharmacological activities can be attributed.

3. Experimental

3.1. Plant material

The roots of *Rumex maritimus* Linn were collected form Savar, Dhaka and taxonomically identified at the herbarium of Department of Botany, University of Dhaka where a voucher specimen (DUH 1208) has been retained. The collected roots were cut into pieces, washed, dried and finally ground to coarse powder (880 g).

3.2. Preparation of extracts

The coarse powder was subjected to a successive cold extraction procedure in distilled *n*-hexane (1.5 L), ethylacetate (1.5 L) and methanol (1.5 L). In each case maceration was done for 3 days and the extract thus obtained was filtered first with clean fine cloth and then with filter paper (Whatman no.1) and finally concentrated *in vacuo*. In this way 3.6 g of hexane extract (HE), 2.8 g of ethylacetate extract (EA) and 6.2 g of methanol extract (ME) were obtained.

3.3. Animals

Colony bred Swiss-Webster mice of either sex (20–25 g body weight) obtained from the Animal Resource Division of the International Center for Diarrhoeal Diseases and Research, Bangladesh (ICDDR, B) were used for the experiments. The animals were maintained in groups of six (each group containing equal number of male and female) at constant room temperature (22.0 ± 1.0 °C), humidity (55–65%) and 12 h light/12 h dark cycle. They had free access to standard rodent food (developed by ICDDR, B) and water *ad libitum.*

3.4. Dose and route of administration

In case of screening for analgesic activity, the doses were 100 and 200 mg/kg body weight *per os*. For evaluation of neuropharmacological effects, the dose was 100 mg/kg body weight administered intraperitoneally. All the extracts were administered as a suspension in 10% DMSO and normal saline.

3.5. Analgesic activity evaluation

3.5.1. Acetic acid induced abdominal writhing assay

The method of Koster et al. [4] was adopted with little modification. The animals were orally fed with the extracts vehicles (for control group) at the specified doses. Thirty min after administration of the extract and the vehicle, each animal was given 0.6% (v/v) solution of acetic acid (0.1 ml/10 g of body weight) intraperitoneally to induce abdominal contractions or writhing. Five min after the administration of acetic acid, the number of writhings for each animal was counted for 15 min. The number of writhings in the control was taken as 100% and percent inhibition was calculated as follows:

$$\% \text{ Inhibition of writhing} = 100 - (\text{treated mean/control mean}) \times 100$$

For comparison, the same experiment was done for a positive control group treated orally with aminopyrine (Sigma, USA) at a dose of 50 mg/kg body weight.

3.5.2. Tail flick test

The central analgesic activity was evaluated by the radiant heat method [5] exploiting the tail flick response of rodents. Mice were orally fed with test materials (extracts and vehicle) at specified doses and after 30 min each mouse was kept into a small plastic cage leaving the proximal third of its tail exposed over a holder having a thin wire of an analgesiometer (Medicraft Co, India). In order to make the wire hot, current was allowed to pass through the wire at a low intensity (4 amperes). Within a few seconds, the animal flicked its tail aside or tried to escape and the time (tail flicking latency) for this tail flick reflex to occur was recorded. The tail flick latency was actually the pain perception time. The data were compared to those of a positive control group treated with morphine (as morphine HCl, 2 mg/kg body weight) intraperitoneally. The tail flick latency for the control group was considered 100% and the percent elongation of tail flick latency was counted in the following way:

$$\% \text{ Elongation of tail flick latency} = \left[\left(\frac{\text{treated mean}}{\text{control mean}}\right) \times 100\right] - 100\,.$$

3.6. Neuropharmacological studies

3.6.1. Open-field tests

This experiment was carried out in accordance with the method of Gupta et al. [6]. The floor of an open field of half square meter was divided into

a series of squares, each alternately colored black and white. The apparatus had a wall of 40 cm. The number of squares visited by the animals was recorded for a period of 2 min.

3.6.2. Hole cross tests

The method of Takagi et al. [7] was adopted. A steel partition was fixed in the middle of a cage $30 \times 20 \times 14$ cm in size. A hole of 3 cm diameter was made at a height of 7.5 cm in the center of the plate. The number of passages of a mouse through the hole from one end of the cage to the other was recorded for a period of 2 min at -60, $+30$, $+60$, $+120$ and $+240$ min. Similar recordings were made for the control animals.

3.6.3. Hole-board tests

This experiment was carried out following the method of Nakama et al. [8]. Sixten holes, each 3 cm in diameter, were presented to the mouse in a flat space of 25 cm square. The number of ambulation (expressed as the number of holes passed), head dripping and defecation was recorded for a period of 2 min.

3.7. Statistical analysis

All data were presented as mean $\pm$ SEM. The level of significance was assessed by the Student's *t*-test for unpaired data using standard application software (SPSS version 10 for Windows^{TM}).

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