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Neuropharmacological study of hot water extract of the seeds of *Vernonia anthelmintica* Kuntze

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SUMMARY

The hot water extract of the seeds of *Vernonia anthelmintica* Kuntze (Compositae) in a dose of 10 ml/kg body weight of mice, showed significant analgesic activity on the hot plate analgesic method throughout the 4 h experimental period. The hole cross scores and the climbing out scores are of lower than the control animals. The hot water extract reduced the defecation in hole board study and significantly reduces the exploratory ambulation and head dipping behaviour. The seed also showed significant depressant activity on the exploratory ambulation of the open field scores. The drug decreased the spontaneous locomotion activity on brick-chip displacement method up to 45 min and also showed the ability to lessen the amphetamine induced hyperactivity up to 20 min. But it didn't show any effect on pentobarbital induced sleeping time test. The extract reduced gastrointestinal motility.

Key words: *Vernonia anthelmintica*; Neuropharmacological; Analgesic; Gastrointestinal

INTRODUCTION

Vernonia anthelmintica Kuntze (Compositae) is traditionally used for the treatment of helminthiasis in Ayurvedic system of medicine in India (Chopra *et al.*, 1934). It is a traditional Chinese medicine as vermicide, also used by Uyghurs for the treatment of vitiligo for several centuries (Tian *et al.*, 2004). The seeds found to contain sterols, flavan glucosides, vernodalin, vernodalol, methylvernosterol, butein, chalcone, β-amyrin, β-sitosterol, β-D-glucoside (daucosterol), vernolic acid, stigmasterol, flavonoids (Smith *et al.*, 1959; Wu *et al.*, 1991; Akihisa *et al.*, 1992; Tian *et al.*, 2004). *V. anthelmintica* could inhibit the immune function of mice and have anthelmintic, antibacterial, activity and anticancer activity (Chopra *et al.*, 1934; Saluja *et al.*, 1979; Deng *et al.*, 2002; Lambertini *et al.*, 2004).

The present study was undertaken to explore the neuropharmacological profile of the hot water extract of *V. anthelmintica* by different mice models.

MATERIALS AND METHODS

Plant material and the extract

The seeds of *Vernonia anthelmintica* Kuntze (SMR) were collected from traditional herbal shops from Dhaka. The seeds were then powdered by a grinder. The hot water extract was prepared by 100 mg of

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the powdered plant materials boiling in 1,600 ml water and it was filtered and evaporated to give 400 ml of hot water extract.

Animals

Nonfasted, male and female mice (*Swiss-Webster* strain, 20 - 25 g body weight) bred in the animal house of the Department of Pharmacy, Jahangirnagar University were used for the experiments. The animals were provided with food and tap water *ad libitum*. The animals were maintained at natural day night cycle. Animals were divided in groups of 6, with each group balanced for sex and body weight. The extract was administered orally at a dose level of 10 ml/kg body weight. The control animals were given equal volume of physiological saline.

Analgesic effect evaluation by hot plate method

The analgesic study of the test drug was conducted by the "Hot Plate" method, described by Wood (1985). Hot plate was maintained at a constant temperature of 55 ± 0.5°C. Each mouse was placed on the hot surface and the time of response to these thermal stimuli, indicated by the licking of hind and/or fore paws or by kicking of the legs or by trying to jump out, was recorded. The observations were made on 0, 30, 60, 120, and 240 min after oral administration of the drug.

Hole cross test

The experiment was carried out using a black cage where a black cardboard partition fixed in the middle of a cage, 30 × 20 × 14 cm. A hole of 3 cm in diameter is made at the height of 7.5 cm in the centre of the plate and a mouse can pass through it only one at a time. The experiment was performed by Takagi *et al.* (1971) method. Spontaneous movement of the animals through the hole from one chamber to the other was counted for 2 min in this test. The observations were made on 0, 30, 60, 120, and 240 min after oral administration of the drug.

Climbing out test

This experiment was carried out by the method of Sandberg (1957) and Hannan *et al.* (2003). The mice were put in a cage with dimension 60 × 50 × 30 cm and having dark walls. Animals were supplied with a ladder and the time taken to climb out of the cage was recorded for the maximum period of 10 min. The observations were made on 0, 30, 60, 120, and 240 min after oral administration of the drug.

Hole board test

This experiment was carried out by the method of Nakama *et al.* (1972). In this experiment a board of 16 holes each 3 cm in diameter were presented to the mouse in a flat space of 25 sq. cm. Each animal was transferred carefully to the corner of the board and the number of holes passed, head dipping and the number of faecal boluses excreted, was recorded for a period of two minutes. The observations were made on 0, 30, 60, 120, and 240 min after oral administration of the drug.

Open field test

In this experiment, the method of Gupta (1971), was employed. The floor of an open field of half square meter was divided into a series of squares, each alternatively colored black and white. The apparatus had a wall of 40 cm, the animals were transferred carefully to the corner of the field and the number of squares travelled by the mouse were recorded for a period of two minutes. The observations were made on 0, 30, 60, 120, and 240 min after oral administration of the drug.

Spontaneous motor activity test

For this experiment, brick chip displacement method (Hannan *et al.*, 2003), a modified version of the sand displacement method of Siegmund and Wolf (1952) was employed. The displaced brick chips, through the wire nettings, due to the spontaneous motor activity of the animals, were weighed with 5 min interval for a period of 1 h.

Table 1. Effect of *V. anthelminticum* on central nervous system on mice[a]

Groups	Observation Time (min) (*P* value)				
	0	30	60	120	240
	Pain perception (Hot-plate) test				
CON	33.0 ± 2.73	23.83 ± 1.11	18.83 ± 1.99	28.83 ± 3.82	26.17 ± 4.03
SMR	40.67 ± 2.89 (0.083)	55.5 ± 2.07 (0.000)***	62.16 ± 1.37 (0.000)***	58.16 ± 2.79 (0.000)***	62.33 ± 1.89 (0.000)***
	Hole cross test				
CON	5.83 ± 1.3	4.0 ± 0.86	2.33 ± 0.76	1.83 ± 0.6	0.67 ± 0.33
SMR	4.50 ± 1.0 (0.434)	1.67 ± 0.95 (0.099)	1.83 ± 0.75 (0.649)	1.5 ± 0.72 (0.729)	1.83 ± 0.47 (0.073)
	Climbing out test				
CON	125.33 ± 62.42	76.17 ± 37.9	76.17 ± 36.71	67.83 ± 32.04	35.0 ± 15.56
SMR	198.0 ± 49.22 (0.382)	30.0 ± 30.0 (0.362)	0.0 ± 0.0 (0.930)	21.67 ± 21.67 (0.260)	9.17 ± 9.17 (0.183)
	Hole board test (Defecation)				
CON	3.33 ± 0.61	0.33 ± 0.33	0.83 ± 0.48	1.83 ± 0.40	1.50 ± 0.34
SMR	1.5 ± 0.43 (0.034)	0.16 ± 0.16 (0.389)	0.17 ± 0.17 (0.217)	0.0 ± 0.0 (0.006)**	0.0 ± 0.0 (0.007)**
	Hole board test (Ambulation)				
CON	25.67 ± 3.75	23.83 ± 6.3	37.83 ± 3.41	29.66 ± 7.03	21.83 ± 3.77
SMR	15.33 ± 3.2 (0.062)	4.16 ± 1.62 (0.028)*	3.67 ± 0.95 (0.000)***	5.0 ± 1.21 (0.017)*	4.0 ± 1.36 (0.001)**
	Hole board test (Head dipping)				
CON	21.67 ± 1.58	4.83 ± 0.65	8.0 ± 1.81	10.0 ± 2.35	19.33 ± 5.44
SMR	16.33 ± 4.59 (0.313)	2.33 ± 0.67 (0.023)*	4.67 ± 1.58 (0.196)	6.83 ± 1.3 (0.266)	2.83 ± 1.54 (0.039)*
	Open field test				
CON	88.33 ± 9.51	56.17 ± 13.43	43.50 ± 10.20	33.33 ± 10.59	24.17 ± 7.37
SMR	72.17 ± 10.6 (0.282)	16.33 ± 9.3 (0.035)*	8.83 ± 5.61 (0.01)**	13.2 ± 6.05 (0.129)	10.83 ± 5.58 (0.180)

[a]Values are expressed as Mean ± S.E.M. (n = 6). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, CON = Control group, SMR = *V. anthelminticum* group.

Amphetamine induced hyperactivity test

This experiment was also carried out by the previously described method of spontaneous motor activity test (Siegmund and Wolf, 1952). The test drug was administered one hour prior to administration of amphetamine at a stimulant dose of 4 mg/kg.

Pentobarbital induced sleeping time test

In this experiment, the method of Tedeschi and Tedeschi (1968) was employed by mouse. The extract was administered intraperitoneally and 60 min later pentobarbital, in a sub-hypnotic dose of 45 mg/kg was administered by the i.p. route. The animals were observed for the onset and the duration of sleeping as evidenced by the observation of loss of righting reflex.

Gastrointestinal motility test with barium sulfate milk

This experiment was carried out by the method described by Chatterjee (1993). Barium sulfate milk (15% barium sulfate in 0.5% NaCMC suspension)

was given to a group of 12 mice (both treated and control) after 15 min of oral administration of the test extract. The treated mice were divided into two groups and were sacrificed after 15 and 30 min after the administration of barium sulfate milk. The distance traversed by milk were measured and expressed as a percentage of the total length of small intestine (from pylorus to the ileoceccal junction).

Statistical analysis

Data obtained from the experiments are expressed as mean and standard error of the mean (Mean ± S.E.M.). Unpaired t-test was performed by computer software SPSS (Statistical Package for Social Science) release 6.0 for Windows, to test the level of significance. Probability (P) value of 0.05 or less ($P < 0.05$) was considered as significant.

RESULTS AND DISCISSION

Vernonia anthelmintica a common medicinal plant in Ayurvedic system of medicine in India has been tested here for its neuropharmacological activity. *Vernonia anthelmintica* showed highly significant ($P < 0.001$) analgesic activity on the hot plate analgesic method throughout the experimental period of 4 h (Table 1). The hole cross scores and the climbing out scores are observed lower than the control animals, but out of the statistical significant level. The lowering begins from 30 min and continued to 2 h. The hot water extract reduced the defecation in the study and interestingly it stops defecation in hole board study after 2 h time and significantly reduces the exploratory ambulation ($P < 0.001$) and head dipping ($P < 0.05$) behaviour starting from 30 min to the end of the 4 h experiment. The seed also reduced the exploratory ambulation of the open field scores, the reduction was significant at 30 min ($P < 0.05$) and 60 min ($P < 0.01$) interval. The data indicates that the extract decreased the spontaneous locomotion activity on brick-chip displacement method up to 45 min (Table 2). The drug also showed the ability to lessen the amphetamine induced hyperactivity of mice up to 20 min. But it didn't show any effect on pentobarbital induced sleeping time test on the onset or duration of sleeping time (Table 3). However, taken together the results of hotplate, hole cross, hole board and climbing out tests the findings suggest that the extract of *Vernonia anthelmintica* have central nervous system depressant activity. The extract decreased the gastrointestinal

Table 2. Effect of *V. anthelminticum* on locomotion activity[a]

Groups	Observation Time (min.)											
	5	10	15	20	25	30	35	40	45	50	55	60
	Spontaneous locomotion activity											
CON	5.75	3.66	3.72	3.05	2.05	1.65	0.75	1.28	0.60	0.26	0.22	0.50
SMR	2.31	1.36	1.34	0.77	0.26	0.36	0.27	0.41	0.23	0.26	0.28	0.55
	Amphetamine induced hyperactivity											
CON	4.75	3.66	3.22	3.41	2.65	2.05	1.43	1.62	0.98	0.60	0.14	0.08
SMR	2.31	1.36	1.25	2.22	3.21	4.2	1.36	2.31	1.35	1.26	2.45	1.56

[a]Values are in gram of brick chips weighed. CON = Control group, SMR = *V. anthelminticum* group

Table 3. Effect of *V. anthelminticum* on pentobarbital induced sleeping time on mice[a]

Groups	Onset of Sleep (P value)	Duration of Sleep (P value)
Control	274.67 ± 17.04	2426.17 ± 220.41
V. anthelminticum	257.33 ± 18.43 (0.506)	2547.0 ± 181.80 (0.681)

[a]Values are expressed as Mean ± S.E.M (n = 6) in seconds. CON = Control group, SMR = *V. anthelminticum* group

Table 4. Effect of *V. anthelminticum* on barium sulfate induced intestinal motility

Groups	% Traversed (*P* value)	
	After 15 min	After 30 min
Control	39.32 ± 2.06	45.66 ± 1.68
V. anthelminticum	31.11 ± 3.24 (0.058)	32.8 ± 2.7 (0.002)

[a]Values are expressed as Mean ± S.E.M. (n = 6)

motility both at 15 min ($P < 0.05$) and 30 min ($P < 0.01$) time interval indicating constipating effect of the drug (Table 4).

In our conclusion the hot water extract of *Vernonia anthelmintica* have showed depressant activity and reduced gut motility in our mice models. Further investigation is required to explore the mechanism and to identify chemical(s) of this activity.

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