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CNS Activities of the Aqueous Extract of *Hydrilla verticillata* in Mice

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Abstract – The aqueous extract of *Hydrilla verticillata* (AEHV) was tested for possible pharmacological effects on experimental animals. AEHV significantly potentiated the sleeping time of mice induced by standard hypnotics *viz.* pentobarbitone sodium, diazepam, and meprobamate in a dose dependent manner. AEHV showed significant analgesic properties as evidenced by the significant reduction in the number of writhes and stretches induced in mice by 1.2% acetic acid solution. It also potentiated analgesia induced by morphine and pethidine in mice. Pretreatment with AEHV caused significant protection against strychnine and leptazol-induced convulsions. The behavioral studies on mice indicate CNS depressant activity of the aqueous extract of *H. verticillata.*
Keywords – *Hydrilla verticillata,* sleeping time, general behavior, analgesic activity, anticonvulsant activity

Introduction

Hydrilla verticillata (Oriya : Chingudiala, English : Tape grass; family : Hydrocharitaceae) is a perennial sedge distributed throughout India, Srilanka, Malaysia, China, and The United States up to the altitude of 2005 ft. It is widely found in water including lakes, ponds, rivers, streams, and marshes and can survive in few inches of water or in depth of up to 20 ft. Various parts of this plant were used in tribal medicines for the diseases like neurological problems, gastrointestinal disturbances, malnutrition, cardiovascular disturbances, and diabetes (Chopra *et al.*, 1992; Mashelkar, 1998). The plant has been found to possess antibacterial properties (Pal *et al.*, 2005). *H. verticillata* on preliminary chemical analysis is found to contain saponins, beta-carotenes, vitamins, minerals, antioxidants, and detoxifying agents (Araki *et al*, 2003; Chopra *et al.*, 1992; Easley *et al.*, 1974; Ulen *et al.*, 2005; Pal *et al.*, 2006, Pal *et al.*, 2006). The aqueous extract of *H. verticillata* (AEHV) showed marked CNS depressant action compared to other extracts of it in preliminary pharmacological screening. However, no work has been reported on the CNS activities of this plant. Keeping this in view, the present study has been undertaken to investigate various CNS activities such as behavioral, sedative-hypnotic, analgesic, and anticonvulsant effects of AEHV in mice to substantiate the folklore claim.

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Experimental

Plant materials – Fresh *H. verticillata* was collected from the Subarnarekha river in the Mayurvanj district of Orissa in the month of August-September and was authenticated by Dr. H. J. Chowdhury, Joint Director, Central National Herbarium, Botanical Survey of India, Howrah, West Bengal. A voucher specimen has been preserved in our laboratory for future references (DNS 1).

Extraction – Shade dried, powdered, and sieved (40 mesh size) plant materials were extracted with distilled water in a soxhlet apparatus. The extract was concentrated to dryness in vacuo. The yield of the aqueous extract was 7.02% (w/w). Phytochemical screening of the extract revealed the presence of steroids and saponins.

Experimental animal – Swiss albino male mice (weighing 25 - 30 g) were used. The animals were fed standard pellet diet and water was provided *ad libitum.* The animals were housed in groups of 10 animals at 25 ± 1 °C. The animals were not provided with food for last 17 h before the commencement of the experiment. The experiment was performed under the guidance of the Institutional Ethical Committee. For the pharmacological testing, the aqueous extract of *H. verticillata* (AEHV) was dissolved in distilled water.

Pharmacological studies

Safety evaluation – An acute toxicity study relating to the determination of the LD_{50} value was performed with different doses of HEAV in albino mice as per the method described by Litchfield and Wilcoxon (1949).

Barbiturate potentiation – Mice were divided into 5 groups, each group containing 6 mice. The animals of group I served as the control (normal saline, 0.9% w/v NaCl, 5 ml/kg,); groups II, III, IV, and V received AEHV at a low, medium, and high dose (25 mg/kg, 50 mg/kg, 75 mg/kg and 100 mg/kg, respectively). Normal saline and the extracts were injected intraperitoneally 30 min prior to the administration of pentobarbitone sodium (40 mg/kg, *i.p.*), diazepam (3 mg/kg, *i.p.*) and meprobamate (100 mg/kg, *i.p*). The sleeping time was noted by recording the interval between the loss and regaining of righting reflex (Bigoniya *et al.*, 2005; Dandiya *et al.*, 1959).

Analgesic activity – The analgesic activity was tested by the following methods:

i) Acetic acid induced writhing (chemical stimulus) method

This method involved *i.p.* injection of freshly prepared 1.2% acetic acid. The number of abdominal constrictions (writhing) and stretching with a jerk at the hind limbs were counted between 5 and 15 min after administering acetic acid (Mandal *et al.*, 2001; Pal *et al.*, 2003; Vedhanayaki *et al.*, 2003). The analgesic effect of the drugs was calculated by the percentage inhibition of writhing episode over that of the control group. The results were compared with those of acetyl salicylic acid (68 mg/kg), paracetamol (68 mg/kg), and morphine sulphate (1.15 mg/kg).

ii) Thermal stimulus by Eddys hot plate method

The analgesic actions were studied using Eddys hot plate method (Eddy *et al.*, 1953). The reaction time was taken as the interval extending from the instant the mouse reached the hot plate till the animal licked its feet or jumped out of the cylinder. The reaction time was recorded at 30, 45, 60, 90, 120, 150, and 180 min after *i.p.* administration of AEHV at doses of 50, 75, and 100 mg/kg. The temperature of the hot plate was maintained at 55 ± 0.5 °C. A cut-off reaction time of 30 s was chosen in order to avoid tissue injury. Morphine and pethidine were used as reference drugs at doses of 5 and 10 mg/kg, *i.p.* respectively. AEHV was given individually and 15 min prior to the administration of reference drugs to investigate the potentiation of morphine and pethidine activity (Gupta *et al*, 1999; Mazumder *et al.*, 1998).

Anticonvulsant activity – The anticonvulsant property of AEHV (50, 75, and 100 mg/kg, *i.p.*) was tested against two standard drugs, strychnine (2 mg/kg, *i.p.*) and leptazol (80 mg/kg, *i.p.*). The average survival time (min) and percentage of mortality after 24 h were recorded (Gitto *et al*, 2004; Gupta *et al.*, 1999; Mazumder *et al.*, 1998; Pal *et al.*, 2005).

Behavioral effects – The effects of AEHV (25, 50, 75, and 100 mg/kg, *i.p.*) on righting reflex, pinna reflex, corneal reflex, awareness, grip strength, touch, and pain responses on mice were observed by conventional methods. Chlorpromazine (5 mg/kg, *i.p.*) was used as a reference drug (Achliya *et al.*, 2005; Mazumder *et al.*, 2005; Murugesan *et al.*, 2001).

Statistical analysis – Results are expressed as mean ± SEM. ANOVA followed by Dunnett's 't' test (Bolton, 1995) was performed as a post hoc test of significance taking vehicle treated animals as control. P value of < 0.05 was considered as statistically significant.

Results

Safety evaluation – Acute toxicity tests in mice established the LD_{50} of AEHV to be 250 mg/kg, *i.p.*

Barbiturate potentiation – Four doses of AEHV (25, 50, 75, and 100 mg/kg) potentiated the sleeping time induced by standard hypnotics *viz* pentobarbitone (61.7%, 99.3%, 146.2%, and 186.7% respectively), diazepam (45.7%, 84.8%, 161.2%, and 220.3% respectively) and meprobamate (30.3%, 53.2%, 84.5%, and 94.4% respectively) (Table 1).

Analgesic activity – AEHV exhibited a dose dependant and significant analgesic activity in the acetic acid

Table 1. Effect of AEHV on sleeping time (min) induced by pentobarbitone, diazepam, and meprobamate in mice

treatment	sleeping time (min) induced by		
	pentobarbitone (40 mg/kg, i.p.)	meprobamate (100 mg/kg, i.p.)	diazepam (3 mg/kg, i.p.)
control (NS, 5 ml/kg, *i.p.*)	40.5 ± 0.95	61.8 ± 0.90	75.0 ± 0.89
AEHV (25 mg/kg, *i.p*)	65.5 ± 1.10[a]	80.5 ± 1.21[a]	109.3 ± 1.47[a]
AEHV (50 mg/kg, *i.p*)	80.7 ± 2.10[a]	94.7 ± 1.98[a]	138.6 ± 3.09[a]
AEHV (75 mg/kg, *i.p*)	99.7 ± 2.15[a]	114.0 ± 2.06[a]	195.9 ± 3.60[a]
AEHV (100 mg/kg, *i.p*)	116.1 ± 2.23[a]	132.6 ± 3.12[a]	240.2 ± 3.89[a]

Values are mean ± SEM from 6 animals in each group; statistical analysis done by ANOVA followed by post hoc test of significance, Dunnett's 't' test. [a]$P < 0.001$ *vs.* vehicle control. NS: normal saline. *i.p.*: intraperitoneal.

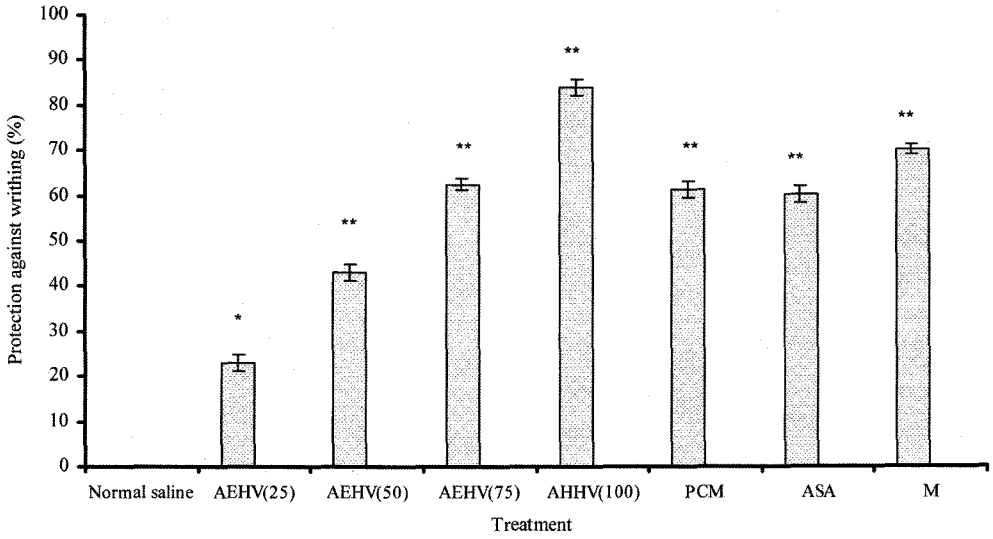

Fig. 1. Influence of AEHV (25, 50, 75, 100 mg/kg, *i.p.*) on the writhing and stretching induced in mice by 1.2% v/v acetic acid (writhing test). The activity was compared with paracetamol (PCM) (68 mg/kg, *i.p.*), acetyl salicylic acid (ASA)(68 mg/kg, *i.p.*), and morphine sulphate (M)(1.15 mg/kg, *i.p.*). Values are mean ± SEM from 6 animals in each group. *P < 0.01, **P < 0.001 *vs.* control (ANOVA followed by Dunnett's 't' test).

Table 2. Effect of AEHV on analgesia induced by morphine and pethidine in mice (by hot plate method)

treatment	resting value	average maximum reaction time (sec) at min							
		15	30	45	60	90	120	150	180
control (NS, 5 ml/kg, *i.p.*)	4.8 ± 0.07	10.1 ± 1.25	7.6 ± 0.06	5.2 ± 0.03	5.1 ± 1.10	4.2 ± 1.02	4.1 ± 0.74	3.7 ± 1.03	3.1 ± 0.33
AEHV (50 mg/kg, *i.p.*)	4.9 ± 0.81	-	19.6 ± 1.00[a]	14.2 ± 1.17[a]	11.1 ± 0.9[a]	9.5 ± 0.84[a]	6.0 ± 0.05	5.0 ± 0.71	4.5 ± 0.67
AEHV (75 mg/kg, *i.p.*)	5.1 ± 0.94	27.2 ± 1.04[a]	24.5 ± 1.33[a]	18.3 ± 1.09[a]	14.1 ± 1.25[a]	12.7 ± 1.38[a]	9.0 ± 0.99[a]	6.5 ± 0.84	5.2 ± 0.71
AEHV (100 mg/kg, *i.p.*)	4.7 ± 0.08	>30[a]	>30[a]	>30[a]	>30[a]	28.3 ± 1.92[a]	25.1 ± 1.42[a]	20.1 ± 0.81[a]	14.1 ± 1.14[a]
morphine (5 mg/kg, *i.p.*)	5.7 ± 0.95	>30[a]	19.3 ± 0.44[a]	18.5 ± 1.04[a]	14.2 ± 0.97[a]	9.9 ± 1.20[a]	8.1 ± 0.83[a]	6.4 ± 0.73	5.0 ± 0.37
AEHV (50 mg/kg, *i.p.*) + morphine	5.3 ± 0.89	>30[a]	>30[a]	27.6 ± 1.52[a]	24.6 ± 0.80[a]	21.2 ± 0.96[a]	12.2 ± 1.31[a]	9.2 ± 0.89[a]	6.8 ± 0.80
AEHV (75 mg/kg, *i.p.*) + morphine	6.0 ± 0.64	>30[a]	>30[a]	>30[a]	28.4 ± 1.10[a]	25.3 ± 1.72[a]	15.9 ± 2.71[a]	11.6 ± 1.25[a]	10.1 ± 0.36[a]
AEHV (100 mg/kg, *i.p*) + morphine	5.9 ± 0.98	>30[a]	>30[a]	>30[a]	>30[a]	>30[a]	>30[a]	28.4 ± 2.01[a]	21.5 ± 1.89[a]
pethidine (10 mg/kg, *i.p.*)	4.8 ± 0.86	25.0 ± 1.29[a]	23.0 ± 1.41[a]	15.5 ± 0.53[a]	11.3 ± 1.05[a]	9.5 ± 0.94[a]	6.0 ± 0.91[a]	4.5 ± 0.95	3.9 ± 0.83
AEHV (50 mg/kg, *i.p*) + pethidine	5.7 ± 0.9	28.3 ± 1.00[a]	27.6 ± 1.23[a]	17.7 ± 1.97[a]	16.0 ± 1.29[a]	16.0 ± 1.45[a]	10.4 ± 1.71[a]	8.3 ± 0.96[a]	6.2 ± 0.71
AEHV (75 mg/kg, *i.p.*) + pethdine	5.5 ± 1.13	>30[a]	>30[a]	28.1 ± 1.49[a]	21.3 ± 1.08[a]	17.5 ± 1.61[a]	14.3 ± 1.19[a]	10.7 ± 1.13[a]	8.1 ± 0.77[a]
AEHV (100 mg/kg, *i.p*) + pethidine	5.8 ± 0.97	>30[a]	>30[a]	>30[a]	>30[a]	>30[a]	28.9 ± 1.59[a]	23.5 ± 2.02[a]	13.5 ± 1.32[a]

Values are mean ± SEM from 6 animals in each group; statistical analysis done by ANOVA followed by post hoc test of significance, Dunnett's 't' test, [a]P < 0.05 *vs.* resting value (average reaction time before treatment). Results of (AEHV + morphine) and (AEHV + pethidine) were significant (P < 0.05) *vs.* AEHV. NS: normal saline; > 30: animals fail to react within 30 s (30-s response latency). *i.p.*: intraperitoneal.

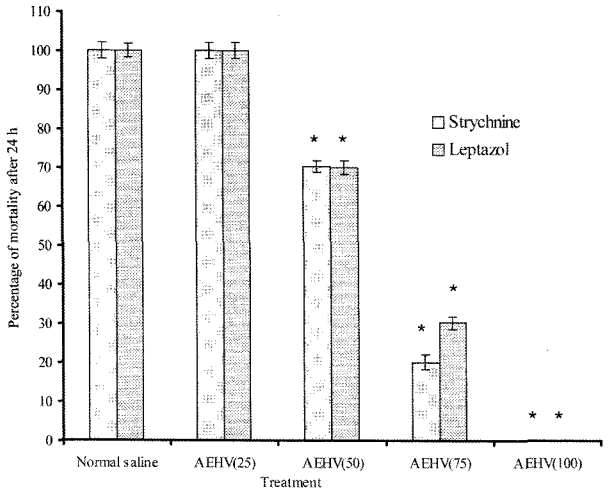

Fig. 2. Anticonvulsant effect of AEHV on strychnine (2 mg/kg, *i.p.*) and leptazol (80 mg/kg, *i.p.*)-induced convulsions in mice. Results are expressed in percentage of mortality. Respective doses of the extracts (mg/kg, b.w.) are in the parenthesis. Values are mean ± SEM from 10 animals in each group. *P < 0.001 *vs.* control (ANOVA followed by Dunnett's 't' test).

induced writhing test. As can be seen in Fig. 1, AEHV with a dose of 25 mg/kg, *i.p.* exhibited percentage of protection 23%. This dose dependant effect reached 84% with a dose of 100 mg/kg, *i.p.* Analgesic compounds acetyl salicylic acid (68 mg/kg), morphine sulphate (1.15 mg/kg), and paracetamol (68 mg/kg) gave 60%, 70%, and 61% protection respectively. From Table 2, it is also found that AEHV not only produced analgesia in mice but also potentiated the analgesic action of morphine and pethidine.

Anticonvulsant activity – Strychnine and leptazol at the doses of 2 mg/kg, *i.p.* and 80 mg/kg, *i.p.* respectively induced tonic type of convulsions with clonus in mice. The degree of convulsions was measured visually. Table 3 and Fig. 2 show that AEHV increased the average survival time and decreased the percentage mortality in a dose dependent manner against strychnine and leptazol- induced convulsions. It was observed that different combinations of strychnine or leptazol with AEHV did not show any significant protective action against convulsions.

Effects on general behavioral profiles – The results obtained from general behavioral profiles are shown in Table 4. It was noted that AEHV depressed awareness and alertness, touch and pain responses, grip strength, altered righting, pinna and corneal reflexes when compared to the control (normal saline 0.9% w/v, 5 ml/kg). However, chlorpromazine hydrochloride (standard) produced a significant depression of these responses in comparison with AEHV.

Table 3. Effect of AEHV on average survival time on strychnine and leptazol- induced convulsions in mice

treatment	survival time (min) after treatment of	
	strychnine (2 mg/kg, *i.p.*)	leptazol (80 mg/kg, *i.p.*)
control (NS, 5 ml/kg, *i.p.*)	6.2 ± 0.95	12.5 ± 1.19
AEHV (15 mg/kg, *i.p.*)	121.3 ± 1.10[a]	132.5 ± 1.05[a]
AEHV (25 mg/kg, *i.p.*)	140.5 ± 1.14[a]	160.0 ± 1.25[a]
AEHV (50 mg/kg, *i.p.*)	185.7 ± 1.76[a]	208.2 ± 1.97[a]
AEHV (75 mg/kg, *i.p.*)	270.8 ± 2.30[a]	318.4 ± 2.96[a]

Values are mean ± SEM from 10 animals in each group; statistical analysis done by ANOVA followed by post hoc test of significance, Dunnett's 't' test. [a]P < 0.001 *vs.* control, NS: normal saline. *i.p.*: intraperitoneal.

Table 4. Effect of AEHV on behavioral profiles in mice

treatment	awareness response	touch response	pain reflex	righting reflex	pinna reflex	corneal reflex	grip strength
control (NS, 5 ml/kg, *i.p.*)	0	0	0	0	0	0	+
chlorpromazine (5 mg/kg, *i.p.*)	4+	4+	4+	4+	4+	4+	4+
AEHV (25 mg/kg, *i.p.*)	+	+	2+	+	+	+	+
AEHV (50 mg/kg, *i.p.*)	+	2+	3+	+	2+	2+	+
AEHV (75 mg/kg, *i.p.*)	2+	3+	4+	2+	+2	3+	2+
AEHV (100 mg/kg, *i.p.*)	3+	3+	4+	3+	3+	4+	3+

Key for scoring: 0, no effect (normal); +, slight depression; 2+, moderate depression; 3+, strong depression; 4+, very strong depression. *i.p.*: intraperitoneal. NS: normal saline. Number of animals used for each group (n = 6). AEHV values were significant (P < 0.05) *vs.* control.

Discussion

Pentobarbitone, diazepam, and meprobamate were used to induce sleep in this study. Benzodiazepines are believed to act at specific binding sites which are closely linked to gamma-aminobutyric acid (GABA) receptors, the binding of benzodiazepines enhancing GABA-ergic transmission. Although the cause of prolongation of diazepam-induced sleeping time is not known, the enhancement of GABA-ergic transmission might be related to its sedative activity. Prolongation of pentobarbitone induced sleeping time might be due to tranquilizing action as well as CNS depressant action. Although the exact mechanism responsible for the sedation action of meprobamate is not clear, it may be due to CNS depressant action or due to enhancement of GABA-ergic transmission (Gupta *et al.*, 1999; Mandal *et al.*, 2001; Mazumder *et al.*, 1998; Murugesan *et al.*, 1999). AEHV potentiated significantly the duration of pentobarbitone, diazepam, and meprobamate-induced sleep in mice, suggesting probable tranquilizing action as well as CNS depressant action (Gupta *et al.*, 2003; Mazumder *et al.*, 2005).

Mazumder *et al.* found that analgesic activity of *Cassia fistula* is probably mediated by inhibition of a post synaptic specific sensitive mechanism either by depleting endogenous levels of nor epinephrine *via* dopamine-β-hydroxylase inhibition or by blocking nor epinephrine effects at the receptor level (Mazumder *et al*, 1998). Analgesic and anticonvulsant activities can also be mediated by other mechanisms. The increase of brain serotonin and GABA level is responsible for analgesic and anticonvulsant activities (Gupta *et al.*, 1999; Gupta *et al.*, 2003; Mazumder *et al.*, 1998). It was found that AEHV increased the brain serotonin and GABA level in mice (unpublished data). Therefore, analgesic and anticonvulsant activities produced by AEHV may be related to the increased brain serotonin and GABA level in mice (Gupta *et al.*, 2003).

Gupta *et al.* established that inhibition of the touch response, righting reflex, and grip strength is probably produced due to a pronounced CNS depressant action (Gupta *et al.*, 1999). Reduction of pinna reflex and awareness may be due to synapses block of the afferent pathway or due to overall CNS depressant action (Rolland *et al.*, 2001). In this study, the mechanism whereby AEHV depressed awareness, touch and pain responses, righting reflex, pinna reflex, corneal reflex, and grip strength may also be due to synapses block of the efferent pathway or by overall CNS depressant action.

Phytochemical tests indicate the presence of saponins in AEHV. Since various saponins have been reported to possess antiepileptic activities (Kar, 2003; Kokate, 2003; Pal *et al*, 2005), the anticonvulsant effects of AEHV on mice might be due to the presence of such compounds.

AEHV enhanced sleeping time, analgesic, and anticonvulsant activities and reduced different behavioral reflexes. It can be concluded from the present discussion that the aqueous extract of *H. verticillata* exhibited strong CNS depressant action.

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