CNS ACTIVITY OF AERIAL PARTS OF *HYBANTHUS ENNEASPERMUS* MULL

D.M.Kar*, L.Maharana, S.P.Rout

Department of Pharmacology, School of Pharmaceutical Sciences, Siksha 'O' Anusandhan University, Kalinga Nagar, Ghatikia, Bhubaneswar, Odisha, India. Pin: 751003
E-mail: dmkar1970@yahoo.com, Telephone: +91 9437276570

Summary

Role of ethanolic and aqueous extract of aerial parts of *Hybanthus enneaspermus* in animal models of general behavioral profiles, maze test (plus, Y-maze), barbiturate and alcohol-induced sleeping time, tail suspension test, despair test, head dip test, locomotor activity, motor co-ordination test was studied. *Hybanthus enneaspermus* (HE) extracts were administered to mice at single doses of 250 and 500 mg/kg each of the extract perorally. While diazepam (1mg/kg), chlorpromazine (5 mg/kg), imipramine (30 mg/kg) were used intraperitoneally as standard drug. The result shown that HE extracts at both dose levels significantly exhibited anxiolytic activity. In another study HE extract significantly decreased the sleeping latency and increased the sleeping time. Tail suspension, head dip and despair test showed that HE extracts (250 and 500 mg/kg) was able to induced a significant increase in the immobility time, like that of imipramine, a recognized antidepressant drug. Phytochemical investigation of ethanolic and aqueous extract for presence of flavonoids, tannins, alkaloids,

terpenoids, sugar, sterols and saponins was also carried out and assumed that one or more than one phyto chemical may contribute for the said CNS activity of the extracts. The biological test reports indicate that the alcoholic and aqueous extracts of aerial parts of *H.enneaspermus* (HE) endowed with CNS depressant activity which is more related towards tranquilizer property.

Key words: Maze, Imipramine, Diazepam, Muscle relaxant, Chlorpromazine.

Introduction

Hybanthus enneaspermus Muell (H.enneaspermus), Syn. *Ionidium suffruticosum* Ging and *Ionidium enneaspermus* DC is a rare ethno medicinal ephemeral herb belongs to family Violaceae (1). The plant is well known as Lakshmisheshata, Padmavati, Padmacharini in Sanskrit and popularly known as Ratnapurus in Hindi. The plant is widely distributed in Africa, Madagascar, Srilanka, China, Austalia and India. It grows 15-30 cm in height with many diffuse or ascending branches and is pubescent in nature (2).The plants are seasonal and appear only few months i.e. in rainy season and soon after, the aerial parts dry up and disappear. The roots and few basal stem stocks retain in the soil and regenerate during rainy season. It is an important plant in the Indian system of medicine. It is a small suffrutescent perennial herb found in war part of India, Ceylon, tropical Asia, Africa and Australia.. Traditionally the plant is used as an aphrodisiac, demulcent, tonic, diuretics, in urinary infect diarrhoea, leucorrhoea, dysuria and sterility (3). The plant has been reported to have anti-inflammatory (4), antitussive (5), antispasmodial (6), anticonvulsant (7) and free radical scavenging activity. Various phyto constituents'viz. dipeptide alkaloids, qurantianmide acetate, isoarrborinol, β-sitosterol and triterpene (8-10). In ancient ayurvedic literature, the plant is reported, to cure conchitions of "Kapha" and "Pitta", painful dysentery urinary calculi, strangury, vomiting, burning sensation, wandering of the mind, urethral discharges, asthma, epilepsy, cough and to give tone to breasts. The aim of the study was to evaluate batteries of behavioral/CNS

activities of alcoholic and aqueous extract of *Hybanthus enneaspermus* Mull, using different standard animal experimental methods.

Materials and Methods

Plant material

Fresh plant of *Hybanthus enneaspermus* were collected from the rural belt of Bhubaneswar, Orissa, during June/July-2007. The plant was authenticated in the Department of Botany, Utkal University, Bhubaneswar. The aerial parts of the plant was collected in bulk and washed with tap water to remove adhering soil and dirt particles and then shade dried. A voucher specimen was deposited at the Department of pharmacy, Utkal University, Vani Vihar, Bhubaneswar. The dried plant materials were coarsely powered and stored in airtight, non-toxic polyethylene bags until used.

Chemicals

Petroleum ether, ethanol were purchased from M/s Qualigens, Mumbai and Diazepam, phenobarbitone were generous gift from Ranboxy.

Animals

Swiss albino mice of either sex weighing between 20-35g procured from, Local Breeder was used. After procuring, all animals were acclimatized for 7 days and housed in groups of six under standard husbandry conditions ($26^0 \pm 2^0$ C, 45-55% RH) (11, 12) and 12:12 hr light/dark cycle. All the animals were fed with synthetic standard diet (Provimi Animal Nutrition India Pvt. Ltd., Bangalore) and water provided *ad libitum* under strict hygienic conditions. After obtaining permission from the Institutional Animal Ethics Committee (IAEC) of School of Pharmaceutical Sciences, Siksha 'O' Anusandhan University, Bhubaneswar, animal studies were performed as per rules and regulations and in accordance to the guidelines of CPCSEA. All experiments were carried out during the light period.

Extraction

The coarse powder of aerial parts of *Hybanthus enneaspermus* (200g) was extracted with petroleum ether 800ml

for 72hr. The defatted plant material was again successively extracted with ethanol and water using soxhlet extractor for 72hr. for each extract. The extracts were concentrated to dryness under vacuum. The yield of the ethanol and aqueous extract measured as 4 and 5 gram respectively.

Phytochemical analysis

The ethanolic and water extracts were subjected to qualitative phytochemical analysis for presence of carbohydrates, proteins, amino acids, tannins, phenolic, flavonoids, alkaloids, anthraquinones, glycosides, saponin and steroidal nucleus using the standard methods.

Acute toxicity study (Determination of LD_{50})

The acute toxicity of ALE and AQE of *H.* enneaspermus was determined in albino mice of either sex maintained under standard husbandry conditions. The animals were fasted 3 hr prior to the experiment. Up and down method (OECD guidelines No. 425) of CPCSEA was adopted for toxicity studies. Animals were administered with single dose of extract and observed for mortality during 48 hr study period (short term toxicity). Based on short-term toxicity profile as per the OECD guideline No. 425. The LD_{50} of the two extracts were calculated using AOT 425 software.

General behavioral profile

The method of Dixit, Verma (13) and Mukherjee et al (14) was followed, in this method albino mice were divided into six groups (n=10). The ALE and AQE extract each (250 and 500 mg/kg, i.p., n=10) were administered to groups III, IV, V, VI , where as Groups I and II were treated with normal saline (10ml/kg) and chlorpromazine (5 mg/kg) and respectively in a similar manner. Activity was observed at 30 min. intervals for 1hr and at 1hr intervals for 4hr. The visual observed response was recorded by average score of 10 mice in the shape of the symbol - , +, ++, +++ and ++++, where these symbols represent, no effect, slight depression, moderate depression, strong depression, very strong depression respectively.

Awareness, alertness and spontaneous activity

These responses were tested by placing the animal in a bell jar. It usually shows a moderate degree of inquisitive behavior.

Touch response- This was noted when the mice were touched with a pencil or forceps (i.e. on the side of the neck, abdomen and groin)

Sound response-Albino mice normally utter no sound; vocalization may indicate a noxious stimulus.

Pain response-This was graded when a small artery clamp was attached to the base of the tail.

Elevated plus maze test

The elevated plus maze test was used to evaluate antianxiety activity, as per Itoh et al.(15) Briefly, the apparatus consisted of two open arm (16 x 5 cm^2) and two enclosed arms (16x5 cm^2). The arms extended from a central platform (5x5 cm^2), and maze was elevated to a height of 25 cm from the floor. The test extracts (ALE and AQE) were administered, ip, in varying doses 60 min before the evaluation of antianxiety activity. At the time of experiment, each mouse was placed at the center of maze, facing one of the enclosed arms. During a 3 min test period the time spent on open arm and in the closed arms was recorded.

Y- Maze test

This experiment was performed in groups of 10 rats, 30, 60, 90, and 120 min after injection of physiological saline (10ml/kg), diazepam (1mg/kg) and test extracts (ALE/AQE) each at 250 and 500 mg/kg body weight. The animals were placed individually in a sym-metrical Y-shaped runway (933 ×38×13 cm) for 3 minutes and number of times a rat entered the arm of the maze was counted as per the method described by Rushton et al (16) and Mukherjee et al (14).

Effect on sleeping time

Potentiation of Phenobarbitone Induced Narcosis

The test was performed as per Vogel (17).Healthy albino mice weighing between 20-35gms were fasted for 24 hrs before

the experiment and were divided into five groups of 6 animals each. Group I was maintained as normal control which was given normal saline (10 ml/kg, i.p.), group II and III were treated intra peritoneally with different doses of ALE (250 and 500 mg/kg) and IV and V were treated with AQE (250 and 500 mg/kg) in a similar manner. After 30minutes of administration of test drugs, phenobarbitone sodium at a dose of 40 mg/kg body weight was administered intra-peritoneal to all groups of animals to measure phenobarbitone (PB) induced sleeping time. The PB induced sleeping time was measured as the time interval between the loss and regain of the righting reflex. The sleep latency is the duration of the time between the administration of the PB and loss of righting reflex. The righting reflex was considered to be lost when the animal was placed on its back and failed to regain its normal posture within 10 sec. The treatment of the experimental mice was made 4 hr before the intraperitonial administration of PB and control mice received only PB (table 4). The percentage effect of the test drug on phenobarbitone sodium and alcohol induced sleeping time was calculated by using formula given below, considering righting reflex in control group as 100%.

$$\% \quad Effect = \frac{Averagedurationof\ loss\ of\ righting reflex in\ Test\ Group}{Averagedurationof\ loss\ of\ righting reflex in\ Control\ Group}$$

Potentiation of Alcohol Induced Narcosis

This test was carried out in a similar manner as per the above but in this experiment alcohol (1gm/kg po) was used instead of phenobarbitone sodium. The time of loss and gain of righting reflex and sleep latency, were observed and recorded in all groups of test animals.

Tail Suspension Test

Antidepressant activity was assessed by tail suspension test as per the procedure of Metkar et al (18) In the pre-test session the mice were suspended by their tail using 50cm long thread for a period of 15 minutes. On the next day, in test session, mice were divided into six groups of six animals each. Group-I, II served control and standard drug treated group and administered with saline (10ml/kg) and imipramine (30mg/kg) respectively through i.p route. The animals of Group-III and IV were given ALE (500mg/kg and 250mg/kg respectively), while animals of Group-V and VI were given AQE in a similar manner. The test is

carried out after 24 hrs of pretest session. Half an hour after treatment, in the test session, each mouse was suspended by its tail for 6minutes and the duration of immobility during last five minutes was recorded with various drug treatments.

Despair Test

This test was performed as per the procedure of Metkar et al (18). In pre-test session mice were forced to swim individually for 15 minutes in a glass beaker (12 cm diameter and 25 cm height) with fresh water up to a height of 9cm at a temperature of 22 ± 1^0C: 24hrs after the pretest session, animals were divided into six groups of six animals each. Group-III and Group-IV were administered with ALE extract in dose level of 250mg/kg and 500mg/kg (b.w.) while Group-V and Group-VI were treated with 250mg/kg and 500mg/kg (b.w.) of AQE respectively by ip route. Group-I was treated with Solvent 10ml/kg (b.w.) and Group-II, treated with standard drug, imipramine (30mg/kg) in a similar manner. Half an hour after treatment, in the test session animals were forced to swim individually in similar manner as pretest session for 6 minutes and the duration of immobility during last five minutes was recorded.

Head dip test

Adult albino mice were divided into six groups (n=6). Thirty minutes after intraperitoneal injection of normal saline (10 ml/kg), imipramine(30mg/kg) and test extracts (ALE and AQE) each at both 250, 500 mg/kg b.wt., the mice were placed singly on a wooden board with 16 evenly spaced holes. The treatments were made as per the above manner. The number of times they dipped their heads into the holes in five minutes was counted, Dorr et al (19).

Effect on motor activity

Locomotor activity (open-field test)

Open field test was studied for recording the locomotor activity as per Verley et al (20). The apparatus (1 m x 1 m) was made up of plywood, surrounded by 40 cm high wall with inside surface painted black. The surface of the floor was equally divided into 25 squares. The open field experiment was performed in a sound proof room by placing the square shaped

box in that room. The animals were placed gently in the centre of the apparatus one after another, where they were free to walk and to get adapted to the new environment. After completion of their training individually, the animals were treated with normal saline/ALE (250mg/kg and 500mg/kg)/AQE (250mg/kg and 500mg/kg) by ip route and 30 min later, the animals were placed individually in the apparatus and the number of squares traveled in five min was considered as locomotor score. The floor of the box was cleaned after every trial.

Motor coordination test by grip-strength

Muscle relaxant activity

Muscle relaxant activity was determined using the traction test, the rotarod test and the 30^0 inclined screen test.

Traction test

This test was conducted as per Rudzik et al (21) in which, in groups of ten animals each, 30 min after injection of either normal saline (10ml/kg), diazepam (1mg/kg) or ALE/AQE each at 250 and 500 mg/kg b.wt. The forepaws were placed on a small twisted wire rigidly supported above with a bench top. Untreated mice grasped the wire with forepaws and placed at least one hind foot on the wire within 5 second. Inability to put at least one hind foot constituted failure in the traction test.

Rotarod test

Untreated mice were placed on a horizontal wooden rod (32 mm diameter) rotating at a speed of 5 revolutions per minute. Only mice remaining on the rod for 3 min or more, in 3 successive trials were selected for the study. The mice were divided into six groups (n=6).The treatment was made in a similar manner as per the Traction test Each group was placed on the rod at intervals of 30, 60, 90, 120 and 150 min. Mice failing more than once to remain on the rotating rod for 3 min constituted a positive result as per the method of Dunham and Miya (22).

30^0 Inclined screen test

Adult albino mice were divided into six groups (n=10). The Group I, II served as control group and were treated with saline (10ml/kg) and diazepam (1mg/kg) respectively. Group III,

IV, V, VI was treated with test extracts as per the above experiment. They were left on the screen for at least 4 hr to observe if the paralysant effect was great enough to cause the mice to slide off the screen as per the method described by Randall (23).

Statistical analysis

Results are expressed as the mean±SEM. Significance was evaluated using Student's *t*-test in all the experiments. The chi-square test was used to assess muscle relaxant activity (Woodson 1987). $P<0.05$ was considered significant.

Results

Phytochemical analysis

The qualitative phytochemical analysis of the extracts of *H. enneaspermus,* revealed the presence of alkaloids, sterols, tannins, flavonoids, isoflavones, terpenoids and saponin. However the extracts are devoid of carbohydrates, anthraquinons, cardiac aglycone, digitoxose.

Acute toxicity study

Acute toxicity of ALE and AQE extracts were determined in mice, as per OECD guidelines No. 425. Both the extracts did not produce any mortality even in greater than 3000 mg/kg dose level.

Effect of extracts on general behavioral profiles

Chlorpromazine (5mg/kg), ALE and AQE of aerial parts of *H.enneaspermus* at different dose levels, affected spontaneous activity, sound and touch responses. The AQE at 500 mg/kg produced moderate or slight depression relating to awareness and alertness. The standard drug chlorpromazine hydrochloride and test extracts produced a marked depression when compared with the solvent control group and in a dose dependent manner (table 1).

Table 1: Effect of extracts of *H. enneaspermus* on general behavioral profiles in mice

Group No	Treatment	Dose	Spontaneous activity	Alertness	Awareness	Sound response	Touch response	Pain response
I	Saline	10 (ml/kg)	-	-	-	-	-	-
II	Chlorpromazine	5 (mg/kg)	++++	++	+	+++	+++	++++
III	ALE	250 (mg/kg)	+	++	+	++	++	++
IV	ALE	500 (mg/kg)	+++	++	++	+++	+++	+++
V	AQE	250 (mg/kg)	+++	++	++	+++	++++	++++
VI	AQE	500 (mg/kg)	++++	+++	++	++++	++++	++++

- No effect, + Slight depression, ++ moderate depression, +++ Strong depression, ++++ Very strong depression, n= 10

Elevated plus maze test

The HE extracts (alcoholic and aqueous) each at 250 and 500 mg/kg, and diazepam (1 mg/kg) ip, induced significant ($p<0.001$) increase in the occupancy in the open arms. The HE extracts and diazepam showed a decreased performance for the closed arms. However, the HE extract was found to have more effective in higher dose level in both ALE and AQE extract (table 2)

Y-maze test

In Y-maze test mice treated with ALE and AQE at tested dose levels showed a significant decrease in exploratory behavior compared with control at all tested time intervals (table 3)

Effect of extract on phenobarbitone and alcohol-induced sleeping time

The test extract except ALE 250 mg/kg, significantly potentiate the phenobarbitone sodium-induced sleeping time ($p< 0.01$) while compared with control. The duration of sleeping time in both alcohol and phenobarbitone induced experimental models increases in a dose dependent manner. The sleep latency on the other hand found to decrease in a similar sequence as that of increase in sleeping time. The percentage effect ranges from 111 to 176 in case of alcohol induced narcosis by keeping solvent control group as 100 percent, while phenobarbitone induced narcosis; the test extract registered 101 to 123 percentage effect in sleeping time (table 4)

Head dip test

The head dip test revealed that ALE and AQE at 500 mg/kg dose level registered 31 and 25 number of head dips on a wooden board with 16 evenly spaced holes within 3 minutes, while standard drug imipramine showed 22 numbers of head dips. The results of the head dip test also showed that the number of head dips decreases in a dose dependent manner while comparing between the tested dose levels of 250 and 500 mg/kg (table 5).

Effect on tail suspension test

The tail suspension test represents the duration of immobility, and found that the duration increases forwardly from ALE to AQE possessing the immobility period of 148 and 167

sec. respectively in 500 mg/kg dose level. The standard drug imipramine at the same time registered 174 seconds of immobility (table 5).

Despair test

In this test report, the duration of immobility is measured and found that both ALE and AQE registered 53 sec. to 99sec. in a dose dependent manner and in a increasing sequence of ALE followed by AQE at the significance rate of $p<0.01$ when compared with saline control (table 5).

Effect on motor activity

Locomotor activity

The ALE and AQE of aerial parts of HE inhibited the locomotor activity of mice in a dose dependent manner. Locomotor activity was decreased significantly at both dose levels in both extracts, except ALE at 250 mg/kg (table 6).

Muscle coordination test by grip strength

Traction test

The traction test performed in mice demonstrates that, mice treated with ALE and AQE showed a significant loss in traction at all doses tested. The AQE measured comparatively more effect than ALE when compared quantitatively (table 7)

Rotarod test

In rotarod test both the test extract at all tested dose levels produced a significant loss in motor co-ordination. The extent of potency registered in a similar extent as like that of traction test report (Table 7)

30^0 Inclined screen test

The 30^0 inclined screen test also showed a significant loss in co-ordination and muscle tone in a similar manner as above. The loss of muscle co-ordination is more in case of AQE when compared with ALE (table 7)

Table 2: Effect of *H.enneaspermus* extract on elevated plus maze test in mice

Group	Treatment	Dose	Time spent in open arms (sec)	Time spent in closed arms (sec)
I	Saline	10 (ml/kg)	24.12±3.21	176.43±4.78
II	Diazepam	1 (mg/kg)	78.37±3.21***	104.62±5.33***
III	ALE	250 (mg/kg)	38.83±3.32**	149.66±5.37**
IV	ALE	500 (mg/kg)	41.33±3.21***	127.61±3.81***
V	AQE	250 (mg/kg)	62.46±4.31***	136.39±2.73***
VI	AQE	500 (mg/kg)	68.73±3.89***	109.57±4.56***

Values expressed as Mean ± SEM, from six observations, *p* values: *<0.05, **<0.01, ***<0.001, when compared to saline control group.

Table 3: Effect of *H.enneaspermous* extracts on exploratory behavioral Potential (Y-Maze test) in rats

Group No.	Treatment	Dose	Number of Entries			
			30 mins	60 mins	90 mins	120 mins
I	Saline	10 (ml/kg)	8.6±0.28	8.5±0.37	8.8±0.54	8.7±0.56
II	Diazepam	1 (mg/kg)	2.9±0.38***	3.1±0.34***	3.3±0.44***	3.3±0.61***
III	ALE	250 (mg/kg)	6.5±0.51**	6.7±0.54*	6.8±0.64*	7.0±0.64**
IV	ALE	500 (mg/kg)	5.4±0.52***	5.5±0.38***	5.6±0.12***	5.8±0.32***
V	AQE	250 (mg/kg)	3.8±0.25***	3.9±0.31***	4.3±0.41***	4.8±0.44***
VI	AQE	500 (mg/kg)	3.4±0.38***	3.5±0.47***	3.7±0.33***	4.1±0.41***

Value are mean ± SEM from 6 animals in each group, *p* values: *<0.05, **<0.01, ***<0.001, when compared to saline control group

Table 4: Effect of *H. enneaspermus* extracts on Phenobarbitone sodium and alcohol induced narcosis in mice

Group No.	Treatment	Dose	Sleep latency (sec)		Sleeping time (min)	
			Phenobarbitone induced	Alcohol induced	Phenobarbitone induced	Alcohol induced
I	Saline	10 (ml/kg)	252±2.31	187±1.89	36.83±0.79	94.83±0.87
II	ALE	250 (mg/kg)	227±1.87***	128±2.61***	41.16±0.47***	96.33±1.14
III	ALE	500 (mg/kg)	173±2.43***	99±2.59***	61.33±1.53***	102.33±1.36***
IV	AQE	250 (mg/kg)	142±1.78***	119±3.22***	42.46±0.71***	97.66±1.40
V	AQE	500 (mg/kg)	93±2.16***	91±2.46***	64.83±1.43***	117.33±1.58***

Values are Mean ± SEM from 6 animals in each group, *p* values: ***<0.001, when compared to saline control group

Table 5: Effect of *H. enneaspermus* extracts on exploratory behavior (head dips, tail suspension, despair test) in mice

Group No.	Treatment	Dose	No. of Head dips	Duration of immobility	
				Tail suspension test	Despair test
I	Saline	10 (ml/kg)	66.5±1.40	109.76±0.43	50.5±0.25
II	Imipramine	30 (mg/kg)	22.16±0.87***	174.32±0.64***	112.26±0.96***
III	ALE	250 (mg/kg)	54.66±0.78***	137.57±0.29***	53.41±0.31***
IV	ALE	500 (mg/kg)	31.5±0.99**	148.01±0.28**	73.33±0.37***
V	AQE	250 (mg/kg)	48.5±0.67**	139.35±0.51**	65.40±0.41***
VI	AQE	500 (mg/kg)	25.16±0.48**	167.24±0.19**	99.81±0.31***

Values are Mean ± SEM from 6 animals in each group, *p* values: ** <0.01, ***<0.001, when compared to saline control group

Table 6: Effect of the extract (ALE and AQE) on locomotor activity

Group	Treatment	Dose	Locomotor activity (No. of squares crossed in 3 min)
I	Saline	10 (ml/kg)	39.41 ± 2.17
II	ALE	250 (mg/kg)	31.72 ± 3.54
III	ALE	500 (mg/kg)	22.33 ± 2.26**
IV	AQE	250 (mg/kg)	18.21 ± 1.35***
V	AQE	500 (mg/kg)	13.12 ± 2.11***

Values expressed as Mean ± SEM, from six observations, *p* values: *<0.05, **<0.01, ***<0.001

Table 7: Percentage effect of *H.enneaspermus* extracts on Muscle relaxant activity in mice.

[Values are percentage animals showing a negative result]

Group No.	Treatment	Dose	Traction test	30^0 Inclined Screen test	Rota rod test
I	Saline	10 (mg/kg)	0	0	0
II	Diazepam	1 (mg/kg)	100	100	100
III	ALE	250 (mg/kg)	59*	44*	84*
IV	ALE	500 (mg/kg)	66*	52*	72*
V	AQE	250 (mg/kg)	64*	63*	65*
VI	AQE	500 (mg/kg)	69*	74*	61*

Significance at * $p<0.05$, compared with control (chi-square)

Discussion

The present study was attempted to delineate CNS activity of ALE and AQE of aerial parts of *Hybanthus enneaspermus*, in several behaviour animal models such as general behavioral study, antianxiety (maze study), barbiturate and alcohol induced sleeping time, motor activity test (locomotor), motor coordination/grip strength activity (30^0 inclined screen test, traction test), antidepressant activity (head dip test, tail suspension test, despair test) in albino mice. These tests are classical models for screening central nervous system (CNS) actions providing information about anxiolytic, psychomotor performances, myorelaxant activity, and anti-depressant.

The results in the present study indicate that, the ALE and AQE influences general behavioral profiles, as evidenced in spontaneous activity, touch, sound and pain responses ranging from slight- moderate to strong to very strong depression. Although rota-rod test results may indicate both central nervous system (CNS) activity and peripheral nervous system (PNS) activity but locomotor activity; and phenobarbitone and alcohol induced sleeping time both are important parameters for determination of CNS activity. Rotarod, traction, 30^0 inclined screen results showed a reduction of grip strength when compared to control mice. Maintenance of grip strength on the rotarod/ inclined screen/twisting string without falling was significantly decreased in extracts treated mice as compared to control. Loss of grip strength may be due to motor incoordination which was influenced by the *H. enneaspermus*. The alcohol and aqueous extract of *H.enneaspermus* inhibited the locomotor activity of mice in a dose dependent manner whereas the extract decreased the latency period of sleep and increased phenobarbitone and alcohol induced sleeping time in the same manner. Locomotor activity of mice decreased significantly in comparison to control values in a dose dependent manner. In case of sleep latency period, the test extracts showed significant decrease in sleep latency, while the test extract increased PB and alcohol induced sleeping time in a significant extent with gradual increase of dose. According to Beninger (24) CNS depressants act via reduction of locomotion and rearing. Reduction of locomotor activity may be a sign of CNS depression as it was evidenced by Ray *et al* (25).

The tail suspension test is considered one of the most widely validated test for assaying new antidepressant agents.

The test extracts of ALE and AQE each at 250 and 500 mg/kg, significantly potentate the phenobarbitone and alcohol-induced sleeping time in a dose dependent manner, possibly through CNS depressant action or tranquilizing action (26, 14). Alcohol in ordinary dose may act primarily on the arousal mechanisms of brain stem reticular formation, inhibitory polysynaptic function and enhancing presynaptic inhibition (27), however barbiturates act throughout the CNS, the site of inhibition is either postsynaptic as at cortical and cerebellar pyramidal cells and in the cuneate nucleus, substantia nigra and thalamic relay neurons or presynaptic as in spinal cord (27). As the test extract registered more duration of sleep in phenobarbitone sodium induced narcosis when compared with alcohol induced narcosis model, therefore it may be suggested that the test extract have potential effect at cortical region or any other region which are affected by phenobarbitone sodium action. The reduction in exploratory behavioral study pertaining to head dip, tail suspension and despair tests revealed the CNS activity of the test extracts. The possible CNS activity of the ethanolic and aqueous extract was further tested against other common psychological tests (i.e. the rotarod test, 30^0 inclined screen test and traction test). A significant lack in motor coordination and muscle relaxant activity were noted in animals treated with the test extract. The results of the present studies reveal that, the ALE and AQE may have CNS depressant activity like major tranquilizers/ psychopharmacological agents. The drug(s) showing positive response in gross behavioral parameters, muscle in-coordination, exploratory behavior, and maze study must have effect on the region of brain (28, 29). It may be suggested that the extracts have effect on the region of brain, which is responsible for the behavioral and other tested parameters. The flavonoid might be the responsible phyto-principle present in the plant extract for the above activity.

Conclusion

The batteries of studies conducted in the work concluded that *Hybanthus enneaspermus* has potent CNS activity towards

depression and tranquilizing property. The said CNS activities demonstrated by the extracts may be due to presence of flavonoids. Further investigations of the mechanism (s) of action of the plant extract, and the active substance(s) responsible for its biological action are necessary.

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