PHARMACOLOGICAL EVALUATION OF *Enhydra fluctuans* AERIAL PARTS FOR CENTRAL NERVOUS SYSTEM DEPRESSANT ACTIVITY

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Summary

In the present study, some neuropharmacological effects of three fractions (Benzene, Chloroform and Ethyl Acetate) of methanolic extract of *Enhydra fluctuans* were studied in mice using various models. The effect of the three fractions (100mg/kg,b.w, i.p) of *Enhydra fluctuans* on central and peripheral nervous system were studied by using spontaneous motor activity, sedative activity, anticonvulsant property, anti-stress activity by tail suspension test (TST) and forced swimming test (FST). Preliminary phytochemical evaluation of extract was also carried out. The methanolic extract of *Enhydra fluctuans* was found to be safe upto a dose of 2000 mg/kg body weight, i. p. on Swiss Albino mice. Only benzene, chloroform and ethyl acetate fractions produced CNS depressant activity. Preliminary phytochemical analysis of the extract revealed the presence of alkaloids, saponins, flavonoids, triterpenoids/steroids, tannins, carbohydrates and glycosides. The results of the present study indicated significant spontaneous motility depressant, sedative, anticonvulsant and anti-stress activity of the different fractions in the tested animal models. Therefore, from the present study it may be concluded that different fractions of *Enhydra fluctuans* aerial parts possess central nervous system depressant activity.

Keywords: *Enhydra fluctuans,* central nervous system, anti-stress, Pentobarbital sodium, Pentylene tetrazole.

Introduction

Enhydra fluctuans Lour var fluctuans (Family: *Asteraceae*) is commonly called Water Cress (English), Helancha (Bengali) and Harkuch (Assamese). Its leaves are used in the treatment of skin diseases, nervous affection and also useful to cure inflammation, leucoderma, bronchitis and biliousness [1, 2].

No major investigative reports were found pertaining to its CNS activity; therefore, the present study was undertaken to determine the neuropharmacological potential of *Enhydra fluctuans* by using mice as the animal model.

Materials and Methods

Collection and extract preparation

The aerial parts of *Enhydra fluctuans* Lour were collected from the markets of Kolkata, West Bengal, India in the month of January, 2010. The aerial parts of *Enhydra fluctuans* were washed thoroughly and shade-dried, cut into small pieces and then pulverized into a coarse powder using dry mixer grinder. Powdered plant material (700 gm) was soxhlet extracted with petroleum ether (60-80^{o}C) followed by methanol. The petroleum ether extract and methanol extract were distilled, and evaporated to dryness under vacuum (yield: 2.14% w/w and 14% w/w respectively) on dry wt. basis. The methanolic extract was suspended in water and successively fractionated using the solvents in order of increasing polarity viz., benzene, chloroform and ethyl acetate. All the fractions were concentrated by distilling the solvent and evaporated to dryness.

Preliminary phytochemical analysis

The individual fractions like petroleum ether, methanol, benzene, chloroform and ethyl acetate were subjected to qualitative chemical investigation for the identification of different phytoconstituents like alkaloids, tannins, flavonoids, saponins, carbohydrates, steroids, triterpenoids and anthraquinone glycosides. Phytochemical screening of the extracts was performed using the standard procedures [3, 4].

Acute toxicity test

Acute toxicity was performed in Swiss Albino mice as per Up and Down method. Overnight fasted healthy albino mice of 2 per group and weighing 20-25 g were administered increased dose (500, 1000 and 2000 mg/kg body wt. i. p.) of the methanolic extract of *Enhydra fluctuans*. After administration of MEEF, the mice were observed for toxic effects for 72 hours of treatment. The number of animals that died during this period was noted.

Animals

Swiss Albino mice *Mus musculus* (18-25 g) of either sex were used in these experiments. Animals were provided with standard food and water *ad libitum* and were maintained at a temperature of 25±2°C, humidity of 55±5% and with 12 h light - dark cycle. All animal procedures have been approved and prior permission from the Institutional Animal Ethical Committee was obtained as per the prescribed guidelines.

The animals were divided into five groups, each containing five mice. The groups of mice were assigned to receive one of the following:- (i) normal control (vehicle: 10%v/v DMSO), (ii) standard drug, (iii) Benzene fraction (100 mg/kg, i.p), (iv) Chloroform fraction (100mg/kg, i.p.) and (v) Ethyl acetate fraction (100mg/kg, i.p); this group pattern was used to assess the neuropharmacological activity.

Neuropharmacological Tests

Spontaneous motility using Photoactometer

The CNS depressant activity of the various fractions of *Enhydra fluctuans* were evaluated by studying spontaneous motility of mice using photoactometer [5]. The mice were placed in groups inside the chamber of photoactometer for 3 minutes and basal activity score was noted before administration of any drugs. The animals were treated as per their treatment design. Standard drug was diazepem (2.5 mg/kg, i.p.). After 30 minutes mice were again placed in photoactometer for 3 minutes and the activity was monitored. This was repeated after 60, 90, 120, 150 and 180 minutes respectively from the time of injection for 3 minutes.

Pentobarbital sodium induced sleeping time

CNS depressant activity was performed as described by Sivaraman and Muralidaran [6]. In this method, mice of either sex were randomly taken and divided into normal control, standard and different test groups, each group containing five animals. The animals were treated as per their treatment design followed by Pentobarbital sodium (40mg/kg, i. p.) 30 minutes later. Standard drug was diazepem (1 mg/kg, i.p.). The duration of sleep was measured for all the groups. The duration of sleep was recorded by time difference between the loss of righting reflex and recovery time.

Anti-convulsant activity using Pentylene tetrazole induced convulsions

The anti-convulsant activities of the fractions were observed against Pentylene tetrazole (75 mg/kg). The duration of tremor (in seconds) and the percentage mortality of the mice were observed after 24 hours. One episode of clonic spasm which persisted for atleast five seconds was considered as one threshold convulsion. Mice of either sex were randomly taken and divided into normal control, standard and different test groups, each group containing five animals. The animals were treated as per their treatment design followed by Pentylene tetrazole (75 mg/kg, i. p.) 30 minutes later. Standard drug was diazepam (2.5 mg/kg, i. p.).

Anti-stress activity using Tail-suspension test (TST)

Tail suspension test is a commonly employed behavioral model for screening antidepressant-like activity in mice [7]. Animals were moved from their housing colony to laboratory in their own cages and allowed to adapt to the laboratory conditions for 1-2 hrs. Briefly, each mouse was individually suspended to the edge of a table, 50cm above the floor, by adhesive tape placed approximately 1cm from the tip of the tail. The total period of mobility was recorded manually during 6 min of testing period. Animals were considered to be immobile when it did not show any body movement, hung passively and completely motionless. Mice of either sex were randomly taken and divided into

normal control, standard and different test groups, each group containing five animals. The animals were pre-treated for 7 days as per their treatment design and the experiment was conducted on the 8th day. Standard drug was imipramine (15 mg/kg, p. o.).

Anti-stress activity using Forced Swim test (FST)

Forced swim test is one of the most frequently used behavioral models for screening antidepressant-like activity in rodents [8]. Animals were moved from their housing colony to laboratory in their own cages and allowed to adapt to the laboratory conditions for 1-2 hours. Briefly, mice were individually forced to swim in open glass chamber (25x15x25 cm^3) containing fresh water to a height of 15 cm and maintained at 26±1°C. At this height of water, animals were not able to support themselves by touching the bottom or the sidewalls of the chambers with their hind-paws or tail. The duration of immobility was manually recorded during the 15 minutes of testing period. Mice were considered to be immobile when they ceased struggling and remained floating motionless in water, making only those movements necessary to keep their head above water. Mice of either sex were randomly taken and divided into normal control, standard and different test groups, each group containing five animals. The animals were pre-treated for 7 days as per their treatment design and the experiment was conducted on the 8th day. Standard drug was imipramine (15 mg/kg, p. o.).

Statistical analysis

The results have been expressed as mean ± standard error mean (S.E.M) and analyzed using GraphPad Prism version 4.00 using ANOVA followed by Dunnett's test.

Results

Preliminary phytochemical analysis

Primarily methanolic extract showed the presence of alkaloids, tannins, triterpenoids, saponins, flavonoids and carbohydrates. Petroleum ether extract contains triterpenoids, flavonoids and carbohydrates. Benzene fraction contains tannins; chloroform fraction contains triterpenes and ethyl acetate fraction contains flavonoids, mainly.

Acute toxicity studies

Intra peritoneal administration of methanolic extract of *Enhydra fluctuans* in mice, at doses from 500-2000 mg/kg produced significant changes in behaviour showing decreased motor activity and calmness. During the experimental period (at dose from 500-2000 mg/kg), no deaths occurred. Finally, the results indicate that the methanolic extract of *Enhydra fluctuans* is safe up to a dose of 2000 mg/kg in i. p. route. This was confirmed by the treatment of 2000 mg/kg in 4 mice and it was found that none of them died within 72 hours. Hence the drug was considered safe for further pharmacological screening. So, according to the Up and Down method for acute oral toxicity, the LD_{50} dose of 2000 mg/kg and above is categorized as unclassified.

Spontaneous motility by photoactometer
Results of spontaneous motility were tabulated in Table 1. The extracts of *Enhydra fluctuans* aerial parts decreased the spontaneous motility in mice. The activity was found to be decreased for ethyl acetate fraction and Diazepam. All the fractions i.e. benzene, chloroform and ethyl acetate and standard drug Diazepam produced significant reduction in spontaneous motility as compared to normal control group.

Pentobarbitone sodium induced sleeping time
The results showed that *Enhydra fluctuans* aerial parts possess CNS depressant activity. Table 2 shows the effect of different extracts of *Enhydra fluctuans* aerial parts. Benzene fraction (100 mg/kg), chloroform fraction (100 mg/kg) and ethyl acetate fraction (100 mg/kg) produced significant prolongation of sleeping time induced by pentobarbitone sodium as compared to control group. Ethyl acetate fraction (100 mg/kg) produced the most potent effect followed by benzene, diazepam and chloroform fraction. All the fractions i.e. benzene, chloroform and ethyl acetate and standard drug Diazepam produced significant potentiation of sleeping time as compared to normal group.

Anti-convulsant activity using Pentylene tetrazole induced convulsions
The results showed that *Enhydra fluctuans* aerial parts possess anti-convulsant activity. Table 3 shows the effect of different extracts of *Enhydra fluctuans* aerial parts. Benzene fraction (100 mg/kg), chloroform fraction (100 mg/kg) and ethyl acetate fraction (100 mg/kg) produced decrease in the duration of tremors induced by pentylene tetrazole. However, none of the fractions produced significant activity compared to the control group except the standard drug Diazepam. Also the extracts decreased the percentage mortality compared to the control group. Percentage mortality was 0% for the standard drug diazepam treated mice, followed by 20% in mice treated with ethyl acetate fraction.

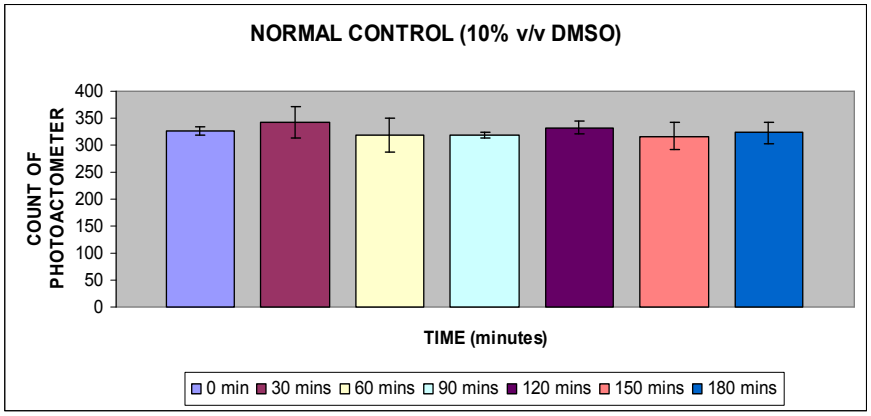
Anti-stress activity using Tail-suspension test (TST)
Table 4 shows the effect of different extracts of *Enhydra fluctuans* aerial parts. Ethyl acetate fraction (100 mg/kg) produced significant decrease in the movements in mice while benzene fraction (100 mg/kg) and chloroform fraction (100 mg/kg) produced significant increase in the movements in mice induced by tail suspension. Thus, the ethyl acetate fraction possesses significant anti-stress activity.

Anti-stress activity using Forced Swim test (FST)
Table 5 shows the effect of different extracts of *Enhydra fluctuans* aerial parts. Ethyl acetate fraction (100 mg/kg) and chloroform fraction (100 mg/kg) produced decrease in the movements in mice while benzene fraction (100 mg/kg) produced increase in the movements in mice induced by forced swim test. The effect produced by ethyl acetate fraction was found to be significant as compared to the control group.
The results of the present study indicated significant spontaneous motility depressant, sedative, anticonvulsant and anti-stress activity of the different fractions of *Enhydra fluctuans* in the tested animal models.

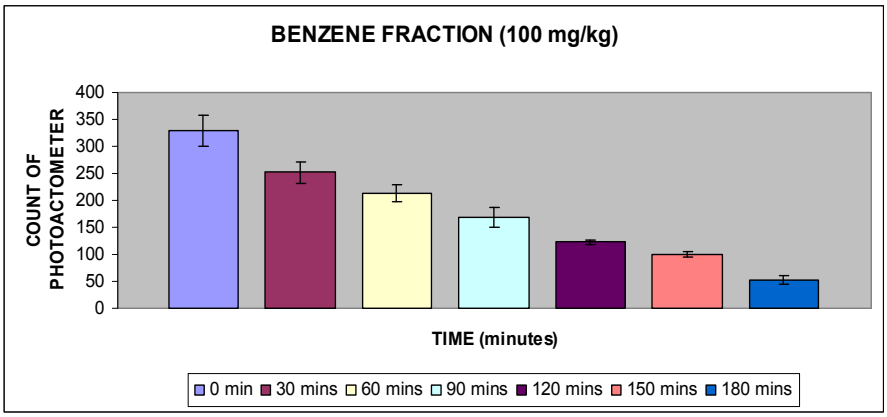
Table 1: Effect of *Enhydra fluctuans* aerial parts extracts on spontaneous motility in mice, accessed using photoactometer.

Groups	Measurement of Photoactometer during 3 minutes						
	0 mins	30 mins	60 mins	90 mins	120 mins	150 mins	180 mins
Normal Control	326.0±7.0	342.0±30.0	319.0±31.0	318.0±6.0	332.5±11.5	316.5±24.5	323.0±19.5
Benzene fraction	329.0±28.0	251.5±19.5**	213.5±16.5**	169.0±19.0**	122.5±3.5**	99.5±4.5**	53.0±7.0**
Chloroform fraction	374.0±23.0	136.0±5.0**	107.0±8.0**	102.0±1.0**	79.0±3.0**	55.5±6.5**	38.5±2.5**
Ethyl acetate fraction	346.0±8.0	71.5±2.5**	51.0±6.0**	48.0±2.0**	38.5±2.5**	31.5±1.5**	23.5±3.5**
Diazepam (2.5 mg/kg, i.p.)	350.0±15.5	60.5±9.0**	40.0±8.5**	30.5±10.0**	26.0±6.5**	20.0±11.5**	17.0±8.0**

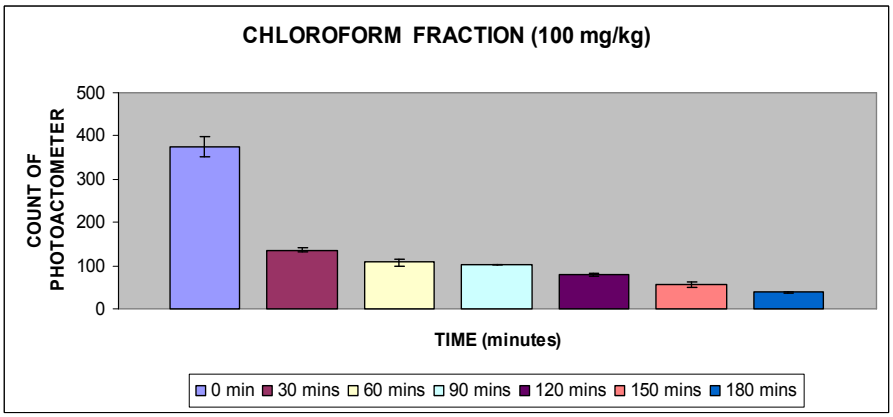
All values are mean ± SEM (n=5); Statistical analysis by one-way ANOVA followed by Dunnet's multiple comparison test; *$P < 0.05$, **$P < 0.01$; Experimental groups compared with Normal Control group.



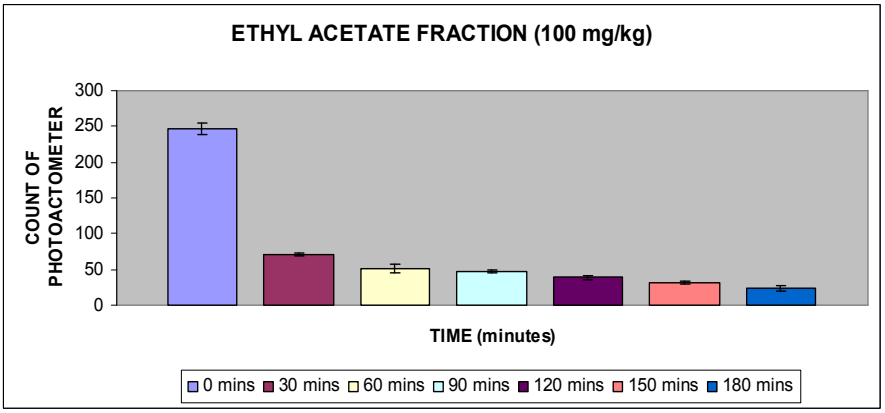
(a) Normal Control (10% v/v DMSO)



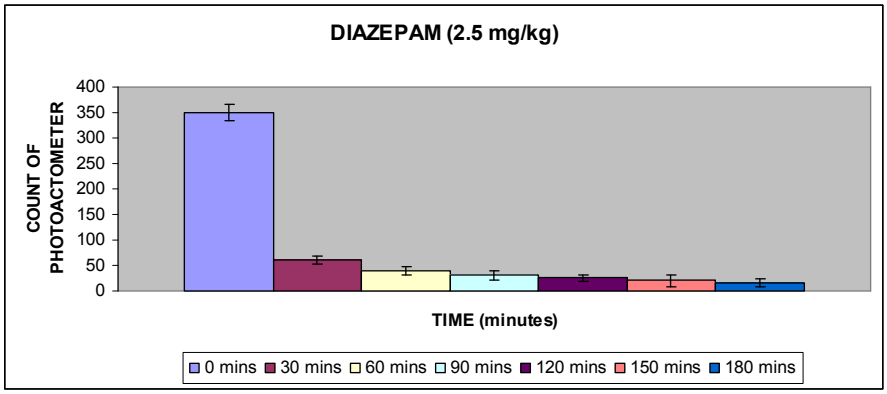
(b) Benzene fraction



(c) Chloroform fraction



(d) Ethyl acetate fraction



(e) Diazepam (Standard)

Figure 1:- Spontaneous motility by different extracts of *Enhydra fluctuans* aerial parts.

Table 2: Effect of *Enhydra fluctuans* aerial parts extracts on pentobarbitone sodium induced sleeping time.

Groups	SLEEPING TIME (minutes)
Normal Control (10% v/v DMSO, i.p.) + Pentobarbital sodium (40mg/kg, i. p.)	**38.8 ± 1.1**
Diazepam (1 mg/kg, i. p.) + Pentobarbital sodium (40mg/kg, i. p.)	**71.0 ± 2.4****
Benzene fraction (100 mg/kg, i.p.) + Pentobarbital sodium (40mg/kg, i. p.)	**73.8 ± 1.7****
Chloroform fraction (100 mg/kg, i.p.) + Pentobarbital sodium (40mg/kg, i. p.)	**52.4 ± 2.9****
Ethyl Acetate fraction (100 mg/kg, i.p.) + Pentobarbital sodium (40mg/kg, i. p.)	**84.8 ± 2.8****

All values are mean ± SEM (n=5); Statistical analysis by one-way ANOVA followed by Dunnet's multiple comparison test; *$P < 0.05$, **$P < 0.01$; Experimental groups compared with Normal Control group.

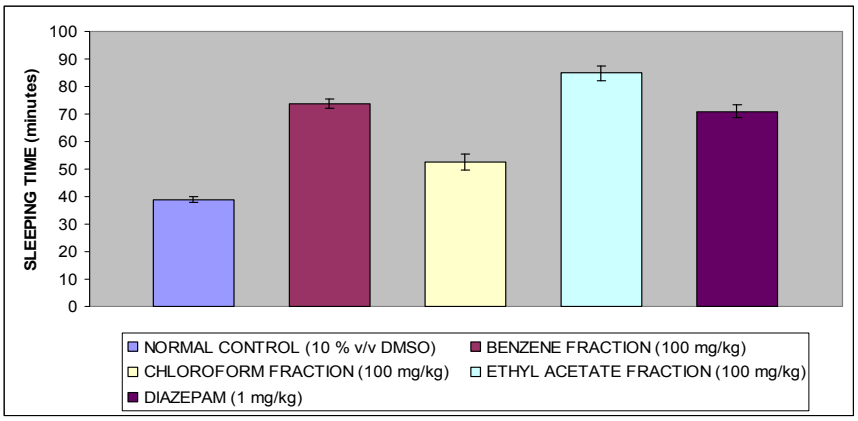


Figure 2:- Pentobarbital Na induced sleeping time of different extracts of *Enhydra fluctuans* aerial parts.

Table 3: Effect of *Enhydra fluctuans* extracts on Pentylene tetrazole (PTZ) induced convulsions in mice.

Groups	DURATION OF TREMORS (seconds)	NO. OF DEATHS
Normal Control (10% v/v DMSO, i.p.) + Pentylene tetrazole (75 mg/kg, i. p.)	**231.0 ± 14.3**	**5 / 5**
Diazepam (2.5 mg/kg, i.p.) + Pentylene tetrazole (75 mg/kg, i. p.)	**144.0 ± 14.7****	**0 / 5**
Benzene fraction (100 mg/kg, i.p.) + Pentylene tetrazole (75 mg/kg, i. p.)	**191.2 ± 14.6**	**4 / 5**
Chloroform fraction (100 mg/kg, i.p.) + Pentylene tetrazole (75 mg/kg, i. p.)	**213.2 ± 26.3**	**3 / 5**
Ethyl Acetate fraction (100 mg/kg, i.p.) + Pentylene tetrazole (75 mg/kg, i. p.)	**177.6 ± 21.2**	**2 / 5**

All values are mean ± SEM (n=5); Statistical analysis by one-way ANOVA followed by Dunnet's multiple comparison test; *$P < 0.05$, **$P < 0.01$; Experimental groups compared with Normal Control group.

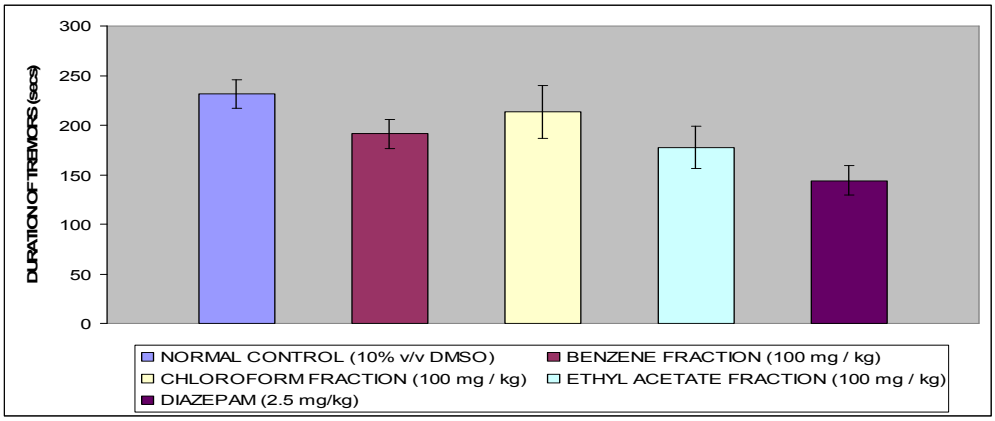


Figure 3:- Duration of tremors by different extracts of *Enhydra fluctuans* aerial parts in Pentylene tetrazole induced convulsions in mice.

Table 4: Effect of *Enhydra fluctuans* aerial parts extracts on tail suspension induced stress in mice.

Groups	DURATION OF MOVEMENT DURING 6 MINUTES (seconds)
Normal Control (10% v/v DMSO, i.p.)	**166.6 ± 3.4**
Benzene fraction (100 mg/kg, i.p.)	**247.2 ± 8.2****
Chloroform fraction (100 mg/kg, i.p.)	**224.8 ± 13.0****
Ethyl Acetate fraction (100 mg/kg, i.p.)	**89.2 ± 10.0****
Imipramine (15 mg/kg, p.o.)	**181.4 ± 4.4**

All values are mean ± SEM (n=5); Statistical analysis by one-way ANOVA followed by Dunnet's multiple comparison test; *$P < 0.05$, **$P < 0.01$; Experimental groups compared with Normal Control group.

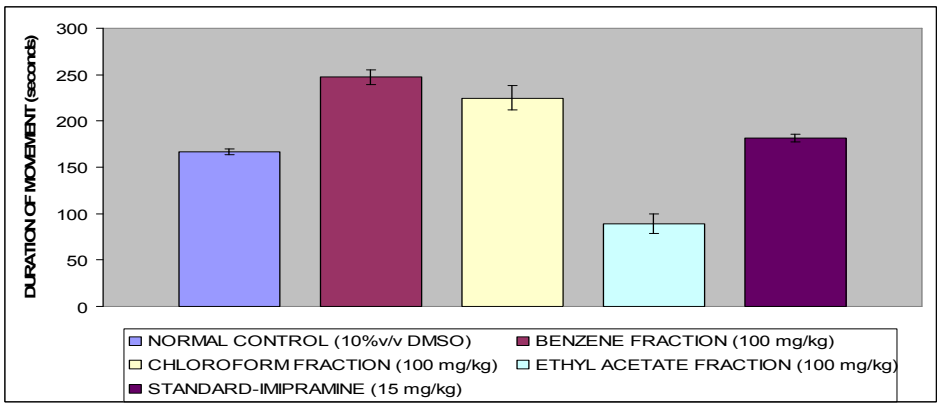


Figure 4:- Duration of movements by different extracts of *Enhydra fluctuans* aerial parts in Tail suspension induced stress in mice.

Table 5: Effect of Enhydra *fluctuans* aerial parts extracts on forced swim induced stress in mice.

Groups	DURATION OF IMMOBILITY DURING 15 MINUTES (seconds)
Normal Control (10% v/v DMSO, i.p.)	**336.2 ± 5.9**
Benzene fraction (100 mg/kg, i.p.)	**318.6 ± 8.0**
Chloroform fraction (100 mg/kg, i.p.)	**357.6 ± 8.4**
Ethyl Acetate fraction (100 mg/kg, i.p.)	**385 ± 4.7****
Imipramine (15 mg/kg, p.o.)	**308.6 ± 3.4***

All values are mean ± SEM (n=5); Statistical analysis by one-way ANOVA followed by Dunnet's multiple comparison test; *P < 0.05, **P < 0.01; Experimental groups compared with Normal Control group.

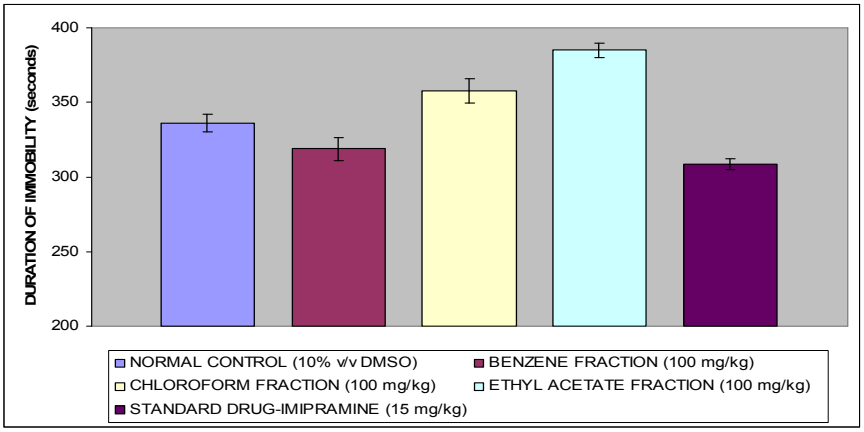


Figure 5:- Duration of movements by different extracts of *Enhydra fluctuans* aerial parts in Forced swimming induced stress in mice.

Discussion and conclusion

The study has established the central nervous system depressant properties of *Enhydra fluctuans* aerial parts. Locomotor activity is considered as an increase in alertness and a decrease in locomotor activity indicates sedative effect [9]. As extracts of *Enhydra fluctuans* aerial parts decreased locomotor activity, this indicates its CNS depressant activity. The study demonstrated that different extracts of *Enhydra fluctuans* aerial parts increased the duration of action of pentobarbitone induced sleeping time significantly (P<0.01).

The study also shows that the extracts decrease the duration of tremors induced by pentylene tetrazole. As pentylene tetrazole causes seizures which increase CNS activity, decrease in the duration of tremors indicates CNS depressant activity.

As tail suspension and forced swimming increases movements as well as stress in mice, decrease in movements in mice indicates possible anti-stress activity.

The neuropharmacological investigations on the fractions of *Enhydra fluctuans* aerial parts indicate that the plant may have active principles with CNS depressant activity [10, 11]. Thus, *Enhydra fluctuans* has a potential clinical application in the management of anxiety and CNS active disorders. Therefore, the enormous neuropharmacological activities of this plant merits further attention. Search on the most active principle as well as elucidation of the exact mechanism of its action is needed.

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