Research Article

Preclinical evaluation of anxiolytic activity of *Enhydra flactuans* leaves on albino Wistar rats

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Abstract

Objective: The goal of the present study was to evaluate an extract of the leaves of *Enhydra flactuans* (EF) for its anxiolytic activity using Albino wistar rat. **Materials and methods:** For this investigation the hydroalcoholic extract of the leaves of the plant *Enhydra fluctuans* was prepared. In present study different models namely Elevated Plus Maze test, Social Interaction test and Rota Rod test were performed to evaluate the anxiolytic activity. Clonazepam was used as a standard drug. **Results:** EF (2.5 and 5 mg/kg, i.p.) was administered to the animals and the results were compared by those elicited by the standard drug clonazepam (4 mg/kg, i.p.) for anxiolytic study. In all the models EF has shown significant anxiolytic property in dose dependent manner. **Conclusion:** By focusing on this it can be assumed that *Enhydra fluctuans* also have the same activity as the folk use support the same.

Keywords: *Enhydra fluctuans*, anxiolytic, elevated plus maze test, social interaction test, rota rod test, Clonazepam

Introduction

Numerous plants and herbs are used to treat anxiety. Anxiety is very common in now a days due to the increase in pressure in work filed, and the uprising competition in various parts of our daily life. Synthetic drugs such as Clonazepam, Diazepam, Lorazepam, Gabapentin etc. are used to treat anxiety but one of the most unavoidable adverse effect causes due to the regular intake of such drugs is drug dependency. To avoid this extremely unwanted event herbal drugs are likely to replace these types of drugs (Karimi et al., 2015).

India is a country with a huge resource of herbal plants that can be pretty effective in the treatment of various severe diseases and illness without causing any or minimum adverse or side effect to the patient. Because of better acceptability, cultural compatibility with the human body, 75–80% of the world populations still use herbal medicine mainly for primary health care.

Enhydra fluctuans, family Asteraceae an annual marsh herb distributed in the topical and sub-topical areas of India. This semi aquatic herbaceous vegetable plant is having serrate leaves which are bitter in taste and equally medicinally important, used to treat different health conditions like skin disease, acidity, liver disorders, sleeplessness, some neurological disorders like anxiety and mania (Ali et al., 2013). *Enhydra fluctuans* contains β-carotene, saponins, kaurol, sesquiterpene, germacranolide, enhydrin, fluctuanin and fluctuandin, myricyl alcohol, cholesterol, sitosterol, stigmasterol, glucoside, other steroids (Ghani, 2003).

The folklore use of *Enhydra fluctuans* commonly known as helencha or hinche is well-known for treating various skin diseases, acidity, liver disorders, sleeplessness, some neurological disorders, obesity, burning stool symptoms etc. Various studies show that it has antioxidant, analgesic, antidiarrhoeal, antidiabetic, anti-inflammatory, central nervous system (CNS) depressant and antimicrobial activity (Sannigrahi et al., 2011; Roy et al., 2011; Kuri et al., 2014).

β carotene is a precursor of vitamin A, an essential vitamin for our body. β carotene used to prevent retina damage, lung and ovarian cancer, osteoarthritis, chronic obstructive pulmonary disease (COPD) and anxiety in human body. The anxiolytic activity of the *Enhydra fluctuans* may be due to the presence of β carotene in it, but still there is a dearth in scientific data on this matter, though used extensively in the treatment of anxiety in folk medicine. Here is an attempt to evaluate the folklore use of *Enhydra fluctuans* as an anxiolytic drug scientifically.

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Materials and methods

Animals

Female albino wistar rats weighing between 50-75 g were used throughout the experiment and placed in polypropylene cages (32 X 24 X 16) cm. The animals were purchased from authorised animal breeder. The animals were kept in CPCSE approved Netaji Subhas Chandra Bose Institute of Pharmacy animal house (approval no:1502/PO/a/11/CPCSEA), well maintained under standard hygienic conditions, at a temperature (22±2°C), 65% relative humidity, and 12-hour light and dark cycle. Commercial food pallets and tap water *ad libitum* were provided. All experiments were conducted between 10 am to 6 pm.

For this study the animals were divided into four groups, each group containing 6 animals. Group I was untreated, Group II was treated with standard drug Clonazepam (4 mg/kg), Group III was treated with 2.5 mg/kg dose of *Enhydra fluctuans* (EFLD) leaf extract, and Group IV was treated with 5 mg/kg *Enhydra fluctuans* (EFHD) dose of the leaf extract.

Preparation of extract formulation

Fresh plants of *Enhydra fluctuans* (family Asteraceae) were selected for this study. The leaves of *Enhydra fluctuans* were collected from the Medicinal Garden of Netaji Subhas Chandra Bose Institute of Pharmacy, Chakdaha, Nadia, West Bengal. They were washed with water and shade dried.

Dried leaves were made coarse by hand crush and subjected to soxhlet extraction by using 70% ethanol for consecutive 48 hours. The extract was dried by using desiccators.

Anxiolytic activity

Elevated plus maze test

The maze consisted of two opposite open arms of 50 cm x 10 cm crossed with two closed arms of the same dimension with walls of 50 cm high. The maze had a central square of 10 cm x 10 cm, and was kept elevated 50 cm above the floor in a dimly-lit room. All the animals were received treatment as per their group. 30 minutes after the administration of drug the rats were placed individually in the centre of the maze, head facing towards the open arm. Then the time spent on the open arm and time spent on the closed arm was noted during the next 3 minutes (Pellow et al., 1985; Kulkarni, 2005; Bhattacharya and Satyan, 1997; Vogel and Vogel, 1997).

Social interaction test

The apparatus used for the detection of changes in social behaviour consist of a perspex open topped box (51 x 51 x 20 cm) with 17 x 17 cm marked area in the floor. All the animals caged separately 1 week prior to the test. On the day of investigation all the animals received treatment as per their groups. After 1 hour two naive rats from different housing were kept together in the apparatus for 7.5 minutes (with 60 W bright illumination 17 cm above) and their social behaviour (sniffing, examining genitals, grooming, boxing, biting, kicking, crawling over or under the partner) was noted and their exploratory motion was measured as the number of crossing of the lines marked in the floor (File and Hyde, 1978; Bhattacharya and Satyan, 1997; Vogel and Vogel, 1997). As each group contained 6 animals, 3 sets of data in each group were obtained.

Rota rod test

The test was used to evaluate the activity of drugs interfering with motor coordination (Dunham and Miya, 1957). The rota rod apparatus (INCO Pvt. Ltd) consisted of a horizontal metal rod coated with rubber with 3 cm diameter attached to a motor. The rod was 75 cm in length in a height of about 50 cm above the table top. The rota rod apparatus was set at 25 rpm. All the groups were received their respective treatment. Group I served as untreated control and not received any treatment. Clonazepam (4 mg/kg i.p. suspended in carboxy methylcellulose) was given to the Group II rats as standard drug, Group III and Group IV received EFLD and EFHD, i.p. 30 minutes after drug administration the animals were placed on the rotating rod one by one. The fall off time was noted down (Bennett et al., 1985; Kulkarni, 2005; Shiotsuki et al., 2010).

Results

Elevated plus maze test

EF (2.5 and 5 mg/kg, i.p.) showed an increase in time spent on the open arms of the elevated plus mage and the decrease on the closed arms, as did the standard drug clonazepam. EFHD showed similar effect of Clonazepam as per as the data derived from the test (Table 1).

Table 1. Effect of *Enhydra fluctuans* extracts and Clonazepam in Elevated plus maze test in rats

Treatment	Time Spent in Closed Arm (Sec.)	Time Spent in Open Arm (Sec.)
Control	173.63±5.678	6.33±0.976
Clonazepam	95.66±1.526***	84.33±8.125***
EFLD	101.45±9.166***	78.45±3.39***
EFHD	74.33±6.122***	105.66±4.625***

All values are mean±SEM, n=6*** p<0.001 vs control.

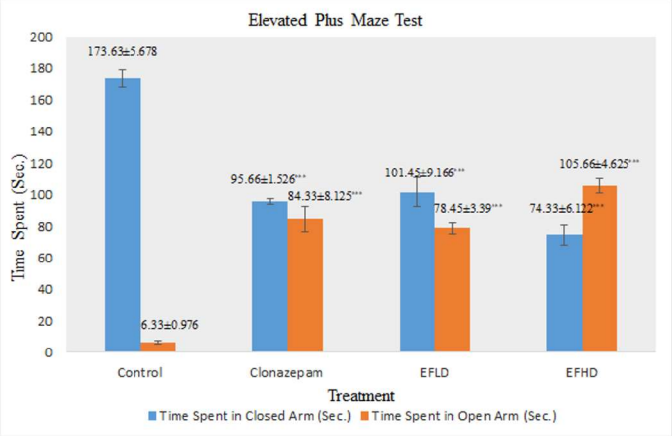

Figure 1. Time spent (sec.) by different extracts of *Enhydra fluctuans* and Clonazepam in elevated plus maze test in rat. All values are mean±SEM, n=6*** p<0.001 vs control.

Social interaction test

EF (2.5 and 5 mg/kg, i.p.) showed an increase in the total time spent by the rat pairs in active social interaction, as did clonazepam. Neither EF nor clonazepam had any significant effect on locomotor activity in the doses used (Table 2).

Rota rod test

Both EFLD and EFHD decreased the fall of time in this test like the standard drug clonazepam. This showed the decrease in the muscle coordination while compared to the control (Table 3).

Discussion

All the psychotic models are having some limitations, extremely difficult to establish the animal model suitable for mental disorder. The criteria proposed by McKinney (1979) are virtually impossible to achieve in the laboratory. Afterward guidelines (Barrett and Miczek, 1995) suggest that the animal models should have validity which may help to identify putative psychotropic agents.

In this study 3 different models of anxiolytic activity were selected to evaluate the anxiolytic activity of the hydroalcoholic leaf extract of *Enhydra fluctuans*. The extract was used in two different doses to evaluate with more precession.

Clonazepam is a benzodiazepines (BDZ) which bind with the BDZ binding site of Gamma amino butyric acid (GABA) receptors and facilitate GABA action followed by cl^- influx in nerves. This produces relaxation followed by sleep. Beta carotene is having CNS depressant action, at the same time EF is having high concentration of Beta carotene. In the folk medicine EF is renowned to use as an anti-anxiety drug. From the above two statement it is obvious to have anti-anxiety property in EF. In all the models EF has shown significant anxiolytic property in dose dependent manner.

In the elevated plus maze model all the animals from

Table 2. Effect of *Enhydra fluctuans* extracts and Clonazepam in Social Interaction test in rats

Treatment	%Time spent in social interaction	Locomotor activity (counts)
Control	25.4±2.3	192.5±19.7
Clonazepam	47.5±3.5***	141.7±25.3***
EFLD	32.5±1.9***	169.1±17.5***
EFHD	39.2±4.2***	155.8±22.6***

All values are mean±SEM, n=6*** p<0.001 vs control.

Table 3. Effect of *Enhydra fluctuans* extracts and Clonazepam in Rota Rod test in rats

Treatment	Fall of time (Sec.)
Control	47.66±4.81
Clonazepam	8.33±0.336***
EFLD	24.33±1.675***
EFHD	17.3±0.867***

All values are mean± SEM, n=6*** p<0.001 vs control.

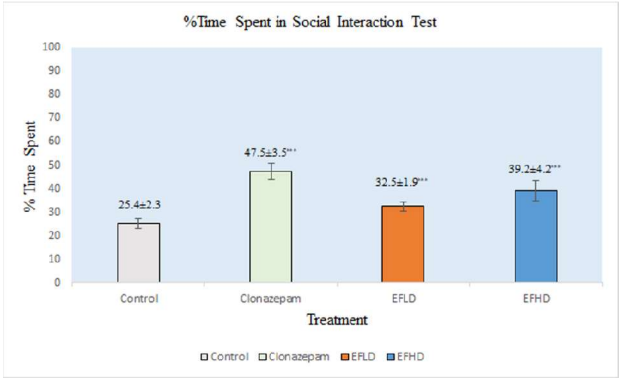

Figure 2(a). %Time spent (sec.) by different extracts of *Enhydra fluctuans* and Clonazepam in social interaction test in rat. All values are mean±SEM, n=6*** p<0.001 vs control.

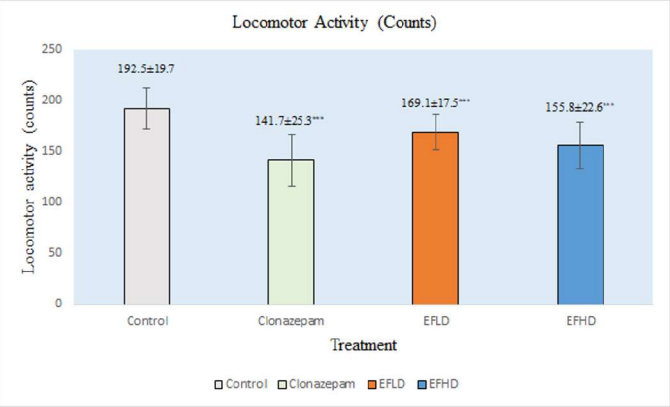

Figure 2(b). Locomotor activity (counts) by different extracts of *Enhydra fluctuans* and Clonazepam in social interaction test in rat. All values are mean±SEM, n=6*** p<0.001 vs control.

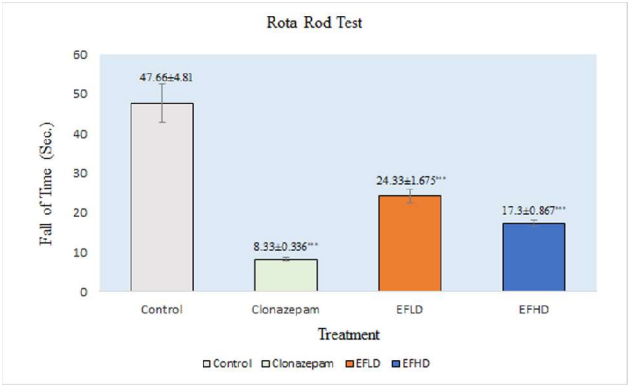

Figure 3. Fall of time (sec.) by different extracts of *Enhydra fluctuans* and Clonazepam in rota rod test in rat. All values are mean±SEM, n=6*** p<0.001 vs control.

different groups were studied separately and the time spent in open arm as well as close arm was measured. Any drug shows anxiolytic activity is used to minimise the level of anxiety on the animal by which the animals tends to move freely in open area to explore. EF high dose showed similar effect of Clonazepam as per as the data obtained from elevated plus maze test.

Social behavior is common on all the animals are belongs to the society, its help to build the community as well as bonding among themselves. Social behavior like sniffing grooming, nipping get suppressed in animals at anxiety, any drug reduce anxiety is known to counteract this suppression.

The motor coordination is generally evaluated by rota rod apparatus. Anxiolytic drugs are used to decrease the motor activity by depressing the motor area of cerebrum, any drug shows anxiolytic activity generally decrease the muscle coordination by suppressing the motor impulses in dose dependant way. Both the EFLD and EFHD significantly decreased the muscle coordination while compared with the untreated control.

Conclusion

In this present study the fresh leaves of *Enhydra fluctuans* were collected and extracted with 70% ethanol by using soxhlet apparatus, the extract was evaluated for its anxiolytic activity by using three different models namely elevated plus maze, social interaction test, rota rod test, clonazepam was served as a standard drug. In all the models *Enhydra fluctuans* showed significant anxiolytic property while compared with control. In all the models the effect shown by EFHD is very similar with the standard drug clonazepam, may be due to the presence of beta carotene in it. This study establish the rationale behind the use of *Enhydra fluctuans* as an anxiolytic in folk medicine, may bring a new hope in the psycho pharmacology.

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Conflicts of interest: The authors have no conflicts of interest.

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