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ANXIOLYTIC AND MOTOR COORDINATION ACTIVITY OF HUPERZINE-A IN RATS

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ABSTRACT: The aim of the present study was to evaluate the anxiolytic effects and motor coordination activity of Huperzine-A in rats. Wistar rats were treated orally with Huperzine-A (0.5, 1, 2 & 4 mg/kg) and diazepam (2 mg/kg p.o) as a standard anxiolytic drug then exposed to Elevated plus Maze (EPM) and Light Dark Box (LDB). The effects of the Huperzine-A on motor coordination were evaluated by actophotometer test. The rats treated with Huperzine-A drug (2mg/kg and 4mg/kg) showed significant increase in the percentage of entries and time spent in open arms of EPM, which is thought to reflect anxiolytic-like effects. Furthermore, Huperzine-A extracts (1, 2 & 4 mg/kg) significantly increased the percentage of time spent and number of transitions in the Light box in LDB test, indicating the anxiolytic effects of the substance. This anxiolytic effect of the huperzine-A was comparable to that of the diazepam, taken as standard. Also the Huperzine-A (2 mg/kg and 4 mg/kg) showed significant alteration in motor coordination compared to that of normal control group of rat. The present study demonstrates that the drug Huperzine-A has anxiolytic activity. The drug might act as NMDA receptor antagonist.

INTRODUCTION: Rapid changes in the global environment have been caused enormous stress and mental disorders [1]. Anxiety is the most common form of psychological CNS disorder in the world [2]. Anxiety is an unpleasant, emotional state which is associated with discomfort and fear [3]. Lifetime prevalence of anxiety disorders is 16.6% in all over the world means nearly one eighth of the worldwide population is affected with anxiety [4, 5]. Benzodiazepines are the first choice, used to treat severe and disabling anxiety. At moderate dosage they exert anxiolytic effects but at higher dosage hypnotic properties occur [6].

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Herbal medication has primary mechanism to involve the modulation of neuronal communication via specific plant metabolites binding to neuromodulator receptors and herbal plants having sedating and stimulating CNS activity [7].

HuperzineA is a Lycopodium alkaloid which can be isolated from whole plant of the *Huperzia serrata* by Jiasen Liu and co-workers at Shanghai Institute of Materia Medica (SIMM), Chinese Academy of Sciences (CAS) [8]. *Huperzia serrata* is the genus belonging to the family Lycopodiaceae. It is a traditional Chinese remedy which is also known as *Qian Ceng Ta* [9]. It is mainly found in India, China, Nepal, Myanmar, Sri Lanka, Japan, Korea, Vietnam, Indonesia, Fiji, Samoa, Mexico, USA, Thailand, Peninsular Malaysia, Russia, Taiwan, Australia and Cuba [10, 11]. Several chemical constituents have been isolated from plants belonging to the category of alkaloids, flavones, Triterpenes and phenolic acids [12-14].

It have been traditionally used as cold, fever, bruises, pain, strains, contusion, stasis swelling and rheumatism etc [15, 16]. Pharmacological activities such as anticonvulsant, anti-inflammatory, anti-nociception, alzheimer, schizophrenia, anti-apoptosis effect, organophosphate poisoning myasthenia gravis, antioxidant and protection of mitochondria [17].

Huperzine-A is used traditionally and pharmacologically for treating various disorders there are no reports of scientific evaluation of its anxiolytic activity as revealed from the literature survey. The present study was envisaged to evaluate the anxiolytic and motor coordination effect of Huperzine-A (*Huperzia serrata*) on the laboratory animals.

MATERIALS AND METHODS:

Animals: The experiments were performed in Wistar rats of either sex weighing around 162-282 gm. The animals were obtained from MM University of Mullana, Ambala, (Haryana, India). Animals were housed in a group of 6 per cage (polycarbonate cage size 29 ×22×14cm) under laboratory condition with alternating dark and light cycle of 12 hr each. The animals have free accessed to food and water. The animals were kept fasted over night before performing experiment. The animals were acclimatized for at least five days before behavioral experiment which were carried out between: 08:00 to 12:00 hr. The experimental protocol was approved (MMCP/IAEC/15/26) by institutional animal ethical committee (IAEC) and animal care was taken as per the guidelines of committee for the purpose of control and supervision of experiment on animal (CPCSEA) Govt. of India.

Drugs and Chemicals:

Drugs: Diazepam was manufactured by neon laboratories limited boisar road, palghar (Thane), M.S. Diazepam dose 2mg/kg, p.o. was used as standard drug.

Huperzine: A was manufactured by lupin ltd, 159 CST road, kalian, Santacruz (E), Mumbai-400098, India, at village Kunja, Rampur road, Pointa -sahib-173025(H.P) India. Huperzine-A was used as test drug.

Chemicals: Potassium permanganate, Silica gel G (S.D Fine chemicals), chloroform and methanol (Ranbaxy), ethanol (Jagjeet industry, Jalandhar).

Thin Layer Chromatography (TLC): Silica gel plates having size 2.5×7.5 cm. Chloroform/ acetone/water (1.0:1.5:1.5) used as developer and 0.3%potassium permanganate solution used as a color reagent. Huperzine-A were dripped using capillary at 1cm away from one end of the plate. After drying, the plate was placed in a developing solution and the solvent was allowed to run up the plate. TLC was analysed at room temperature and TLC was dried natural. After solvent fully volatized, the color reagent was sprayed onto the plate by using a sprayer. Then the plate was blown dry, the number of spots was recorded. R_f value was determined.

Experimental Protocol:

Groups for elevated plus maze test, light/ dark transition test, actophotometer: Six groups of rat, each group comprising of six rats were employed in the present study.

Group I: Control group (vehicle treated, p.o).

Group II: Diazepam (2mg/kg) was administered orally 45 min before test.

Group III: Huperzine-A (0.5mg/kg p.o) as administered 45 min before test.

Group IV: Huperzine-A (1mg/kg p.o) as administered 45 min before test.

Group V: Huperzine-A (2mg/kg p.o) as administered 45 min before test.

Group VI: Huperzine-A (4mg/kg p.o) as administered 45 min before test.

Vehicle: DMSO (Dimethyl sulphoxide). Test drugs at different doses (0.5, 1, 2, 4 mg/kg) suspended in DMSO.

Anxiolytic Study:

Elevated plus maze: Elevated plus maze is the most widely used experimental model of anxiety [18] to assess the anxiolytic and anxiogenic effects of drugs [19]. EPM is composed of two open arms (50×10cm) and two enclosed arms (50×10×40cm) with an open roof and is elevated to height of 50

cm [20]. Weighing and numbering of animals was done. Then they were divided into six groups, each consisting of 6 rats. Vehicle was administered to the Group I (control group). Diazepam was administered to Group II and Group III – VI received Huperzine-A according to the treatment regimen. Administrate the dose of drug 45 min prior to the test. After 45 min animals were placed individually in the centre of the maze and the following parameters were recorded: Time spent in open arm; Latency to enter the open arm. Total number of entries in open arm during 5 minutes session [21]. Clean the apparatus with 70% ethanol before starting the test or to remove any dirt or smell accumulated on the apparatus. Afterwards, the next rat can be placed in the apparatus [22].

Light/ dark exploration test: In 1980 Crawley and Goodwin was described experimental model light and dark box for anxiety [23]. The apparatus consist of a polypropylene cage (44×21×23 cm^3) separated into two compartments by a partition which had a small aperture (12×5 cm^2) at the floor level. The larger compartment (28 cm long) was open-topped, transparent. The smaller compartment (14 cm long) was close topped and painted black [24].

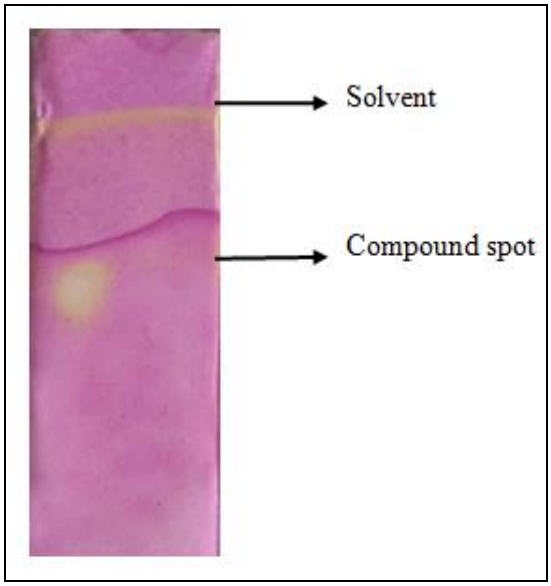
Weighing and numbering of animals was done. Then they were divided into six groups, each consisting of 6 rats. One group was used as control and second group for standard drug (Diazepam) treatment and other groups for test drug (Huperzine-A). Each rat was placed in the middle of the light area and following observation was recorded. The number of transitions and time spent in the light and dark area were recorded for 5 min. Clean the apparatus before every trial [25].

Actophotometer: Actophotometer can be used to measure the locomotor activity [26]. It is equipped with 6 photocells in the outer wall of the bottom so that single animal can block only one beam. Weighing and numbering of animals was done. Then they were divided into six groups, each consisting of 6 rats. One group was used as control and second group for standard drug (Diazepam) treatment and other groups for test drug (Huperzine-A). The animal was placed individually in the actophotometer. Now actophotometer was turned ON and animal was observed for 5 min [27].

Statistical Analyses: The results were analysed via analysis of variance (ANOVA) followed by Dunnett's method. The data were expressed as the mean ± S.E.M. The difference were considered statistically significant when $p < 0.05$.

RESULTS:

Thin layer chromatography of Huperzine-A:



$$\text{Retention factor } (R_f) = \frac{\text{Distance travelled by compound}}{\text{Distance travelled by solvent}}$$

$$\text{Retention factor } (R_f) = \frac{3.5}{5.5} = 0.6$$

Elevated Plus Maze: The performance time period of Elevated Plus Maze test was five minutes (300 sec). Huperzine-A significantly (P <0.05) increased the cumulative time spent in the open arms relative to the control at doses of 1, 2 and 4 mg/kg. The most pronounced effect of Huperzine-A was produced at the dose of 4mg/kg. In this case the cumulative time spent in the open arms was 146.8± 4.1 sec (48.66 %) compared to a value of 34.2 ± 1.7 sec (11.3 %) for control.

This effect was greater and also significantly different from that effect produced by diazepam, 96.4± 3.3 sec (32.13 %). At dose of 4 mg/kg, the effect of Huperzine-A also increasing the no. of entries (4.1±0.87) in open arm when compare with control (2.8±0.31).

TABLE 1: EFFECT OF HUPERZINE-A ON TIME SPENT IN OPEN ARMS AS MEASURED ON EPM IN RATS

S.no	Treatment	Dose (mg/kg)	Average time spent in open arms Mean ± SEM	No. of Entries in open
1	Control		34.2 ± 1.7	2.8±0.31
2	Diazepam	2	96.4± 3.3*	3.6±0.56
3	HuperzineA	0.5	55.2± 2.4	3.4±0.65
4	HuperzineA	1	90.4± 3.0*	4.2±0.60*
5	HuperzineA	2	139.2± 3.5*	4.3±0.74*
6	HuperzineA	4	146.8± 4.1*	4.1±0.87*

SEM= Standard error of mean. Values are Mean ± SEM (n=6). P<0.05 as compared to control. (n=6)

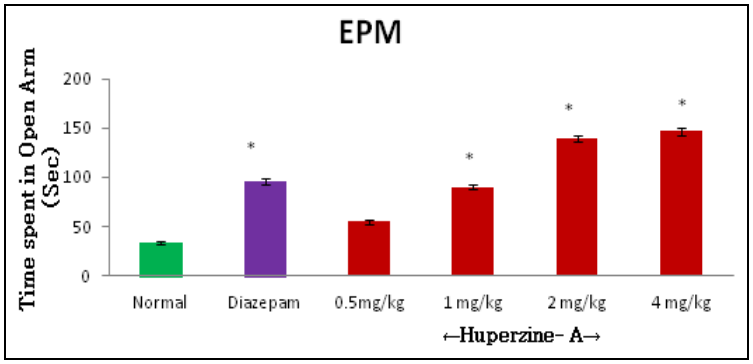


FIG. 1: EFFECT OF HUPERZINE-A ON TIME SPENT IN OPEN ARM USING EPM TEST. BARS REPRESENT MEAN ± S.E.M. (*$P<0.05$). THE DATA WAS ANALYZED BY ONE WAY ANOVA FOLLOWED BY DUNNETT'S METHOD

Light-dark model: The performance time period of Light and Dark Test test was five minutes (300 sec). Huperzine-A (1mg/kg, 2mg/kg and 4 mg/kg doses) induced a significant ($P < 0.05$) increment of the time spent and no. of entries by rat on the illuminated (light) side of the light–dark apparatus. Huperzine-A showed maximum time spent (85.3± 2.1) and number of entries (4.6±0.8) on the illuminated (light) side at dose 2mg/kg when compared to control group (30.4± 1.3). This effect showed relatively high time spent (18%) as compared to standard drug diazepam (60.4±2.9), standard anxiolytic treated group shown in **Table 2** and **Fig. 2**.

TABLE 2: EFFECT OF HUPERZINE-A ON TIME SPENT IN LIGHT COMPARTMENT AS MEASURED IN LIGHT DARK EXPLORATION TESTS IN RATS (n=6)

S.no	Treatment	Dose (mg/kg)	Average time spent in open arms Mean ± SEM	No. of Entries in open
1	Control		30.4± 1.3	2.8±0.5
2	Diazepam	2	60.4± 2.9*	4.4±1.1*
3	HuperzineA	0.5	51.6± 1.5	3.8±0.7
4	HuperzineA	1	62.8± 1.8*	4.3±0.9
5	HuperzineA	2	85.3± 2.1*	4.6±0.8*
6	HuperzineA	4	81.6± 2.3*	4.2±0.9*

Values are Mean ± SEM (n=6). P<0.05 as compared to control

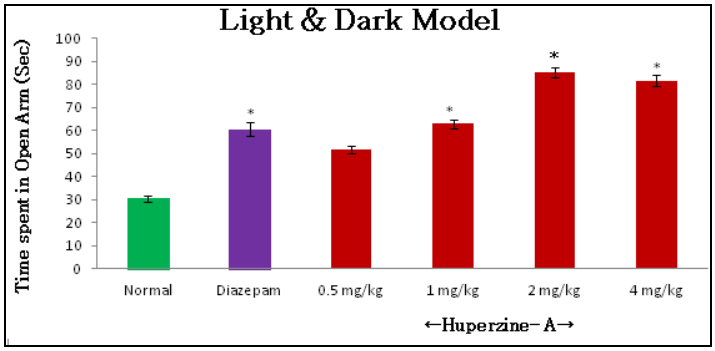


FIG. 2: TIME SPENT IN LIGHT COMPARTMENT IN LIGHT-DARK MODEL. BARS REPRESENT MEAN ± S.E.M. (*$P<0.05$). THE DATA WAS ANALYZED BY ONE WAY ANOVA FOLLOWED BY DUNNETT'S METHOD

Actophotometer: The results of the parameters taken during five minutes from actophotometer and the results are Huperzine-A (1, 2, 4 mg/kg) treated rats significantly ($P < 0.05$) increased the locomotor activity count measured for 5 min. The increase in locomotor activity was not observed with the dose of the 0.5 mg/kg (**Fig. 3**). Diazepam on the other hand significantly decreased the locomotor activity (sedation) at three times intervals.

TABLE 3: EFFECT OF HUPERZINE-A ON LOCOMOTOR ACTIVITY IS MEASURED BY ACTOPHOTOMETER IN RATS

S.no	Treatment	Dose (mg/kg)	Number of animal (n)	Reading/counting Mean ± SEM
1	Control		6	107± 6.8
2	Diazepam	2	6	274± 9.3*
3	HuperzineA	0.5	6	217± 9.1
4	HuperzineA	1	6	263± 10.8*
5	HuperzineA	2	6	326± 13.4*
6	HuperzineA	4	6	369±13.9*

Values are Mean ± SEM (n=6). P < 0.05 as compared to control.

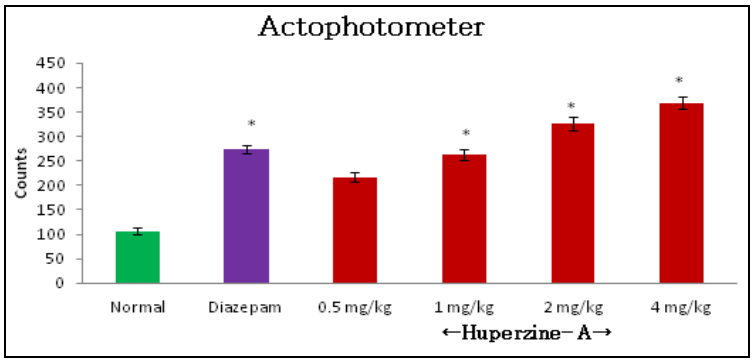


FIG. 3: LOCOMOTOR ACTIVITY IN ACTOPHOTOMETER. BARS REPRESENT MEAN ± S.E.M. (*$P<0.05$). THE DATA WAS ANALYZED BY ONE WAY ANOVA FOLLOWED BY DUNNETT'S METHOD

DISCUSSION: In this decade anxiety has become a very important area of research in psychopharmacology. This increases rapid growth of scientific studies and discovery of new drugs which alters anxiety in animal models [28]. The present work has evaluated the anxiolytic activity of various doses of the alkaloidal content of Huperzine-A in rats employing two non-conditioned behavioral animal models of anxiety; EPM model and LDM. Diazepam a standard anxiolytic agent used clinically and is also employed in behavioral pharmacology as a reference compound for inducing anxiolytic-like effects, even when the compound being screened does not act via benzodiazepine receptors. As expected standard diazepam significantly increased the number of entries and time spent in open arm [29, 30].

Oral administration of Huperzine-A at the doses (2mg/kg and 4mg/kg) significant increase in both number of entries and time spent in open arms, compared to the closed arm in the elevated plus maze model. In light and dark test model, results showed that Huperzine-A at dose (Huperzine-A 2mg/kg) induce significant increase in time spent in light compartment as compared to the dark compartment. These results are compared with that observed in control group as well as with group treated with diazepam (2mg/kg) standard anxiolytic drug.

In actophotometer, Huperzine-A at the dose of (Huperzine-A 4mg/kg) shows significant increase in motor coordination activity. No sedative effects were observed at any tested dose.

Non-competitive NMDA receptor antagonist also shows anxiolytic effects in rodents. For example, phencyclidine reduces anxiety in rat on elevated plus maze [31]. AP5 (2-amino-5-phosphonoheptanoate) and AP7 (2-amino-7phosphonoheptanoate) are two competitive NMDA antagonist exert anxiolytic effects on several animal models of anxiety [32]. NMDA receptor antagonist shows anxiolytic effects through the blockade of NMDA receptor in ventral

hippocampus in different animal models and laboratory tests of anxiety for example: CPP [3-(2carboxy piperazine-4yl) propyl-1-phosphonic acid] increases time spent in open arms on elevated plus maze with reduction of anxiety [33].

Further pharmacological and chemical investigations are required to elucidate the exact cell based mechanism of action of Huperzine-A that responsible for such effects.

CONCLUSION: From the results it was concluded that Huperzine-A shows anxiolytic effects because NMDA receptor antagonist shows anxiolytic effects through the blockade of NMDA receptor in ventral hippocampus in different animal models and laboratory tests of anxiety. Huperzine-A acts as a selective NMDA receptor antagonist [34].

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CONFLICT OF INTEREST: The authors confirm that this research article content has no conflict of interest.

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