International Standard Serial Number (ISSN): 2319-8141
International Journal of Universal Pharmacy and Bio Sciences 3(5): September-October 2014
INTERNATIONAL JOURNAL OF UNIVERSAL PHARMACY AND BIO SCIENCES
*IMPACT FACTOR 2.093****
*ICV 5.13****
Pharmaceutical Sciences
RESEARCH ARTICLE……!!!

AMELIORATIVE EFFECT OF HORDEUM VULGARE LEAF EXTRACT USING LIGHT/ DARK MODEL AND SOCIAL INTERACTION MODEL IN ANXIETY

Yash Prashar[1*], N.S Gill[1], Daman Jit Kaur[1]
[*1]Rayat Institute of Pharmacy, Railmajra, SBS Nagar, Punjab, India.

KEYWORDS:

Hordeum vulgare; Social interaction test; Light and dark arena model.

For Correspondence:
Damanjit Kaur *
Address:
Rayat Institute of Pharmacy, Railmajra, SBS Nagar, Punjab, India.
E Mail: damansaini990@gmail.com

ABSTRACT

Description of the Plant: *Hordeum vulgare* is an important medicinal plant in the folk medicine of India, Australia, South Africa, Nepal and USA for the treatment of various disorders. **Materials and Methods:** Powdered materials of the plant part were subjected to aqueous extraction with water. Extracts were evaluated for their anxiolytic effects using social interaction, light/dark arena tests in rats. **Results:** In the present study, it was found that the aqueous extracts (250 and 500 mg/kg) of leaf plant of *Hordeum Vulgare* and fluoxetine shows Increase in the no. of crossings and time spent in light compartment while decrease in the time spent in the dark compartment which shows the reduction in the anxiety in light dark model. In the Social Interaction, the sniffing and crawling parameters both are increased by the extract and fluoxetine groups as compared to the control vehicle, but there is decreased in the aggressive behaviour of the animals. This demonstrates the reduction in the anxiety. These allied parameters helped to assess the anxiolytic potential of *Hordeum Vulgare*. **Conclusion:** Results indicate that AEHV has a wide range of anxiolytic properties and avers a new drug evaluation on anxiety.

INTRODUCTION:

Anxiety is a complex progressive behavioural and physiological alteration of the organism which ultimately leads to wide variety of CNS disorders if remain untreated. In addition to individual genetic factors also external influences such as nutrition, smoking, alcohol, socioeconomic status, environmental conditions etc. can strongly contribute to its anticipated appearance[(1)]. Anxiety effects one-eighth population globally and has become an important research area of brain disorders and psychiatric disorders[(2)]. Excessive anxiety can debilitate and damage the quality of healthy and wealthy life. Anxiolytic drugs are among the most frequently prescribed drugs as the disease is highly prevalent in the society.

In the clinical treatment, benzodiazepines, $GABA_A$ receptor agonist and buspirone, $5\text{-}HT_{1A}$ [(3,4)] receptor agonist are mainly used in the treatment of anxiety and regular results in physical and pharmacological dependence along with other serious concerns arise some problems such as rebound insomnia, sedation, muscle relaxation, withdrawal and tolerance (BZD's, barbiturates and alcohol), sexual dysfunction. Anti-cholinergic, antihistaminic effects (TCA's) have limited their use in patients [(5)]. Pharmacological research in the treatment of anxiety and stress is very much influenced by the availability of anxiolytic drugs[(6)].

Allopathic drug treatments that are the available for anxiety, other brain and psychiatric disorders may possesses many side effects like hallucinations, sleeping, dizziness, nausea, headache etc. to overcome these problems many plant derived drug treatments are preffered[(6)].

India has a rich biodiversity and knowledge in the use of "folk" herbal medicines to cure many ailments in various cultures and tribes[(5)]. *Hordeum vulgare* belonging to family Graminae, is one of the important plant in Indian history, but according to previous studies it can be used in the treatment of depression, psychological and brain disorders and historically has been used as an anti-inflammatory, anti-ulcerous actions, anti-obesity, anti-diabetic, anti-hypertensive, antifungal, antibacterial etc[(8)].

The grains and leaves contain several alkaloids and volatile oil, minerals, proteins, flavonoids, glycosides, terpenoids, etc. *Hordeum vulgare* contains proteins prolamins, glutens, and albumin hordein, hordenin which are the precursors of essential amino acids[(9)]. It also contains ferulic acid, flavons, anthocyaninis, flavanols and flavanones, cyanogenic glycosides, linalool and β-caryophylline and lignans were reported[(10)].

 International Standard Serial Number (ISSN): 2319-8141

MATERIALS AND METHODS:

Plant material:

The plant was collected from District Ropar, Punjab, India. Plant was identified and authenticated by Dr. Sunita Garg chief scientist at Raw Material Herbarium and Museum, Delhi (RHMD), CSIR-National Institute of Science Communication and Information Resources- 110012 voucher specimen no: (NISCAIR/RHMD/Consult/2014/2413/10).

Drying and size reduction:

Plant leaves were washed in cold water and then subjected to shade drying for about 1 week. The dried plant material was further crushed to powder and the powder was passed through sieve mesh 40 and stored in air tight container for further analysis.

Extraction of plant material:

The aqueous extract of barley was daily prepared by diluting one volume of well grinded plant to 10 volume of water at 80 °C in stopper flask. After shaking well, it is allowed to stand for 10 minutes then cooled and filtered to be used within 12 hours[(11)].

Animals:

Experiments were carried out on Wistar rats. Healthy adult rats were used for pharmacological evaluation. The animals were housed in polypropylene cages. Paddy husk was provided as bedding material, which was changed every day. The cages were maintained clean and hygienic. The rats were kept 4 per cage. They were fed with standard pellet diet and water *ad libitum.* They were kept in a well-aerated room and a 12-hour light and dark cycle was maintained. The room temperature was maintained at 22+2^{o}C.

Grouping of animals:

Group 1: Control/ saline treated- 0.9% saline (10ml/kg/p.o)**.**

Group 2: Standard/ Fluoxetine (10 mg/kg/p.o).

Group 3: Test-1/ AEHV (250 mg/kg/ p.o).

Group 4: Test-2/ AEHV (500mg/kg/ p.o)

5 animals were used for each dosage. Each group was treated for 7 days before stress induced.

Chemicals used

Fluoxetine: Cyper Pharma**.**

Acute toxicity studies:

The acute toxicity was performed according to OECD (Organization for Economic Co-operation) 423 guideline. The paste of extract , at the dose of 300 & 2000 mg/kg body weight, was administered to the rats and they were subsequently observed closely for the first 4 hours for any

untoward symptoms such as tremors, convulsion, salivation, diarrhoea and lethargy followed by observation for a 14 days. At the end of the experimental period, the animals were observed for any changes in behavioural pattern and mortality.

Phytochemical studies:

Phytochemical evaluation of *Hordeum vulgarae* was carried out as per standard methods (Table-1) [15].

Experimental models:

Evaluation:

Light Dark Model:

1. Time spent in light and dark compartment
2. No. of crossings
3. Immobility

Social Interaction in Rats:

1. Sniffing
2. Crawling
3. Aggressive behavior

In-vivo studies:

Light Dark Model

The apparatus consisted of an open top wooden box. Two distinct chambers, a black chamber (20×30×35 cm) painted black and illuminated with dimmed red light and a bright chamber (30×30×35 cm) painted white and brightly illuminated with 100 W white light sources, were located 17 cm above the box. The two chambers were connected through a small open doorway (7.5×5 cm) situated on the floor level at the center of the partition. Each animal was in bright and dark arena paradigm. Study was carried out; 60 min. after the drug administration [fluoxetine (10 mg/kg, po), test drugs (250 and 500 mg/kg, p.o.)] and vehicle administration, the animal was placed at the center of the brightly lit arena in the light and dark box. Similarly, study was carried out on 1st, 3rd and 7th day.

The followings parameters were noted down:

The time spent in the light arena, time spent in dark arena, number of crossing, duration of immobility was noted for 10 min for each trial. Following each trial, the apparatus were cleaned to mask the odor left by the animal in the previous experiment[5,613,14].

Social Interaction in Rats:

Male Sprague-Dawley rats (225–275 g body weight) were housed in groups of 5 animals The apparatus used for the detection of changes in social behaviour and exploratory behaviour consists

of a Perspex open-topped box (51×51 cm and 20 cm high) with 17×17 cm marked areas on the floor. One hour prior to the test, two naive rats from separate housing cages were treated with the standard drug (fluoxetin 10 mg/kg, p.o) and test drug (250 mg/kg and 500 mg/kg, p.o). They were placed into the box (with 60 W bright illumination 17 cm above) and their behaviour is observed over a 10-mins of period and two test conditions were performed: high light unfamiliar arena (HU) and high light familiar arena (HF) .Three types of behaviour can be noted: social interaction between the animals is determined by timing the sniffing of partner, crawling under or climbing over the partner, and aggressive behaviour. Five pairs are used for each dose[5,6,16].

Statistical analysis:

All observations were presented as Mean ± SEM (standard error mean) and were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's test [*$p<0.05$, **$p<0.01$, ***$p<0.001$ and non-significant (ns)]. P values lower than 0.05 were considered statistically significant.

Results:

Light & Dark Model

Effect of AEHV on time spent in each compartment and duration of immobility on animals in light/dark model.

In the L/D model, the standard group showed significant increase ($p<0.01$) in time spent **light compartment** as compared to control. In order to determine the anxiolytic activity of AEHV on L/D model at the dose of 250mg/kg and 500mg/kg were tested. AEHV at the dose of 250 mg/kg showed non-significant increase on 3rd day but significant increase ($p<0.05$) on 1st and 7th day, while AEHV at the dose of 500 mg/kg (1st, 3rd, 7th day) showed significant increase ($p<0.01$, $p<0.05$) in time spent in light compt. as compared to control group **(Fig-1)**.

Standard group showed significant decrease ($p<0.01$) in time spent in **dark compt**. as compared to control group which indicates reduction in the anxiety. AEHV at the dose of 250 mg/kg showed non-significant decrease on 3rd day but significant increase ($p<0.05$) on 1st and 7th day, while AEHV at the dose of 500 mg/kg (1st, 3rd, 7th day) showed significant decrease ($p<0.01$, $p<0.05$) in time spent in dark compt. as compared to control group **(Fig-2)**.

Standard group showed significant decrease ($p<0.01$) in duration of **immobility** as compared to control group which indicates reduction in the anxiety. Insignificant decrease in duration of immobility was observed by AEHV (250 mg/kg/p.o) on (1st, 3rd, 7th) while significant decrease was observed by AEHV (500 mg/kg/p.o) ($p<0.05$, $p<0.01$) respectively on comparison with control group which revealed the anti anxiety effect **(Fig-3)**.

Standard drug showed significant increase ($p< 0.01$) in **no. of crossings** as compared to control group which indicates reduction in the anxiety. Non-significant increase in number of crossings was observed at the dose of AEHV (250 mg/kg) on (1^{st}, 3^{rd}, 7^{th} day) and non-significant increase on 1^{st} day and significant increase ($p< 0.01$, $p<0.05$) in duration of immobility at the dose of 500 mg/kg on 3^{rd} and 7^{th} day on comparison to control group which showed reduction in anxiety **(Fig-4)**.

Social Interaction in rats

Effect of AEHV on Social Interaction in Rats:

In the high light, unfamiliar test condition there was a significant drug-induced increase in social interaction [**p < 0.01]. Further analysis confirmed that both 250 and 500 mg/kg/p.o. doses of AEHV and fluoxetine significantly and non-significantly increased the social interaction time in comparison to the control group and also markedly enhanced active social interaction (**P < 0.01). In the high light, familiar test condition there was again a significant and non-significantly increase in social interaction [*p < 0.05] at the dose of 250 mg/kg of AEHV, although standard group also increased social interaction time. The data of varied behavioural categories are shown in **(fig 5,6)**. The results displayed that the increase of social interaction time was due to the enhancing duration of "sniffing and crawling and climbing" for rats significant and non significant (**p < 0.01or *p < 0.05). The significant and non-significant decrease in the duration of aggressive behaviours with AEHV at 250 and 500 mg/kg (**p < 0.01 or *p < 0.05) in both HU and HF test conditions. However, standard group also decreased the duration of aggressive behaviour.

Discussion:

Anxiety is a complex progressive behavioural and physiological alteration of the organism which ultimately leads to wide variety of CNS disorders if remain untreated. Animal models help to understand the information about molecular mechanisms involved in anxiety, and are useful for screening and developing new medications for their treatment that would be impossible in humans. Numerous psychological and brain disorder research studies have been conducted using traditional medicinal plants in the form of herbal extracts and combinations to treat specific diseases, including anxiety, depression, insomnia, Alzheimer's, convulsions, Parkinson's, etc. in an effort to discover new therapeutic agents that lack the toxic side effects associated with the current agents.

The light/dark test is widely used rodent anxiety model for screening anxiolytic or anxiogenic like drugs. The social interaction test of anxiety was developed to provide an ethologically based test that was sensitive to both anxiolytic and anxiogenic effects. Generally speaking, an increase in social interaction is indicative of an anxiolytic effect, whereas a specific decrease in social interaction indicates an anxiogenic effect.

Synthetic drugs including SSRI's is used in the treatment of anxiety, however some side effects like psychological dependency, physiological dependency, and withdrawal symptoms are major hurdles for their use in long term treatment of diseases which is always associated with any kind of CNS disorder including anxiety. The present study was designed to prove the anxiolytic activity of aqueous extract of *Hordeum Vulgare* leaves. This plant was evaluated for its effect on animals in:

Light Dark Model: Treatment with fluoxetine significantly increased the time spent in the light arena as well as number of crossing between the light and dark arena, whereas the time spent in dark arena and duration of immobility were reduced. The AEHV at dose 250 and 500 mg/kg treated rat also showed increase in the time spent in the light arena and the number of crossing between the light and dark arena. However, the time spent in dark arena and duration of immobility was reduced as compared to control.

Social Interaction Method: In the high light, unfamiliar test condition there was a significant drug-induced increase in social interaction. Further analysis confirmed that both 250 and 500 mg/kg doses of AEHV and fluoxetine (10 mg/kg, p.o.) increased social interaction time in comparison to the control group and also markedly enhanced active social interaction. In the high light, familiar test condition there was again increase in social interaction at the dose of 250 mg/kg of AEHV, although fluoxetine (10 mg/kg) also increased social interaction time but it was found to be less than the test drug at a dose of 500 mg/kg of AEHV. The results displayed that the increase of social interaction time was due to the enhancing duration of "sniffing and crawling" .On the other hand, ANOVA followed by Dunnet's test revealed a significant decrease in the duration of aggressive behaviours with AEHV at 250 and 500 mg/kg in both HU and HF test conditions. However, fluoxetine also decreased the duration of aggressive behaviours.

Conclusion

Aqueous extract of leaves of *Hordeum Vulgare* (AEHV) was found to possess a therapeutic effect against anxiety disorder. Further studies with different extracts and their fractions are encouraged to identify the chemical constituents respectively responsible for anti-anxiety activity. Biochemical estimations of brain homogenate which are responsible in the anxiety can also be done to check out the effect of the plant. Also clinical studies to prove this effect is also needed for its applicability in humans for treatment of anxiety disorder. Thus all the results pave a way towards further research must be carried out to explore the golden use of plant in psychological disorders.

REFERENCES:

1. Kulkarni SK, Reddy DS. (1996). Animal behavioural models for testing anti-anxiety agents. Meth Find Exp Clin Pharmacol.;18 (3):219-30.

2. Kumar D, Bhat ZA, Kumar V, Shah M.Y. (2013) Coumarins from *Angelica archangelica* Linn and their effects on anxiety-like behavior. Progress in Neuro-Psychopharmacology & Biological Psychiatry; 40: 180–6.
3. Graeff FG, Guimares FS, de Andrade TG, Deakin JF., (1996). Role of 5-HT in stress, anxiety and depression. Pharmacol. Biochem. Behav. 54, 129–141.
4. Griebel, G (1995) 5-hydroxytryptamine pathways in anxiety and its treatment. Pharmacol. Ther. 66, 103–148.
5. Kumar D, Bhat ZA, Shah MY. (2012). Effect of successive extracts of Stachys tibetica Vatke (Lamiaceae) in anxiety. Orient Pharm Exp Med; DOI 10.1007/s13596-012-0083.
6. Kumar D, Bhat ZA, Shah MY. (2012). Anti-anxiety activity of successive extracts of angelica archangelica linn on the elevated t-maze and forced swimming tests in rats. J Tradit Chin Med;32(3):423-9.
7. Rabbani M, Sajjadi SE and Mohammadi A. (2008) Evaluation of the anxiolytic effect of *Nepeta persica* Boiss in mice. eCAM ;5(2):181–6.
8. Al-dalain S., El-kutry M. S. and Ibrahim H.S., (2008). Inhibitory Effect of Aqueous Extracts of Barley and Fenugreek on Ulcer Induction in Rats. 5 (3): 332-339.
9. Sastri B.N, (2501), "An Dictionary of Indian Raw Materials And Production"; The Wealth of India; volume-5, page no. 117-131.
10. Dykes.L and Rooney W.L., (2007). Phenolic Compounds in Cereal Grains and Their Health Benefits. 53(2), 105-111.
11. Darwish E Inas, Maklad M Hala, Diab H Iman., (2013) "Behavioral and neuronal biochemical possible effects in experimental induced chronic mild stress in male albino rats under the effect of oral barley administration in comparison to venlafaxine" Int. J Physiol Pathophysiol Pharmacol; 5(2):128-136.
12. Vogel H.G.Vogel W.H., Sandow J., Vogel W.F (2012) Drug Discovery and Evaluation, second edition. 25 (64), 430-432.
13. Vogel H.G.Vogel W.H., Sandow J., Vogel W.F (2002) Drug Discovery and Evaluation, second edition. 25 (64), 430-432.
14. K.Trivedi, P.K. Gupta, (2010). "An overview of Indian research in anxiety disorders", Indian Journal of Psychiatri, volume-52, page no. 210-218.
15. Kokate C.K., Purohit A.P., Ghokhale S.B., (2008). The Text Book of Pharmacognosy,; A.1-A-6.

16. File SE., (1980). The use of social interaction as a method for detecting anxiolytic activity of chlordiazepoxide like drugs. J Neurosci Methods; 2:219–238.

Table-1 Phytochemical Screening

Sr. No.	Extract Constituents	Present (+) /Absent (-)
1)	Alkaloids	+
2)	Volatile oils	+
3)	Phenolic/Tannins	+
4)	Proteins	+
5)	Glycosides	+
6)	Flavanoids	+
7)	Saponins	-

+ indicates presence of compounds; - indicates absence of compounds

Light Dark Model:

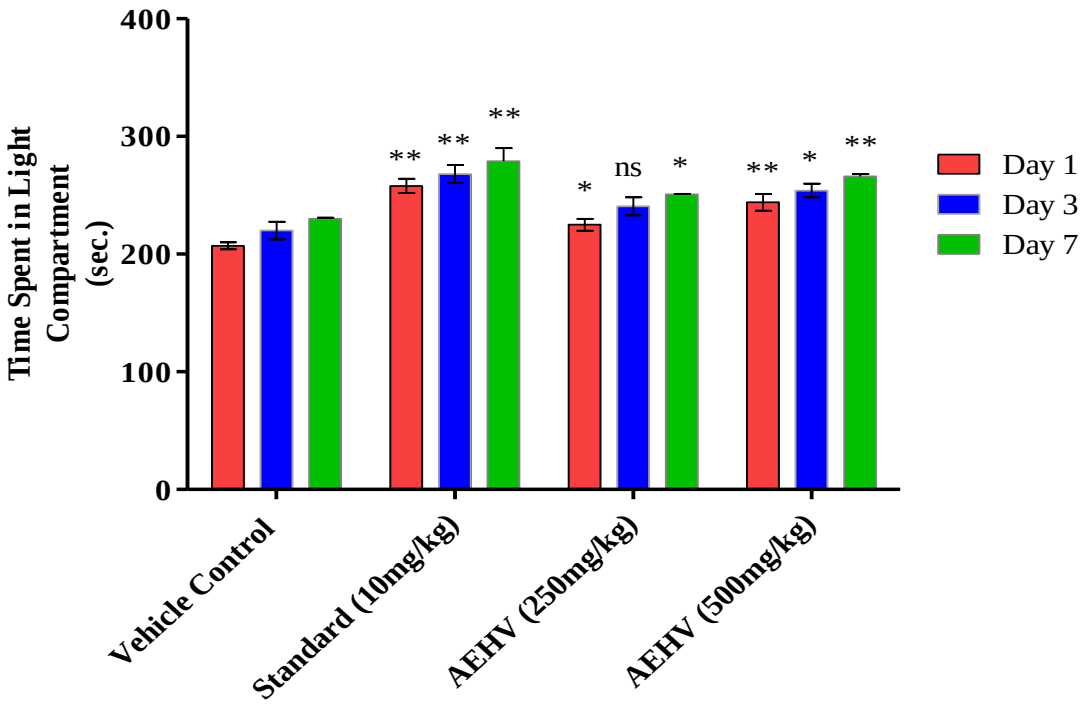

Figure-1: Effect of AEHV on time spent in light compt. on animals in L/D model.

Comparison between control vs. standard and test groups.

Statistical significance test for comparison was done by ANOVA followed by Dunnet's test:

*p<0.05; **p<0.01 and ns (non- significant)

 International Standard Serial Number (ISSN): 2319-8141

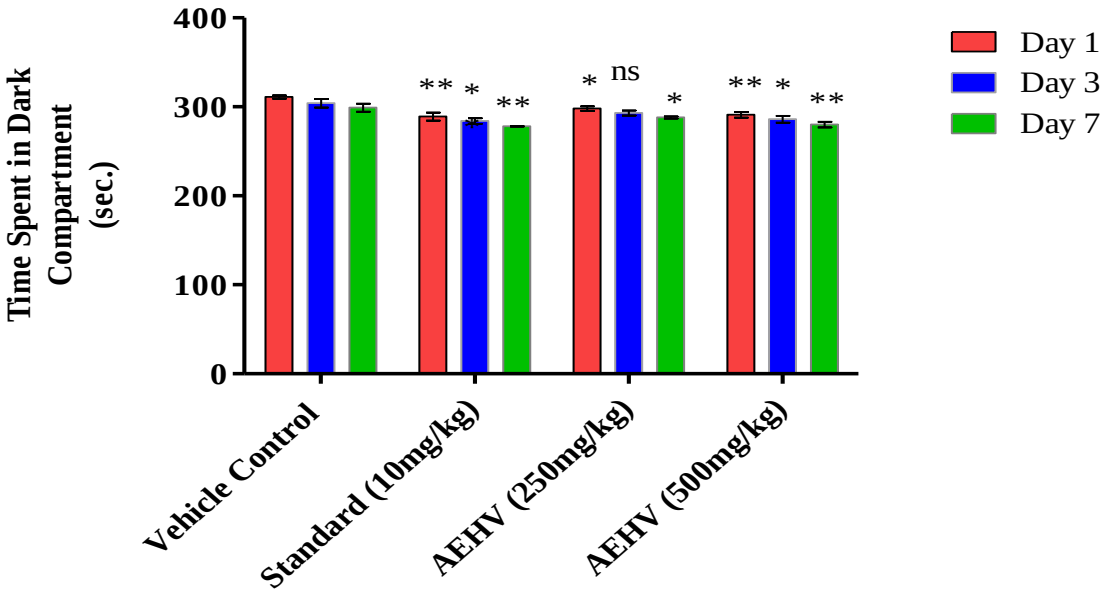

Figure- 2: Effect of AEHV on time spent in dark compt. on animals in L/D model.
Comparison between control vs. standard and test groups.
Statistical significance test for comparison was done by ANOVA followed by Dunnet's test: *p<0.05; **p<0.01 and ns (non- significant)

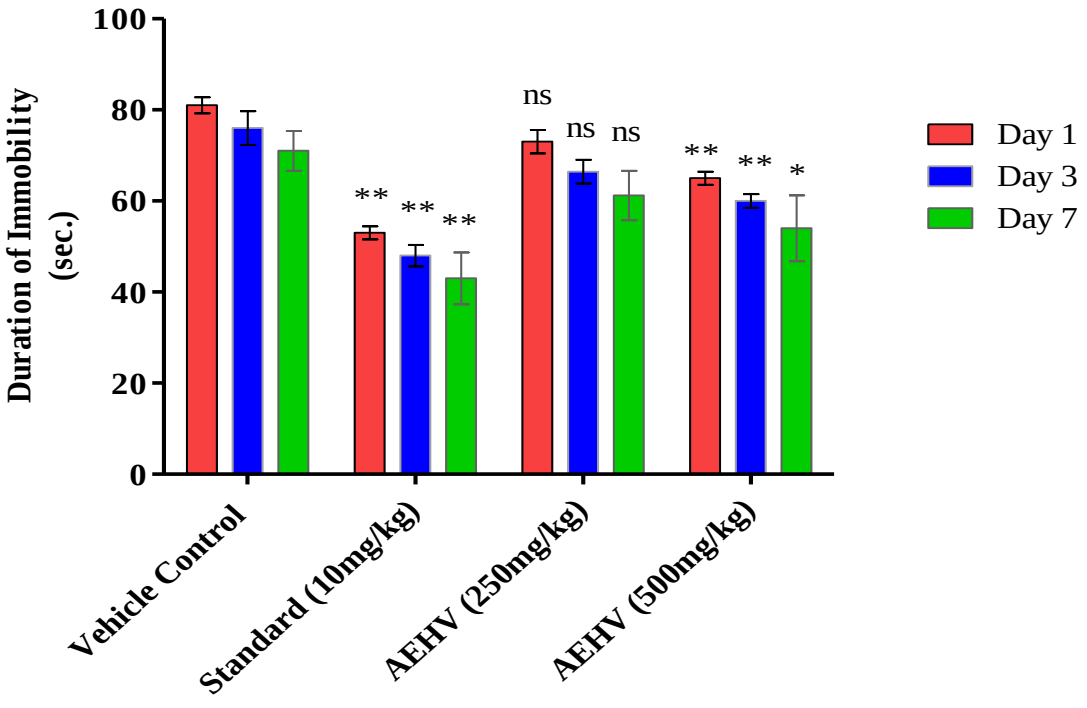

Figure-3: Effect of AEHV on duration of immobility on animals in L/D model
Comparison between control vs. standard and test groups.
Statistical significance test for comparison was done by ANOVA followed by Dunnet's test: *p<0.05; **p<0.01 and ns (non- significant)

 International Standard Serial Number (ISSN): 2319-8141

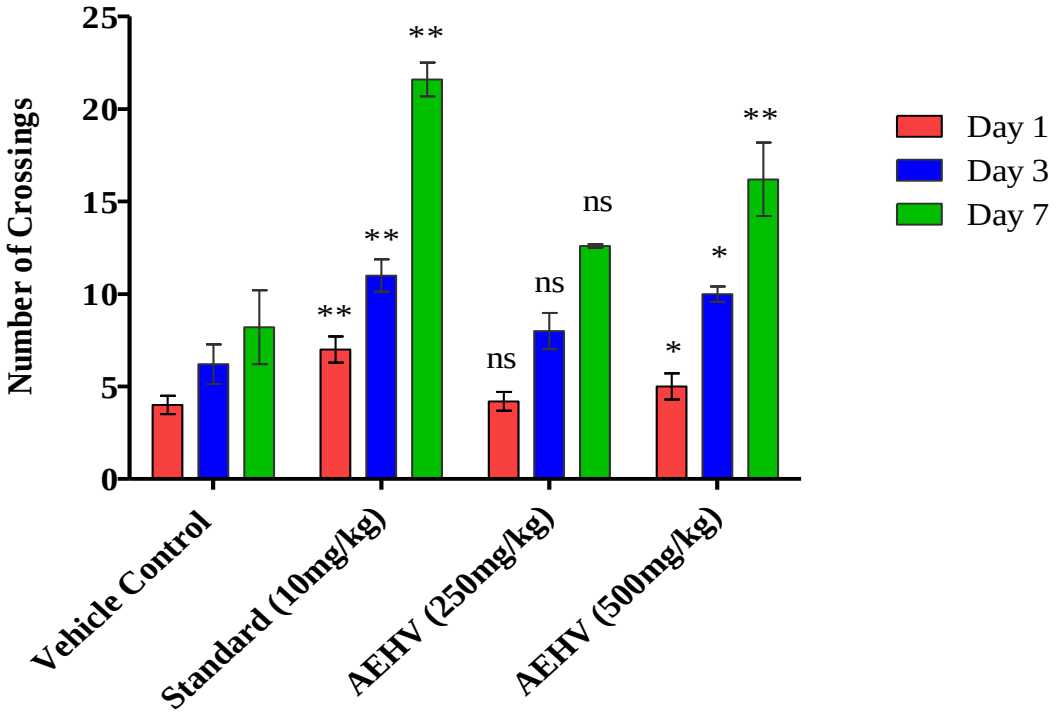

Figure--4: Effect of AEHV on no. of crossings of animals in L/D model.

Comparison between control vs. standard and test groups.

Statistical significance test for comparison was done by ANOVA followed by Dunnet's test: *p<0.05; **p<0.01 and ns (non- significant).

Social Interaction Model:

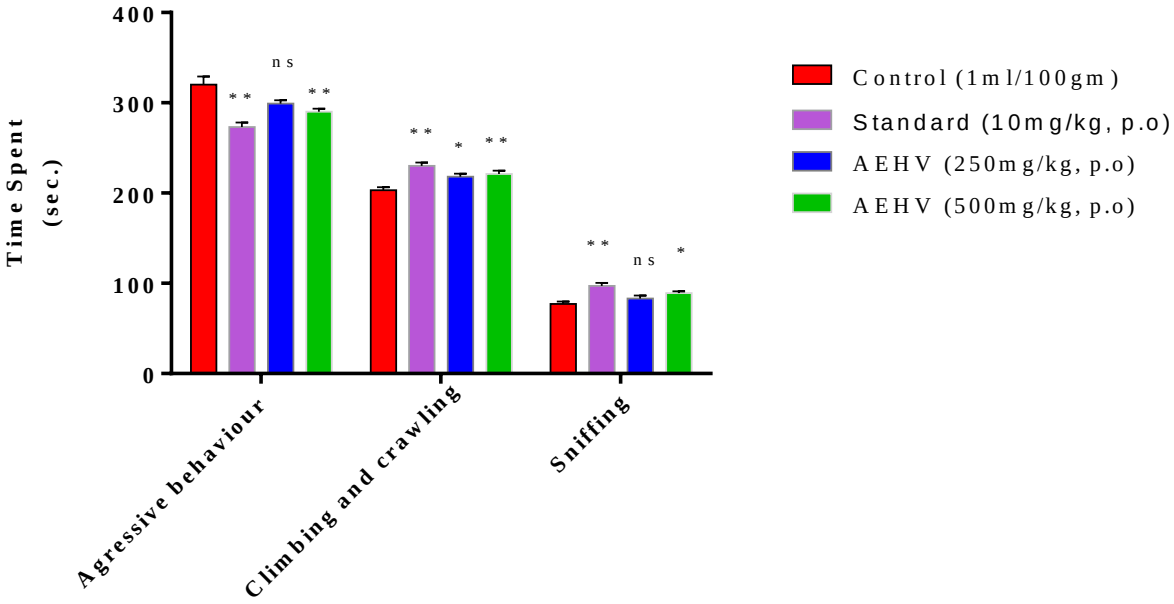

Figure-5: Effect of AEHV on time spent in social interaction in high familiar light.

Comparison between control vs. standard and test groups.

Statistical significance test for comparison was done by ANOVA followed by Dunnet's test: *p<0.05; **p<0.01 and ns (non- significant)

 International Standard Serial Number (ISSN): 2319-8141

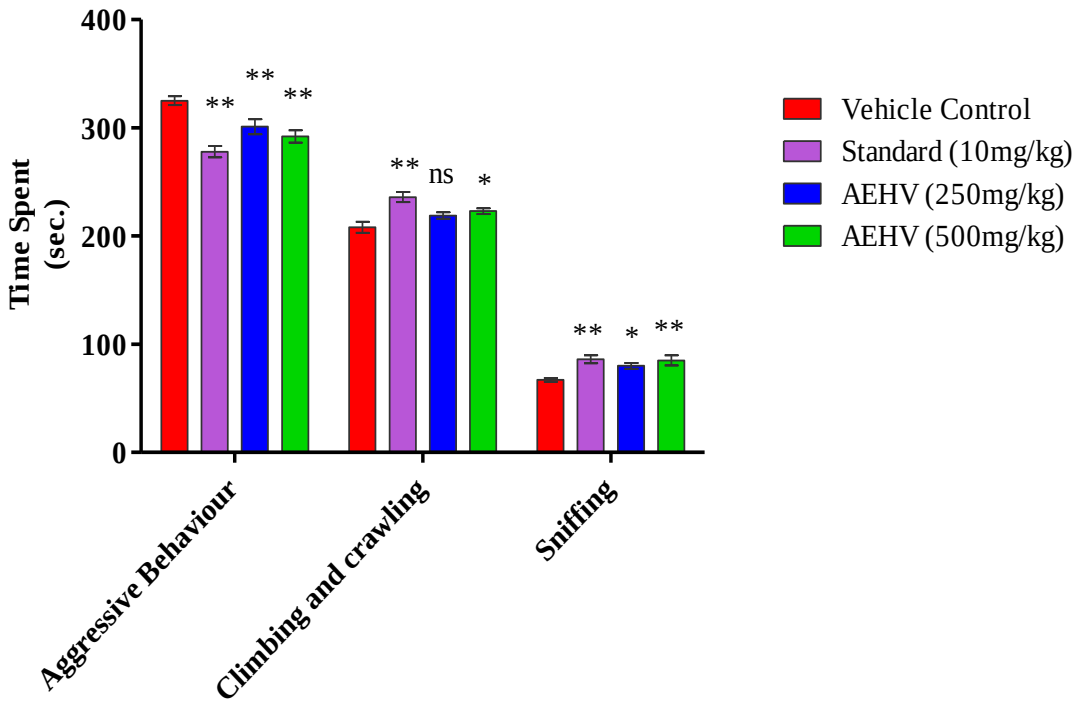

Figure-6: Effect of AEHV on time spent in social interaction in high unfamiliar light.

Comparison between control vs. standard and test groups

Statistical significance test for comparison was done by ANOVA followed by Dunnet's test: *p<0.05; **p<0.01 and ns (non- significant)