

EVALUATION OF ANTIANXIETY AND ANTIOXIDANT ACTIVITY OF AERIAL PARTS AND ROOT OF *EREMURUS HIMALAICUS* BAKER

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ABSTRACT

The aim of the present study was to determine the *in-vitro* antioxidant and *in-vivo* antianxiety activity of crude extracts of *Eremurus himalaicus* (Asphodelaceae) introduced orally at the dose of 200 mg/kg once daily for a period of 1 week. The anxiolytic activity was determined after single dose administration, 3rd and the 7th day of treatment using elevated plus maze and light and dark arena models. Results of antioxidant activity demonstrated significant DPPH radical scavenging and reducing power of methanolic and aqueous extracts compared to BHT (Butylated Hydroxy Toulene). Results of the antianxiety study of extract treated groups (except chloroform extract) showed a statistically significant increase in the open arm activity and time spent in light box in elevated plus maze and light and dark arena models respectively.

Keywords: *Eremurus himalaicus*, antioxidant, antianxiety activity.

1. INTRODUCTION

WHO defines health as a state of complete physical, mental and social well-being and not merely the absence of diseases (Hassan A and Shafi A, 2013). Anxiety is mental illness and a state of excessive fear. It is characterized by hyper sympathetic activity, worry, uneasiness, and vigilance syndrome which may leads to a wide variety of CNS disorders if not treated (Ninan PT, 2001). As anxiety leads to other psychiatric disorders and medical conditions, the quality of life of the patients suffering from anxiety deteriorates, and the social disadvantage becomes comparable to that of chronic somatic disorders (Pasquini M and Berardelli I, 2009).

Global scenario of the anxiety disorders reveals that it affects one-eighth of total world population (Shri R, 2010). In the general population, anxiety disorders are frequent, with a lifetime prevalence rate ranging from 13.6% to 28.8% (Pasquini M and Berardelli I, 2009). Benzodiazepines, SSRIs, azapirones, TCAs and MAOIs are the most frequently prescribed drugs used for the treatment of anxiety (Katzung GB, 2004), however, the side effects related to these drugs, tolerance and withdrawal symptoms especially associated

with benzodiazepines (Lader MH et al., 1999), have urged researchers to evaluate newer compounds for the treatment of anxiety, especially the plant based ones, as the plant based compounds are believed to be more safe, more compatible with human body, besides being cheap and locally available (Gupta LM & Raina R, 1998; Kala, Prakash & Sajwan, 2007).

E. himalaicus (Himalayan desert candle) is found in Himalayas, grows from Afghanistan to Himachal Pradesh. Young leaves of *E. himalaicus* are edible. It has beautiful white flowers so this plant has great horticultural potential (Shilaji T, 2011). Besides this, *E. himalaicus* is used in anemia, as galactagogue, It also promotes digestion. It has been reported to possess antibacterial property. A herbal formulation containing *E. himalaicus* Baker has been used in the treatment of migraine with insomnia indicating its effect on CNS, therefore the present study has been undertaken to explore the anxiolytic potential of this plant (Tramboo AM, 2013).

In addition to the antianxiety activity, antioxidant potential of the plant material was also evaluated because the stress promotes free radical and lipid peroxidation damage to a number of areas in brain, thus promoting the

chances of serious diseases like anxiety, depression, multiple sclerosis, Alzheimer's and Parkinson's disease. A large number of studies have shown that antioxidants protect the brain enzyme system from harmful effects induced by stress (Devasagayam et. al 2004; Blaylock RL, 2006).

2. MATERIALS AND METHODS

2.1. Plant material and preparation of extract

The plant material was collected from Harwan area of district Srinagar, J&K, India, identified and authenticated by Prof. A. R. Naqshi, Department of Botany, University of Kashmir. Specimen voucher number is KUAA12. After collection and authentication, the plant material were air dried and powdered and subjected to successive extraction using the solvents of increasing polarity, aqueous extract was prepared by decoction method.

2.2. Determination of antioxidant activity

2.2.1. Determination of total phenol

The amount of phenol in the methanolic and aqueous extracts of *E. himalaicus* was determined with Folin-Ciocalteu reagent. 10% Folin-Ciocalteu reagent (2.5 ml) and 2% Na₂CO₃ w/v (2ml) was added to 0.5 ml of each sample of plant extract solution (1 mg/ml). The resulting mixture was incubated at 45°C for 15 min with occasional shaking. The absorbance of the samples was measured at 765 nm using spectrophotometer. Results were expressed as milligrams of Gallic acid (0-0.5 mg/ml) dissolved in distilled water (Aiyegoro OA, Okoh AI, 2010).

2.2.2. Estimation of total flavonoids

Flavonoid determination was carried out using Aluminum chloride colorimetric method. 1 ml of 1 mg/ml sample was mixed with, 0.2 ml of 10% aluminum chloride, 3 ml of methanol, 0.2 ml of 1 M potassium acetate and 5.6 ml of distilled water and remains at room temperature for 30 min. The absorbance was measured at 420 nm with UV visible spectrophotometer. The flavonoid content in sample was determined from extrapolation of calibration curve which was made by preparing gallic acid solution (0-0.8 mg/ml) in distilled water. The concentration of flavonoid was expressed in terms of mg/ml (Aiyegoro OA, Okoh AI, 2010).

2.2.3. Determination of reducing power

To 1.0 ml of the extract dissolved in distilled water, 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of 1%w/v K₃Fe(CN)₆ was added. The resulting mixture was incubated

for 20 min at 50°C, followed by the addition of 2.5 ml of (10% w/v) tricarboxylic acid. The mixture was centrifuged at 3000 rpm for 10 min. Upper layer of the solution was collected. To this 2.5ml distilled water and 0.5 ml of 0.1%, w/v ferric chloride was added. The absorbance was then measured against blank sample at 700 nm (Saeed N, Khan MR, 2012; Aiyegoro OA, Okoh AI, 2010).

2.2.4. DPPH (2, 2-Diphenyl-1-Picrylhydrazyl) assay

Aqueous extract ranging from 0.2-0.8 mg/ml was prepared. To 1.0 ml of aqueous extract one ml of 0.135 mM DPPH prepared in methanol was added. The mixture was then vortexed thoroughly and left in dark at room temperature for half an hour. The absorbance was measured spectrophotometrically at 517 nm. The DPPH scavenging ability of the plant extract was calculated using this equation;

$$\text{DPPH Scavenging activity (\%)} = \frac{[(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})]}{(\text{Abs}_{\text{control}})} \times 100$$

Where; Abs_{control} is the absorbance of DPPH + methanol;

Abs_{sample} is the absorbance of DPPH radical + sample (i.e. extract or standard) (Aiyegoro OA, Okoh AI, 2010).

2.3. Procurement of animals

Swiss albino mice of either sex weighing 25-30 g were used for the anxiolytic study. The albino mice were obtained from animal house of Indian Institute of Integrative Medicine - Jammu, Jammu and Kashmir, India. The protocol for present study was approved by Institutional Animal Ethical Committee [Approval no. F-IAEC (Pharm. Sc.) Approval/2013/07]. The animals were housed in polypropylene cages and maintained under standard environmental conditions: 25±2°C, 12:12 h light: dark cycle and 45-55% humidity, with free access to food and water *ad libitum*. All experiments were carried out during the life period (8.00-18.00 h).

2.4. Acute toxicity testing

The acute toxicity study was performed as per Organization for Economic Cooperation and Development (OECD) guidelines no 425. Since the plant is locally used as vegetable therefore only limit test was performed as per the guidelines.

2.5. Evaluation of antianxiety activity

The animals were divided into ten groups, with six animals in each group.

Group I serves as control receiving 0.5% CMC by oral route, Group II serves as standard group receiving diazepam at the dose of 0.5mg/kg, intraperitoneally, and remaining groups serve as test groups receiving petroleum ether, chloroform, methanol and aqueous extract of both aerial parts and roots of *E. himalaicus*.

Two models were used for the antianxiety studies: elevated plus maze & light and dark arena.

2.5.1. Elevated plus-maze (EPM)

The EPM apparatus consists of two open arms and two closed arms that extend from a common central platform elevated to a height of 50 cm above floor level (Bourin M, 2007). Mice were given vehicle, diazepam and the oral doses of plant extracts as per the groups assigned 30 min before their placement on the EPM. The number of entries and the time spent in the open and closed arms was recorded during a 5 minute test period. Similar procedure was carried out on the 3rd and 7th day for the chronic study.

2.5.2. Light and dark test

Light and dark arena consists of a rectangular box, partitioned into two compartments connected by an opening in the wall between compartments. One compartment is painted black and the other compartment is brightly illuminated bulb located above the box (Bourin M, Hascoe't M, 2002). Mice were given vehicle, diazepam and oral doses of plant extracts according to the group assigned and after a period of 30 minutes, each animal was placed in the center of the light compartment and observed for 5 min. The time spent in the each compartment and the number of transitions was recorded. Similar procedure was carried out on the 3rd and 7th day for the chronic study.

2.6. Statistical analysis

All observations were presented as mean±SEM and were analyzed using one-way analysis of variance (ANOVA) followed by Dunnet's test. *P<0.05, **P<0.01, ***P<0.001, ⁿP>0.05 are considered as significant, highly significant, extremely significant and insignificant respectively compared with control.

3.0. RESULTS

All the results are given at the end of paper in graphical form.

4.0. DISCUSSION

Neural tissue are highly susceptible to oxidative stress because of high content of lipids particularly unsaturated fatty acids in them hence any agent possessing free radicle scavenging activity could be protective against oxidative damage and neuronal damage (Devasagayam TPA et al. 2004). Phenolics and flavonoids are free radicle scavenging agents thus protective against oxidative damage (Saeed, N., Khan, M.R., Shabbir, M., 2012). Total phenolic and flavonoid content are given in fig. All the extracts showed significant DPPH anion scavenging power comparable to scavenging power of BHT. In the reducing power assay, yellow colour of the test solution changes to green solution due to the reduction of Fe³⁺ to Fe²⁺ depending on the redox potential of the test sample. Increasing absorbance indicates an increase in reductive ability (Aiyegoro, O.A., Okoh, A.I., 2010; Saeed, N., Khan, M.R., 2012). The results showed that there was an increase in the reducing power of plant extract as the concentration increased.

Results of antioxidant activity reveal that extracts of *Eremurus himalaicus* showed significant antioxidant activity which is protective against free radical damage and oxidative stress.

EPM is currently one of the most widely used models of animal anxiety. EPM is based on the natural aversion of rodents for open spaces. It is based on the natural tendency of rodents to explore novel environment and their innate fear for elevated places. (Cryan, J.F., Holmes, A., 2005). Increase in open arm entries and time spent in open arm are the most important parameters of anxiolytic activity (Blanco et al., 2009). Extract groups of *E. himalaicus* i.e. methanol, aqueous and petroleum ether both of aerial and root part increased open arm entries and the time spent in open arm compared to the control group however, chloroform group of aerial as well as root had no significant effect on the open arm activity. Each group was retested on the 3rd and 7th day of the study after chronic treatment. Results reveal that compared to the first day, there was a marked decrease in open arm exploration of the diazepam group and the extract groups on the 3rd day and 7th day of study. Chloroform groups had no significant effect on number of open arm entries and time spent in open arm even on the 3rd or 7th of study indicating lack of anxiolytic activity of these extract. The reduction in the efficacy upon retesting on EPM has been confirmed by other studies also (Gonzalez and File, 1997; Lily, S.M., Tietz, E.I., 2000; File, S.E., 2001; Costa et al.,

2011). The reduction in efficacy upon retesting could be due to the possibility of greater anxiety evoked in rodents due to repeated exposure to EPM (Rodgers and Shepherd 1991).

Another significant parameter for the evaluation of anxiolytic behavior is light and dark arena which is based on the natural aversion of rodents to the bright light. In this test, the number of transition between the light and dark compartments as well as the time spent in the light box is recognized as anxiety indices (Blanco et al., 2009). In this model of anxiolytic study, results reveal that there was a significant increase in the time spent in light box in case of methanolic, aqueous and petroleum ether group of both the aerial and root part compared to the control group, however, the activity of extracts was less effective than diazepam group. Results of repeated dose study revealed that there was a decrease in time spent in light box in case of diazepam group on the 3rd and 7th of study compared to day 1, but there was no marked change in case of extract groups on the 3rd or 7th day of study. However, the overall number of transitions increased in the extract groups (methanol, aqueous and petroleum ether) on the chronic dosing indicating anxiolytic activity of the extract groups. Chloroform groups had

no significant effect on the time spent in light box compared to control group on the first day or repeated dose study.

5.0. CONCLUSION

In the present study, analysis of free radical scavenging activity and total phenolic and flavonoid content showed that methanolic and aqueous extract of aerial parts of *E. himalaicus* can be the source of natural antioxidants which can counteract oxidative stress. The results of elevated plus maze and light and dark arena suggest the anxiolytic activity of the methanolic, aqueous and petroleum ether extracts of *E. himalaicus* both in the acute as well as chronic study, although effect was decreased which could be due to greater fear induced in mice during trail 2 and 3 or contingent upon previous exposure to EPM and light and dark arena.

As the current study was carried out using crude extracts, thus further studies are needed to ascertain the main phytoconstituents responsible for activity and also to elucidate the molecular mechanism responsible for the pharmacological action.

6.0. Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

3.0. RESULTS

3.1. Results of antioxidant activity

3.1.1. Determination of total phenolic content

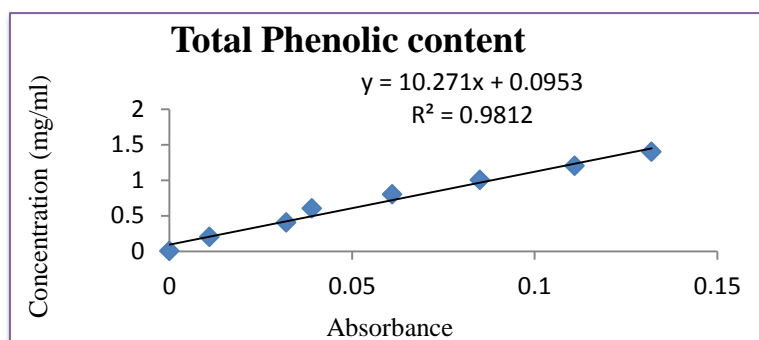


Fig. 1: Standard curve of gallic acid for the determination of total phenolic content

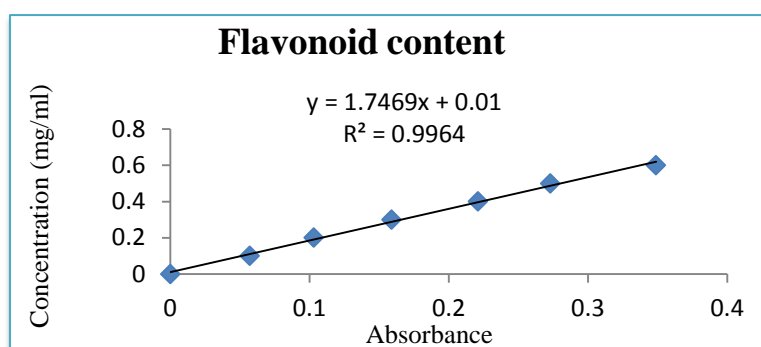


Fig. 2: Standard curve of gallic acid for the determination of total flavonoid content of various extract of *E. himalaicus*.

3.1.2. Determination of total flavonoid content

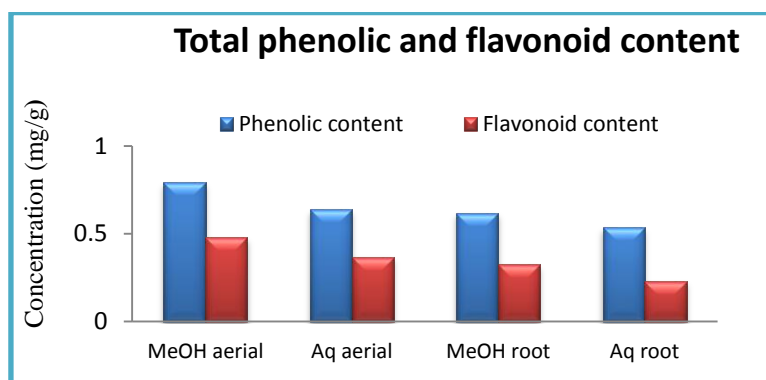


Fig. 3: Total phenolic and flavonoid content of various extracts of *E. himalaicus*

3.1.3. DPPH radical scavenging activity

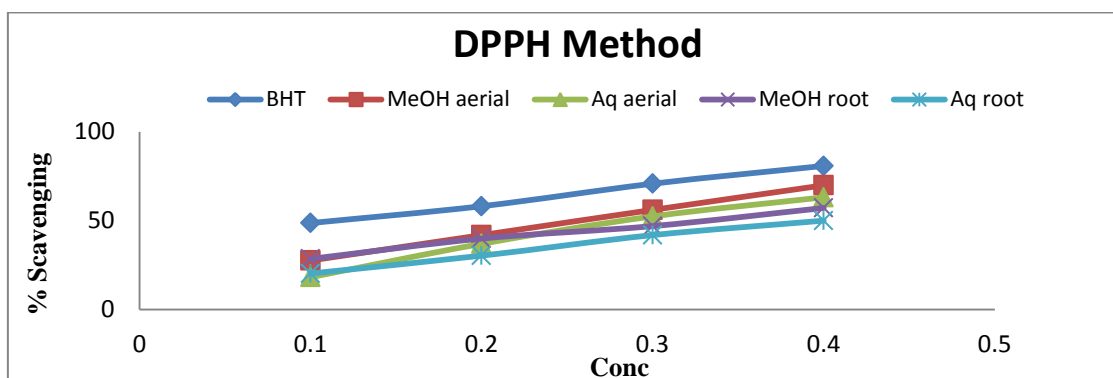


Fig. 4: DPPH radical scavenging activity of BHT and various extracts of *E. himalaicus*.

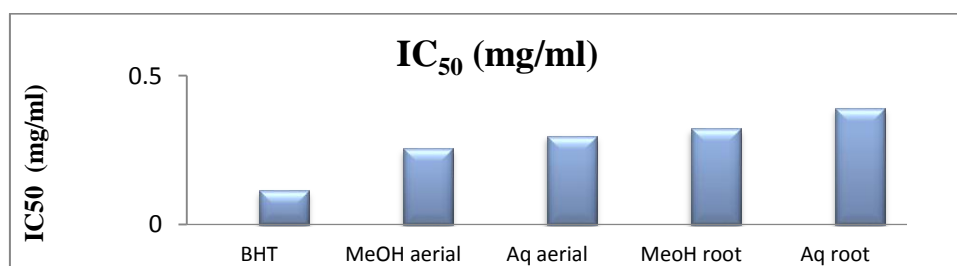


Fig. 5: IC₅₀ of BHT, methanolic and aqueous extract of aerial parts and root of *E. himalaicus*.

3.1.4. Determination of reducing power

The results showed that there was increase in reducing power of the plant extract as the extract concentration increases.

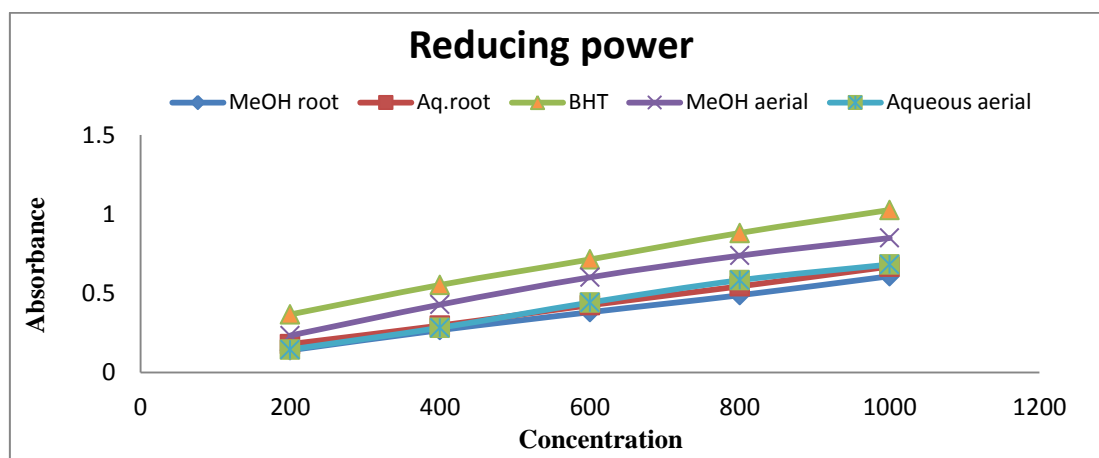


Fig. 6: Reducing power of BHT and various extracts of *E. himalaicus*.

3.2. Results of acute oral toxicity studies

Since no mortality or any moribund state was observed in any of the animal during the entire period of study, hence the dose administered is considered to be safe and LD50 is more than 2000mg/kg.

3.3 Results of antianxiety activity

3.3.1 Results of elevated plus maze

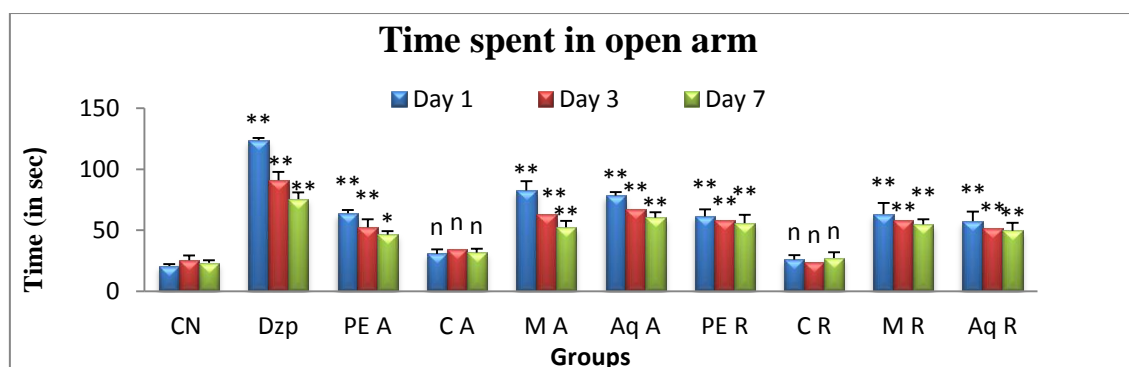


Fig. 7: Graphical representation of effect of various extracts of *E. himalaicus* on the time spent in open arm.

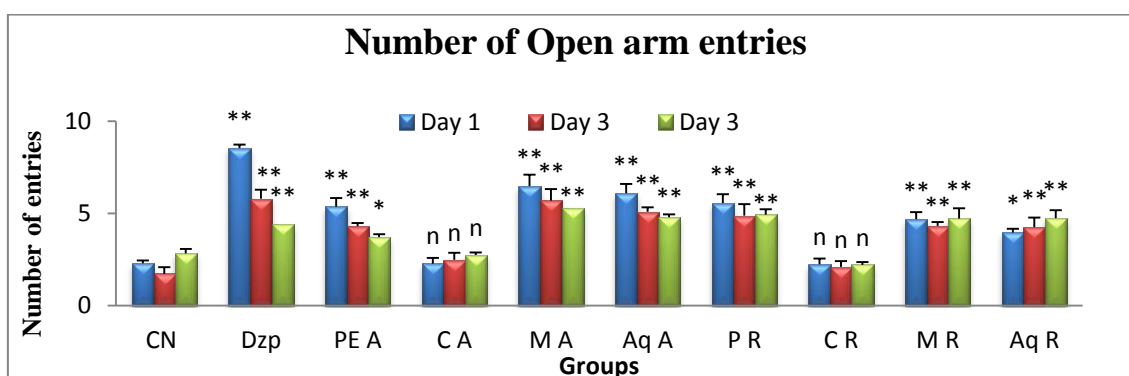


Fig. 8: Graphical representation of effect of various extracts of *E. himalaicus* on the number of open arm entries.

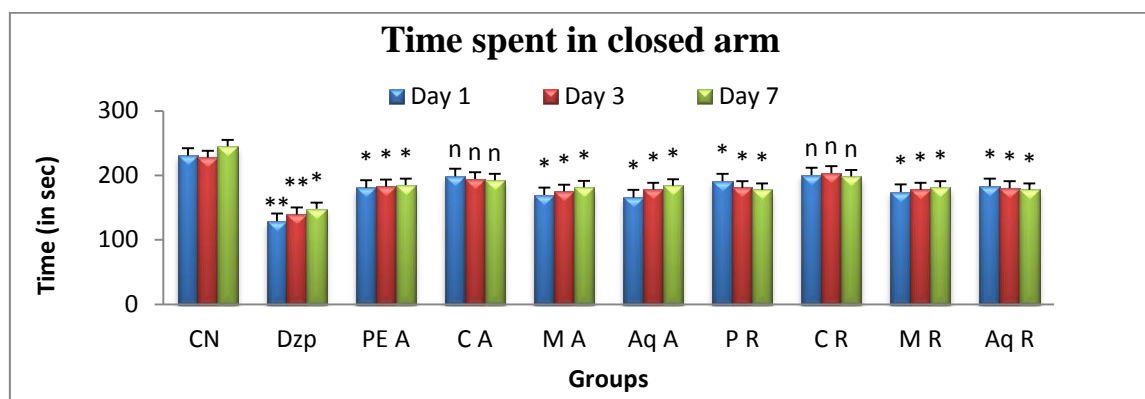


Fig. 9: Graphical representation of effect of various extracts of *E. himalaicus* on the time spent in closed arm.

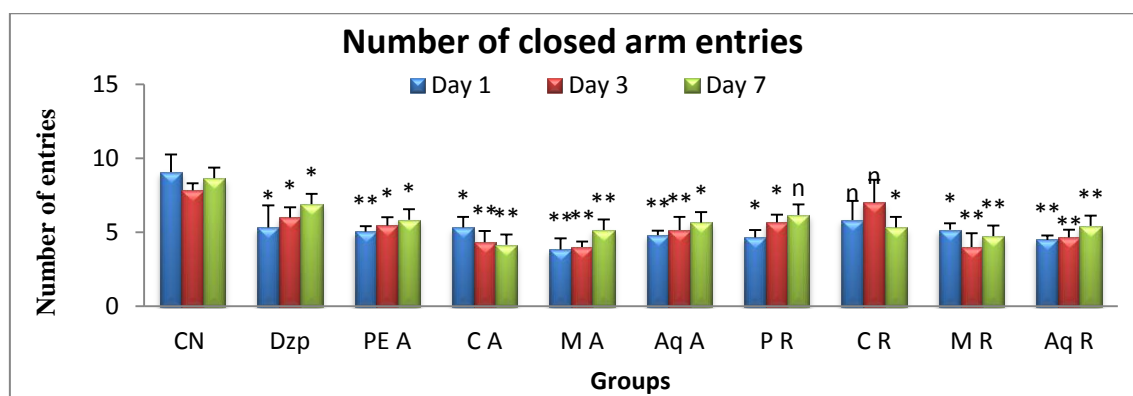


Fig. 10: Graphical representation of effect of various extracts of *E. himalaicus* on the number of closed arm entries.

3.3.2. Results of Light and dark arena

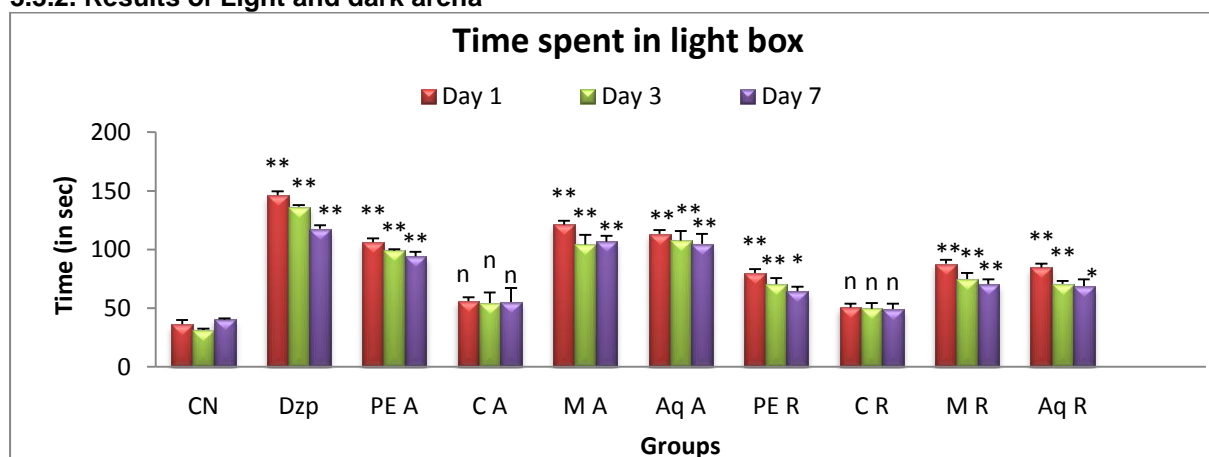


Fig. 11: Graphical representation of effect of various extracts of *E. himalaicus* on the time spent in light box.

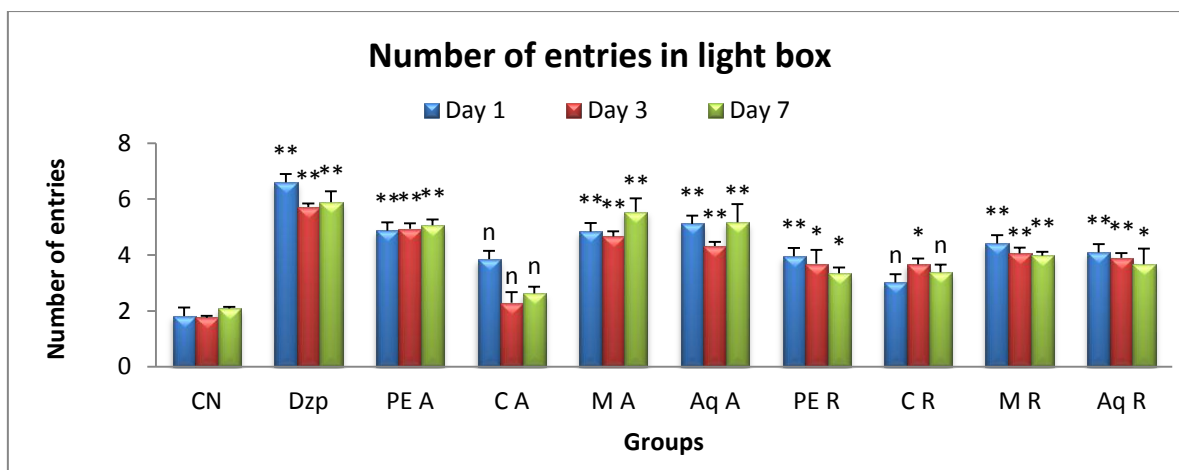


Fig. 12: Graphical representation of effect of various extracts of *E. himalaicus* on the number of entries in light box.

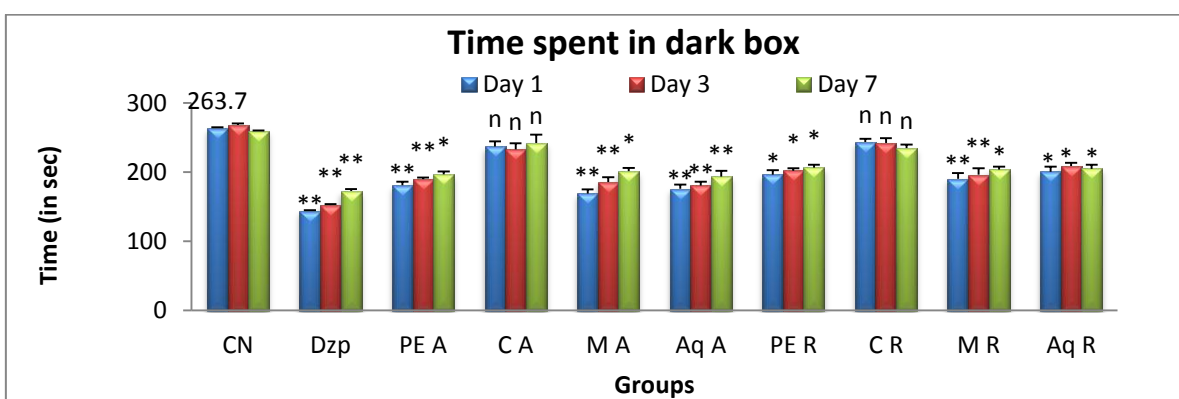


Fig. 13: Graphical representation of effect of various extracts of *E. himalaicus* on the time spent in dark box.

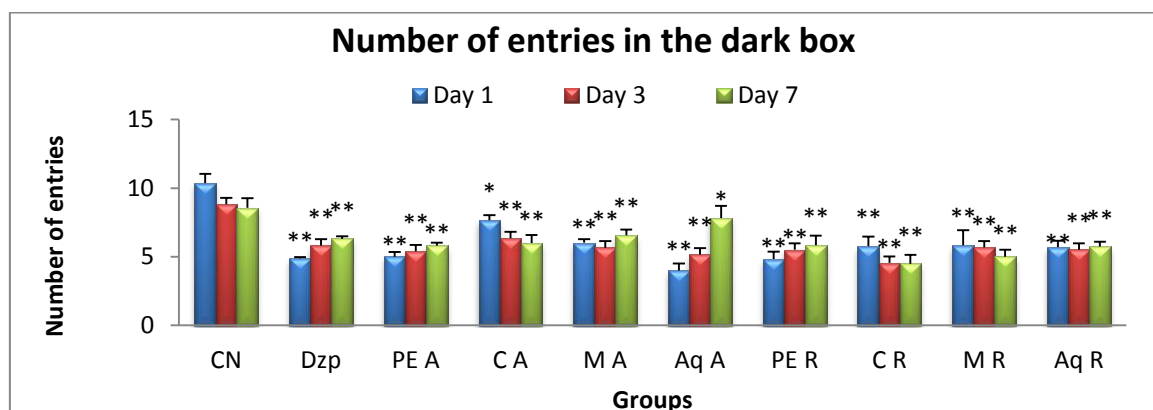


Fig. 14: Graphical representation of effect of various extracts of *E. himalaicus* on the number of entries in dark box.

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