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Research Article

EVALUATION OF ANXIOLYTIC ACTIVITY OF AERIAL PARTS OF *SARCOSTIGMA KLEINII* WIGHT AND ARN.

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ABSTRACT

Current therapeutic treatment for anxiety is associated with a wild variety of side effects. The traditional use of plant extract to health care can indicate an important source of new pharmaceuticals. The present study was designed to evaluate the anxiolytic effect of *Sarcostigma kleinii* through the ethanolic extract of its aerial parts. The study duration was 5 days, and Swiss Albino mice were subjected to dosage of 400 mg/kg. The anxiolytic effect of the ethanolic extract was evaluated in light and dark, elevated plus maze, and stair case models. The ethanolic extract was effective in inducing anxiolytic effects when compared with the control group. Finally, neurotransmitters such as serotonin (5-HT) and gamma-aminobutyric acid (GABA) was estimated in mice brain. Decreased serotonin (5-HT) and increased level of GABA was observed in ethanolic extract, when compared with the control group. The alkaloids, flavonoids, and other chemical constituents are speculated to account for the observed pharmacological effects of the plant extract in the experimental animal paradigms used. These findings suggest that the ethanolic extract from the aerial parts of *S. kleinii* exhibits significant anxiolytic activity.

Keywords: *Sarcostigma kleinii*, Anxiolytic activity, Gamma-aminobutyric acid, Serotonin.

INTRODUCTION

Human anxiety is defined as a feeling of apprehension, uncertainty or tension stemming from the anticipation of imagined or unreal threat [1]. Anxiety disorder is increasingly recognized as a highly prevalent and a chronic disorder with onset during the teenage years, with an incidence of 18.1% and in lifetime prevalence of 28.8%. The disorder is associated with a significant disability (including educational and occupational), which has a negative impact on the quality of life. Pharmacotherapeutic approaches for the management of anxiety disorders include psychotropic drugs, but these agents are limited by their side effects profile, the need for dietary precautions and drug interactions. Regular use of benzodiazepines causes deterioration of cognitive functioning, addiction, psychomotor impairment, confusion, aggression, excitement, anterograde amnesis, physical dependence and tolerance [2]. Numerous traditionally used plants exhibit pharmacological properties with great potential for therapeutic applications in the treatment of central nervous system disorders [3]. *Sarcostigma klenii* Wight and Arn (Icacinaceae) a large perennial woody climber grows up on large trees. Mainly found in sacred groves. The plant is also termed as odal, vellayodal, etc., plant possess various ayurvedic medicinal properties. Plant pacifies vitiated vata, arthritis, anorexia, worm infestation, skin diseases, hysteria, epilepsy, ulcers, and headache [4]. Based on the literature search, no study has been carried out to scientifically validate the folkloric uses of *Sarcostigma kleinii* Wight and Arn in the treatment of anxiety disorder. Hence, this study was carried out to investigate the anxiolytic effects of the *S. kleinii* Wight and Arn in mice and also the biochemical estimation of neurotransmitters in the anxiety state.

METHODS

Plant material

Collection and authentication of plant materials

Aerial parts of the plant *S. kleinii* Wight and Arn were collected from the place Pala, Kottayam district, Kerala during the month of February and authenticated by Asst. Prof. Rogimon P. Thomas, Department of Botany, C.M.S College, Kottayam, Kerala. The voucher specimens of the plant were kept in the library with register number DPS/MGU/RIMSR/HERB7, for further reference.

Processing of sample

The aerial parts of the plant were collected, cleaned thoroughly with distilled water and the desired plant parts were dried under shade for 30 days. The shade dried aerial parts were pulverized in a mechanical grinder to obtain coarse powder.

Preparation of extracts

The powdered plant was subjected to extraction by the soxhlet method using ethyl alcohol as a solvent. Evaporation of solvent from the extract was done by distillation method. A sticky mass were obtained after evaporation of the solvent. The samples were stored at 10°C till further use. At the time of administration, a suspension was prepared using the extract in 1% w/v of sodium carboxymethyl cellulose (sodium CMC).

Phytochemical analysis

Ethanolic extract of S. kleinii Wight and Arn

Ethanolic extract of *S. kleinii* Wight and Arn were subjected to preliminary phytochemical screening [5-7].

Experimental animals

Swiss Albino mice of either sex 20-30 g of body weight obtained from animal house, Department of Pharmacology, RIMSR, Kottayam. Animals were kept in standard animal house condition. Mice were housed in groups of six per cage. All the animals were maintained under standard conditions; that is room temperature 26±1°C, relative humidity 45-55% and 12:12 hrs light-dark cycle. The cages were maintained clean, and all experiments were conducted between 9 am and 4 pm.

Acute toxicity study

Female Swiss Albino mice (20-25 g weight) were used for acute oral toxicity study. The study was carried out as per the guidelines set by OECD 423 and animals were observed for mortality and behavioral changes [8].

Ethical approval
The experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC) of Regional Institute of Medical Sciences and Research Centre, Kottayam. (1702/po/c/06/CPCSEA2014) and all the experiments were conducted according to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Drugs and chemicals
Diazepam (Abbott Laboratory Limited), ethanol (Spectrum Chemicals), gamma-aminobutyric acid (GABA) (Sigma Aldrich), serotonin (Sigma Aldrich), O-phthaldialdehyde (OPT) (Hi Media), heptane (Spectrum Chemicals).

Pharmacological screening

Anxiolytic activity

Light and dark model
The apparatus consisted of a light and dark unit, consisting of a box with two distinct chambers, black and white, connected by a small open door way. The dark compartment (15 cm×40 cm) had its sidewalls and floor covered by a smooth black colored sun mica material. The bright compartment (40 cm×40 cm) had its side walls and floor covered by white colored sun mica material; it was brightly illuminated with a 60 W milky white bulb located 28 cm above the floor on the partition wall. An opening of (10 cm×10 cm) was located at a floor level in the center of the partition to allow access between two compartments for the animal [9]. Swiss albino mice (20-25 g) were taken and divided into three groups, each group comprised of six animals. Group A served as control and was administered with 1% sodium CMC, Group B with diazepam (2 mg/kg P.O) and served as a standard. Group C with extract (400 mg/kg P.O) for 5 days. On the 5th day 1 hr after oral administration of 1% sodium CMC/standard/extract to respective groups. Mouse is placed in the middle of the illuminated part of the cage.

The following parameters [10] are counted during 5 minutes period.

Percentage time spent in the light compartment was determined as follows:

$$\% = \frac{100 \times \text{Number of seconds spent in light compartment}}{300 \text{ total seconds (5 minutes observation time}}$$

Elevated plus maze model
The apparatus consist of two open arms (5 cm×30 cm) and two closed arms (5 cm×15 cm×30 cm) radiating from a platform (5 cm×5 cm) to form a plus-sign figure. The apparatus was situated 40 cm above the floor. The open-arm edges were 1 cm in height to keep the mice from falling, and the closed-arm edges were 15 cm in height [11]. Swiss albino mice (20-25 g) were taken and divided into three groups, each group comprised of six animals. Group A served as control and was administered with 1% sodium CMC, Group B with diazepam (2 mg/kg P.O) and served as a standard. Group C with extract (400 mg/kg P.O) for 5 days. On the 5th day 1 hr after oral administration of 1% sodium CMC/standard/extract to respective groups. Animal was placed at the center facing the open arm. During 5 minutes test period the following measures [10] are taken:

Percentage time spent on the open arms was determined:

- Percentage entries in open arms was determined:

$$\% = \frac{100 \times \text{Number of seconds spent on open arms}}{300 \text{ total seconds (5 minutes) observation time}}$$

- Percentage entries in open arms was determined:

$$\% = \frac{100 \times \text{Number of open arms entries}}{\text{Total entries in open and closed arms}}$$

Stair case model
Staircase consists of five identical steps 2 cm high, 10 cm wide and 7.5 cm deep. The internal height of the walls is constant along the whole length of the staircase [12]. Swiss albino mice (20-25 g) were taken and divided into three groups, each group comprised of six animals. Group A served as control and was administered with 1% sodium CMC, Group B with diazepam (2 mg/kg P.O) and served as a standard. Group C with extract (400 mg/kg P.O) for 5 days. On the 5th day 1 hr after oral administration of 1% sodium CMC/standard/extract to respective groups. Then animal is placed on the floor of the box with its back to the staircase. The number of steps climbed and the number of rears are counted over a 3 minutes period. A step is considered to be climbed only if the mouse has placed all four paws on the step. After every observation, the box is cleaned in order to eliminate any olfactory cues, which might modify the behavior of the next animal.

- The number of steps climbed and the number of rears are counted over a 3 minutes period.

Estimation of amino acids in the brain

Estimation of GABA

The level of GABA was estimated by multiple development paper chromatography method.

Experimental design

Swiss albino mice of either sex with body weight between 20 and 25 g were divided randomly into three groups of six animals each.

Group 1: Control; 1% sodium CMC (P.O)
Group 2: Standard; diazepam (2 mg/kg, P.O)
Group 3: EESK; (400 mg/kg, P.O).

All the treatment groups are pretreated with respective drug and dosage for 5 days. On the 5th day after oral administration of control/standard/EESK was given 60 minutes prior to scarification of animals for *ex-vivo* studies.

Assay-procedure
1.0 ml of the supernatant from brain homogenate was evaporated to dryness at 70°C in an oven and the residue is reconstituted in 100 ml of distilled water. Standard solution of GABA at a concentration of 2 μM along with the sample is spotted on Whatman no. 1 chromatography paper using a micropipette. It was placed on a chamber containing butanol:acetic acid:water (12 v/v:3 v/v:5 v/v) as solvent. When the solvent front reached the top of the paper, it was removed and dried. A second run is performed similarly, after which the papers are dried sprayed with ninhydrin reagent and placed in an oven at 100°C for 4 minutes. The portions, which carry GABA corresponding with the standard are cut and eluted with 0.005% $CuSo_4$ in 75% ethanol. Their absorbance is read against blank at 515 nm in a spectrophotometer and using statistical analysis graph was plotted with corresponding values [13].

Estimation of serotonin

Experimental design

Swiss Albino mice of either sex with body weight between 20 and 25 g were divided randomly into three groups of six animals each.

Group 1: Control; 1% sodium CMC (P.O)
Group 2: Standard; diazepam (2 mg/kg, P.O)
Group 3: EESK; (400 mg/kg, P.O)

All the treatment groups are pretreated with respective drug and dosage for 5 days. On the 5th day after oral administration of control/standard/EESK was given 60 minutes prior to scarification of animals for *ex-vivo* studies.

Preparation of tissue extracts

On the day of the experiment mice were sacrificed, whole brain was dissected out and the sub-cortical region (including the striatum) was separated. Wet tissue was weight and was homogenized in 5 ml HCl-butanol for about 1 minute. The sample was then centrifuged for 10 minutes at 2000 rpm. An aliquot supernatant phase (1 ml) was removed and added to centrifuge tube containing 2.5 ml heptane and 0.31 ml HCl of 0.1 M. After 10 minutes of vigorous shaking, the tube was centrifuged under the same conditions as above in order to separate the two phases, and the overlaying organic phase was discarded. The aqueous phase (0.2 ml) was then taken for 5-HT. All steps were carried out at 0°C.

Assay

Reagents: OPT reagent: (20 mg in 100 ml conc. HCl)

Procedure

To 0.2 ml, aqueous extract 0.25 ml of OPT reagent was added. The fluorophore was developed by heating to 100°C for 10 minutes. After the samples reached equilibrium with the ambient temperature, readings were taken at 360-470 nm in the spectrofluorimeter. Tissue blank were prepared by adding, 0.25 ml conc. HCI without OPT reagent. Internal standard: (500 µg/ml of serotonin are prepared in distilled water) HCl - butanol in 1:2 ratio [14].

Statistical analysis

All data were represented as mean±standard error mean values. Data were analyzed by one-way ANOVA. Whenever ANOVA was significant, further comparison was made against the vehicle treated groups were performed using the Dunnett's - tests.

RESULTS

Acute toxicity

Acute toxicity study for EESK was performed according to OECD guidelines 423 using female Swiss Albino mice. At 2000 mg/kg, the extract was neither produced mortality nor the signs of morbidity. Hence, the dose 400 mg/kg (1/5th of 2000 mg/kg) was selected for further studies.

Phytochemical analysis

The results of the chemical tests performed in the screening revealed the presences of flavonoids, alkaloids, tannins, carbohydrates, glycosides in the ethanolic extract of aerial parts of *S. kleinii* Wight and Arn (Table 1).

Assessment of anxiolytic activity

Light and dark model

In light and dark model, the ethanolic extract of aerial parts of *S. kleinii* at a dose of 400 mg/kg P.O and standard drug diazepam 2 mg/kg P.O, significantly increased the percentage of time spent in light side, when compared to control group (Fig. 1).

Elevated plus maze model

The ethanolic extract of aerial parts of *S. kleinii* at a dose (400 mg/kg P.O) and standard drug (diazepam 2 mg/kg P.O). Significantly increased the percentage time and percentage of entries into open arms as compared to the control group (Figs. 2 and 3).

Stair case model

The ethanolic extract of *S. kleinii* 400 mg/kg, P.O, and standard drug (diazepam 2 mg/kg, P.O) both were significantly reduced the number of rearing as well as the number of steps climbed, when compared to control (1% CMC) (Fig. 4).

Ex-vivo methods

Estimation of GABA in mice brain

The ethanolic extract of *S. kleinii* 400 mg/kg, P.O, and standard drug (diazepam 2 mg/kg, P.O) showed a significant increase in the GABA level, when compared to control (1% CMC) and the caliberation curve shows linearity (Table 2 and Fig. 5).

Table 1: Phytochemical analysis of aerial parts of *S. kleinii*

Phytochemicals	Ethanolic extract
Flavonoids	+
Alkaloids	+
Tannins	+
Phenols	+
Glycosides	+
Triterpenoids	+
Amino acids	–
Saponins	–

"+" Indicates presence, and "–" indicates absences of the phytochemical constituents. *S. kleinii: Sarcostigma kleinii*

Table 2: Effect of ethanolic extract of *S. kleinii* (400 mg/kg) showed significant increase in the GABA level in the mice brain, diazepam (2 mg/kg) showed more significant increase in the GABA levels in the brain

S.No	Treatment group	Amount of GABA in µg/g of Ww tissue
1	Control (1% CMC)	14.3±0.51
2	Standard (diazepam 2 mg/kg)	58.6±1.36**
3	EESK 400 mg/kg	35.1±0.98**

*p<0.05, **p<0.01, when compared to control. GABA: Gamma-aminobutyric acid, *S. kleinii: Sarcostigma kleinii*

Table 3: Effect of ethanolic extract of *S. kleinii* (400 mg/kg) and standard drug diazepam (2 mg/kg) showed a significant decrease in serotonin level in the mice brains

S.No	Treatment group	Amount of serotonin in µg/g of Ww tissue
1.	Control (1% CMC)	0.13±0.006
2.	Standard (diazepam 2 mg/kg)	0.013±0.0018**
3.	EESK 400 mg/kg (day 5)	0.056±0.0048**

*p<0.05, **p<0.01, when compared to control. CMC: Carboxy methyl cellulose, *S. kleinii: Sarcostigma kleinii*

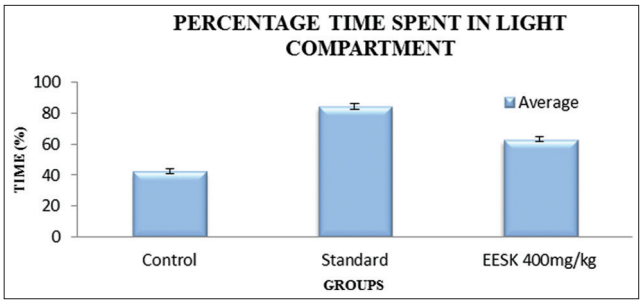

Fig. 1: Effect of ethanolic extract of *Sarcostigma kleinii* (400 mg/kg) and standard (diazepam 2 mg/kg) on percentage time spent by the mice on light area in light and dark model, *p<0.05, **p<0.01 when compared to control

Estimation of serotonin in mice brain

The ethanolic extract of *S. kleinii* 400 mg/kg, P.O, and standard drug (diazepam 2 mg/kg, P.O) showed a significant decrease in the serotonin level, when compared to control (1% CMC) and the caliberation curve shows linearity (Table 3 and Fig. 6).

DISCUSSION

Transitions have been reported to be an index of activity exploration because of habituation overtime and the time spent in each compartment to be a reflection of aversion. The percentage of

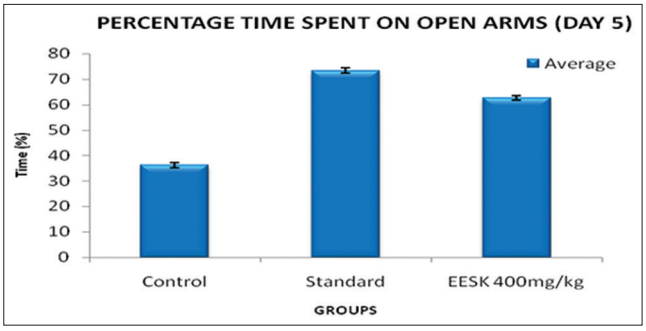

Fig. 2: Effect of ethanolic extract of *Sarcostigma kleinii* (400 mg/kg) and standard (diazepam 2 mg/kg) on percentage time spent by the mice in open arm in elevated plus maze, *p<0.05, **p<0.01 when compared to control

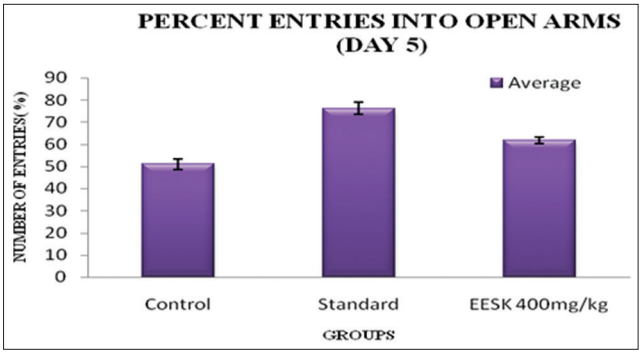

Fig. 3: Effect of ethanolic extract of *Sarcostigma kleinii* (400 mg/kg) and standard (diazepam 2 mg/kg), percentage entries into open arms by the mice in elevated plus maze, *p<0.05, **p<0.01 when compared to control

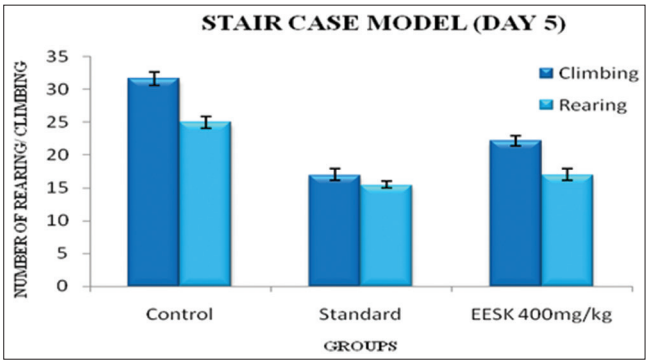

Fig. 4: Effect of ethanolic extract of *Sarcostigma kleinii* (400 mg/kg) and standard (diazepam 2 mg/kg) on number climbing and rearing by the mice in stair case apparatus, *p<0.05, **p<0.01 when compared to control

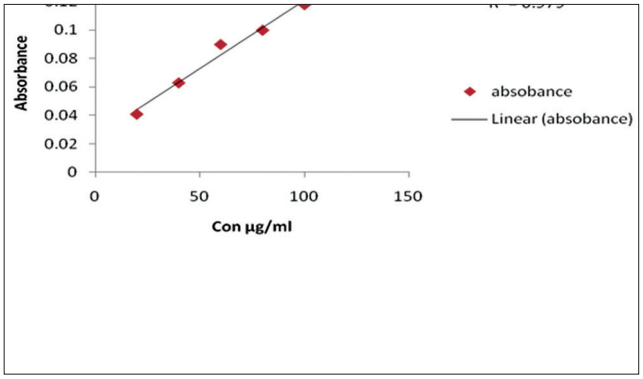

Fig. 5: The calibration curve for gamma-aminobutyric acid was plotted with concentration against absorbance at 550 nm

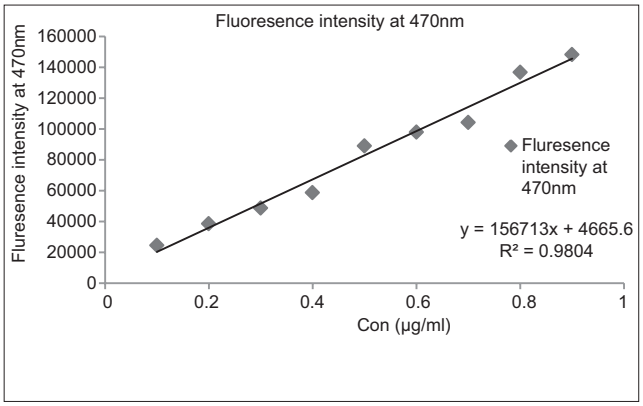

Fig. 6: The calibration curve for serotonin with concentration against fluorescence intensity a linear graph was plotted

time spent in the lit compartment is an index of the anxiety-related behavior. Anxiety is considered to be high if the percentage of time spent in the lit compartment is low. Following repeated administration of ethanolic extract of *S. kleinii* significantly increased the percentage time spent in the lit compartment, compared to the control group. The sub-chronic administration of EESK, in the elevated plus-maze test-induced an increase in the exploration and the percentage time spent (i.e. anxiolytic-like action) and the percentage number of entries into the open arms did not changed by the oral treatment with the EESK in comparison to the control values. The elevated plus-maze is a well-accepted, experimental animal model typically used to test the effectiveness of anxiolytics drugs. When animal introduced into a novel environment, rodents experience a conflict between anxiety and exploratory behavior manifested by increased vigilance and behavioral activity. In the staircase paradigm, step climbing is purported to reflect exploratory or locomotor activity or sedative activity while rearing behavior is an index of anxiety state. The present investigation successfully detected the anxiolytic-like effects of repeated administration of ethanolic extract of *S. kleinii* and diazepam both significantly decreased the number of rearing, and number of steps climbed, compared to control. The neurotransmitters such as GABA, and serotonin involved in the development of anxiety disorders. Estimation of GABA levels by multiple development paper chromatography follows that the principle of different partition coefficients that can be obtained from a stationary cellulose phase with a mobile solvent phase for different amino acids, which aid in their separation. Ethanolic extract of *S. kleinii* and standard drug diazepam (2 mg/kg) showed a significant increase in the GABA level in the brain when compared with the control animals. Estimation of serotonin by the spectrofluorometric method discuss that activation and fluorescence spectra derived from 5-HT was described by the formation of 5-HT - OPT complex. Ethanolic extract of *S. kleinii* (400 mg/kg) and standard drug diazepam (2 mg/kg) showed a significant decrease in the serotonin level in the brain when compared with the control animals.

CONCLUSION

The chemical investigation of the plant was not able to identify specific bioactive compounds, but the phytochemical group was associated with the medicinal effects. It can be tannins, alkaloids, flavonoids, terpenoids and glycosides. Many of these phytochemical groups are known individually to have specific health benefits. Based on these, the effect of ethanolic extract of *S. kleinii* was studied in different animal models. The results of the work provided evidence that the ethanolic extract

of *S. kleinii* may contain phytoconstituents that can suppress anxiety. Thus, *S. kleinii* may be a promising candidate for future development as a new anxiolytic drug.

REFERENCES

1. Kulkarni SK, Reddy DS. Animal behavioral models for testing antianxiety agents. Methods Find Exp Clin Pharmacol 1996;18(3):219-30.
2. Suresh K, Anupam S. Apigenin. The anxiolytic constituent of *Tumera aphrodisiaca.* Pharm Biol 2006;44:84-90.
3. Carlini EA. Plants and the central nervous system. Pharmacol Biochem Behav 2003;75:501-12.
4. Warrier PK, Nambiar VP, Ramankutty C. Indian Medicinal Plants A Compendium of Species. Vol. 5. Hyderabad: Orient Longman; 1993. p. 76-9.
5. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy. 29th ed. Pune: Nirali Prakasan; 2005. p. 225.
6. Mukherjee PK. Qualitative analysis of plant extract for phytochemical constituents. Pharmacognosy. 1st ed. Horizon Pharmaceutical Publishers; 2002.
7. Evan WC. Trease and Evans Pharmacognosy. 14th ed. India: Harcourt Brace and Company; 1998.
8. OECD Guideline 423, for Testing of Chemicals. Acute Oral Toxicity – Acute Toxic Class Method. December, 2001.
9. Ankitkumar A, Ashok M, Jyothsna V, Radhakrishna B, Gowda KP. Evaluation of anxiolytic activity of aqueous and alcoholic extracts of leaves of *Crataegus oxycantha* in mice. Int J Pharm Biomed Sci 2008;2(3):86-9.
10. Gilhotra N, Dhingra D. Possible involvement of GABAergic and nitriergic systems for antianxiety-like activity of piperine in unstressed and stressed mice. Pharmacol Rep 2014;66(5):885-91.
11. Baretta IP, Felizardo RA, Bimbato VF, dos Santos MG, Kassuya CA, Gasparotto Junior A, *et al.* Anxiolytic-like effects of acute and chronic treatment with *Achillea millefolium* L. extract. J Ethnopharmacol 2012;140(1):46-54.
12. Kavya Sree K, Vijusha M, Rajani A, Hemamalini K. Screening of behavioural, muscle co-ordination and anxiolytic activities of methanolic extract of *Holoptelea integrifolia* (ROXB). Int Res J Pharm 2013;4(11):90-4.
13. Rahman H, Muralidharan P, Anand M. Inhibition of AChE and antioxidant activities are probable mechanism of *Nardostacys jatamansi* DC in sleep deprived Alzheimer's mice model. Int J Pharm Technol Res 2011;3(3):1807-16.
14. Curzon G, Green AR. A rapid and sensitive method for measuring 5-hydroxytryptamine and 5-hydroxyindoleacetic acid, using O-phthaldehyde and L-cysteine, is presented, enabling both compounds to be measured in small areas of rat brain. Br J Pharm 1970;39:653-5.