

## EVALUATION OF ANTI-ANXIETY ACTIVITY OF SILICA GEL ENTRAPPED TRADITIONAL MEDICINE

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### ABSTRACT

**Objectives:** The present study has been designed to compare the anxiolytic property of Makaradhwaja (a well-known traditional Ayurvedic medicine) and gel encapsulated Makaradhwaja in laboratory animals.

**Methods:** Makaradhwaja has been encapsulated into silica gel matrix to enhance the bioavailability and potency. The proper formation of the gel has been evidenced by the transmission electron microscopy micrograph and Fourier transform infrared (FTIR) spectrometry. Due to the presence of Makaradhwaja into silica gel matrix broad peaks at  $3474\text{ cm}^{-1}$  and  $1637\text{ cm}^{-1}$  were detected in FTIR spectrum. The UV-visible spectroscopic analysis reveals sustainable and controlled release (up to 50%) of the drug for more than 240 hrs. The anti-anxiety activity was studied by exposing the mice into two different anti-anxiety models like elevated plus maze (EPM) and actophotometer.

**Results:** The gel encapsulated Makaradhwaja has shown more potent anxiolytic effect than the crude Makaradhwaja in the EPM (significant increase in the number of entries and time spent in the open arm). The number of locomotor score in actophotometer has also been significantly increased by the gel encapsulated Makaradhwaja.

**Conclusion:** Present study confirms that the gel encapsulated Makaradhwaja has significant anxiolytic activity which is comparable with standard anxiolytic diazepam.

**Keywords:** Actophotometer, Makaradhwaja, Elevated plus maze.

### INTRODUCTION

Anxiety is extremely dramatic, debilitating, and complex disorder. This widespread psychiatric disorder (affecting one-eighth of the worldwide population) [1] is mostly affecting the elderly population. Human anxiety is defined as a feeling of apprehension, uncertainty or tension stemming from the anticipation of imagined or unreal threat [2]. Number of scientific works has been done on the various neurobiological aspects of anxiety.

Benzodiazepines are the one of the most common medicines that is being widely used in anxiety disorders. But the clinical applications of benzodiazepines as anti-anxiety agents are limited because of unwanted side effects. Due to this many pharmaceutical companies are conducting studies to find out alternative medicines or plant-derived medications with more specific anxiolytic effects [3]. The traditional medicine of Ayurveda has a close relationship with philosophy since antiquity. The great scholars of Ayurveda in the ancient era used to believe in the role of psychology in the generation of diseases. According to traditional medicine Makaradhwaja (a compound of purified mercury, sulfur and gold) is very much effective in degenerative diseases, diabetes, cardiac and respiratory diseases, acute and chronic infections, insomnia, and mental anxiety [4]. It also has the property of stress adaptation. It can enhance the power of immunity and also can be used as a rejuvenating agent [5-7]. This medicine is odorless, tasteless, fine powder with bright red color [5]. In the present study, a sustainable and controlled release drug delivery system had been developed by entrapment of Makaradhwaja powder into silica gel matrix [8,9]. For last few years, controlled drug delivery systems have become an important area for the researcher. Controlled drug delivery would be the optimal way for drug administration if the drug could be precisely matched with physiological needs [10] Makaradhwaja entrapped into the gel matrix is biologically more active in comparison to the crude drug and no significant physical and behavioral change has

been observed in the treated animals in acute oral toxicity study [8]. After encapsulation, characterization has been done with the help of different analytical methods such as Fourier transform infrared (FTIR) spectrometry, transmission electron microscopy (TEM), and UV-visible spectroscopy [9]. The objective of adopting analytical methods was to authenticate the method of encapsulation. Despite a long tradition of use, no work has been carried out to evaluate central nervous system depressant properties of the silica gel encapsulated Makaradhwaja. Thus, the present study has been done to determine the anti-anxiety activity of silica gel entrapped Makaradhwaja. In compare to powder Makaradhwaja by using two different anti-anxiety models i.e. Elevated plus maze (EPM) and actophotometer. Diazepam (Ranbaxy Laboratories Limited) was used as the standard anxiolytic drug [11].

### METHODS

#### Source of the crude drug (Makaradhwaja)

The powder Makaradhwaja used in this study has been kindly supplied by the Dr. P. K. Prajapati of Department of Rasashastra and Bhaishajya Kalpana, Gujrat Ayurved University, Jamnagar.

#### Encapsulation of Makaradhwaja into silica gel matrix

The silica gel encapsulated Makaradhwaja used in this study has been prepared in the Department of Metallurgical and Material Engineering at Jadavpur University, Kolkata-700032. The hydrolysis and condensation reactions are the two main steps for the synthesis of silica gel. The hydrolysis reaction (that can be either acid or base catalyzed) replaces oxide groups with hydroxyl groups. Siloxane bonds (Si-O-Si) are formed during the condensation reaction. Alcohol and water are the by-products of the condensation reaction and both evaporate during drying. All silica gels were synthesized by a room temperature process. Tetraethyl orthosilicate (TEOS, purchased from Sigma Aldrich) has been used as precursor of silica. Addition of the aqueous solution of Makaradhwaja during the formation of the oxide backbone at room

temperature facilitates their encapsulation within the oxide matrix leading to the production of composite gel with the active ingredient homogeneously distributed throughout the gel. The prepared gel was subjected to drying until the weight (of the gel) became constant. The processes of gel formation, condensation and drying, produce a glassy solid gel matrix (Fig. 1). In this way, Makaradhwaja were entrapped into silica gels [8,9].

#### Characterization of silica gel entrapped Makaradhwaja

The TEM analysis of the synthesized gel has been done by using HRTEM JEOL-TEM 2100 to obtain the image of drug entrapped silica gel matrix. The optical absorption spectra of gel entrapped Makaradhwaja over 300-800 nm were taken (to study the percentage of drug release against time) by using Perkin Elmer, Lambda 35 UV-Visible spectrophotometer and the FTIR spectrum was taken using Shimadzu (Model: Prestige 22) FTIR spectrophotometer.

#### Standard drug

Diazepam (Ranbaxy Laboratories Limited) was used as the standard anxiolytic drugs. Distilled water was used as vehicle [12].

#### Animals

Animal studies were conducted according to the Institutional Animal Ethical Committee regulations and were approved by the Committee for the purpose of the control and supervision of experiments on animals. The experiments were conducted on swiss albino mice (weighing about 25-35 g) of either sex. The animals were divided into four groups, each consisting six mice and were used in all sets of experiments. The animals were maintained in a well-ventilated room under standard laboratory conditions (temperature  $25\pm 2^\circ\text{C}$ ) with 12:12 hr light/dark cycle in polypropylene cages. Standard pellet feed and drinking water was provided *ad libitum*. Animals were acclimatized to laboratory conditions 1 week prior to initiation of experiments [11,12].

#### Acute toxicity study

The acute oral toxicity study of silica gel entrapped Makaradhwaja has been performed on laboratory animals. Swiss albino mice of either sex (weight about 25-35 g) were used for the study. The experimental procedures related to the animals were authorized by the Institutional Animal Ethical Committee (Proposal No. IAEC/2012/08). The mice were observed scrupulously for any indications of toxicity effect for a period of 14 days. Visual observations for mortality, behavioral pattern, changes in physical appearance and signs of illness were conducted daily during this period [8,11,12].

#### Anti-anxiety activity study

The test drugs i.e. Makaradhwaja and silica gel entrapped Makaradhwaja were tested for anti-anxiety activity by using EPM and actophotometer.

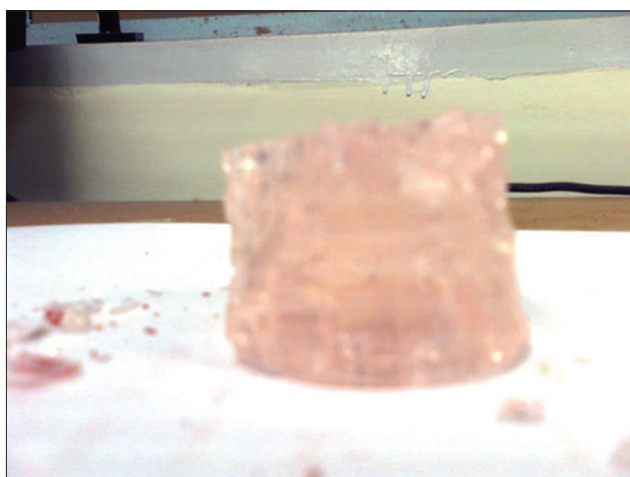


Fig. 1: The image of the synthesized gel

#### Preparation of test doses

Therapeutic dose of Makaradhwaja is 2 mg/kg body weight daily in case of the human being [13]. The suitable dose for mice has been calculated, and the same amount of Makaradhwaja and active ingredient of gel entrapped Makaradhwaja were used in the present study. The test drugs (i.e. Makaradhwaja and silica gel entrapped Makaradhwaja) were suspended in the vehicle and prepared for administration through the oral route. Animals were divided into four groups each group having six animals. Group 1 was a control group and was treated with vehicle i.e. Distilled water; Animals of Group 2 were treated with standard drug, diazepam (2 mg/kg, orally), suspended in the vehicle. Animals of Group 3 and Group 4 were treated with test drug Makaradhwaja and silica gel entrapped Makaradhwaja, respectively. All the behavioral tests were performed 60 minutes after the administration of respective treatments [12].

#### EPM model

The anti-anxiety activity was evaluated using EPM model in Swiss albino mice of either sex (weight about 25-35 g). It is a well-established animal model for testing anxiolytic drugs. The EPM apparatus, consisting two open arms (16 cm  $\times$  5 cm) and two closed arms (16 cm  $\times$  5 cm  $\times$  12 cm) extended from a common central platform (5 cm  $\times$  5 cm). The entire maze is elevated (25 cm) from the floor having an open roof. After proper treatment, each mouse was placed at the center of the maze with its head facing the open arm.

The stopwatch was started, and following parameters were noted for 5 minutes. (a) First preference of mice to open and closed arm. (b) Number of entries in open and closed arms (an arm entry defined as the entry of four paws into the arm) (c) Average time each animal spends in each arm (average time = total duration in the arm/number of entries). The open-arm entries and open-arm time were used as indices of anxiety [2,11,14].

#### Locomotor activity

The locomotor activity was measured by using an actophotometer. Six lights and six photocells are placed in the outer periphery of the bottom in such a way that a single mouse can block only one beam. Technically, its principle is that a photocell is activated when the rays of light falling on photocells are cut off by animals crossing the beam of light. Photocells are connected to an electronic automatic counter device which counts the number of "cut-offs." When the movement of the animal interrupts a beam of light falling on a photocell, a count was recorded and displayed digitally. After proper treatment, the animals were kept in the actophotometer individually, and the basal activity score of each animal were recorded for a period of 10 minutes [2,15].

## RESULTS AND DISCUSSION

#### Characterization of silica gel entrapped Makaradhwaja

Fig. 2 represents the TEM image of the synthesized gel. The TEM image of the sample is the proof of the formation of a silica matrix with network-like porous structures. Pores of the silica gel act as the host of the drug molecule. Rigid framework like structure of the gel matrix stabilizes the entrapped drug molecule and prevents their leakage. Release kinetics in a simulated body fluid (SBF) has been subsequently observed. The concentration of SBF buffer is nearly equal to human blood plasma. The UV-visible spectroscopy reveals up to 50% release of the drug molecule in the SBF in 240 hrs (Fig. 3). In FTIR spectrum of the gel entrapped Makaradhwaja (Fig. 4) the broad peaks at  $3474\text{ cm}^{-1}$  and  $1637\text{ cm}^{-1}$  were formed due to the characteristic OH stretching ( $\gamma\text{-OH}$ ) and H OH bending ( $\delta\text{ OH}$ ) vibrational band due to adsorbed water in the sample.

#### Acute toxicity study

Acute oral toxicity studies revealed the nontoxic nature of test drugs. There was no morbidity observed or any profound toxic reactions found which indirectly pronouns the safety profile of the test drugs. The test

drug did not produce any mortality up to the ten times of its therapeutic dose (2 mg/kg body weight, orally) even after encapsulation in porous gel matrix. Hence, it can be concluded that the test drug is without any toxic potential at ten times of its therapeutic dose even after encapsulation [8].

**EPM model**

The fear due to height induces anxiety in the animals when placed on the EPM. The ultimate manifestation of anxiety and fear in the animals is exhibited by decrease in the motor activity and preference to remain at safer places. Anti-anxiety agents are expected to increase the motor activity [16], which is measured by the time spent by the animal in the open arms. The conventional EPM is highly sensitive to the influence of both anxiolytic and anxiogenic drugs acting at the GABA<sub>A</sub>-benzodiazepine complex. This animal model is considered one of the most widely validated tests for assaying sedative and anxiolytic substances like the benzodiazepines. In EPM, naïve mice will normally prefer to spend much of their allotted time in the closed arms. This preference appears to reflect an aversion towards open arms that is generated by the fears of the open spaces [17]. Drugs that increase open arm exploration are considered as anxiolytics [12]. The data of Anti-anxiety activity of silica gel entrapped Makaradhwaja and powder Makaradhwaja evaluated by elevated plus-maze have been presented in Table 1. The Anti-anxiety activity study showed that both the number of open arm entries and time spent in the open arms were significantly increased in case of the animals treated with silica gel

entrapped Makaradhwaja compare to the animals treated with powder Makaradhwaja thereby producing anti-anxiety activity. The silica gel entrapped Makaradhwaja exhibit significant anti-anxiety effect which is comparable with the effect of standard drug Diazepam. In the other hand, the powder Makaradhwaja has produced minimal anti-anxiety effect. Figs. 5 and 6 show the comparison of the anti-anxiety effects in between the silica gel entrapped Makaradhwaja and powder Makaradhwaja and standard drug diazepam.

**Table 1: Effect of gel encapsulated Makaradhwaja and Makaradhwaja in laboratory animals in EPM model**

Group	Treatment	Time spent (sec) in open arm (Mean±SE)	Number of entries in open arm (Mean±SE)
1	Control	13±0.96	3.2±0.48
2	Diazepam	103±0.84***	14.2±0.48***
3	Makaradhwaja	18±0.96**	7±0.56***
4	Gel entrapped Makaradhwaja	64.8±1.44***	12.8±0.48***

SE: Standard error, EPM: Elevated plus maze, SEM: Standard error of the mean. Values are expressed as mean ± SEM; n=6; \*\*p<0.01- significant, \*\*\*p<0.001 - very significant

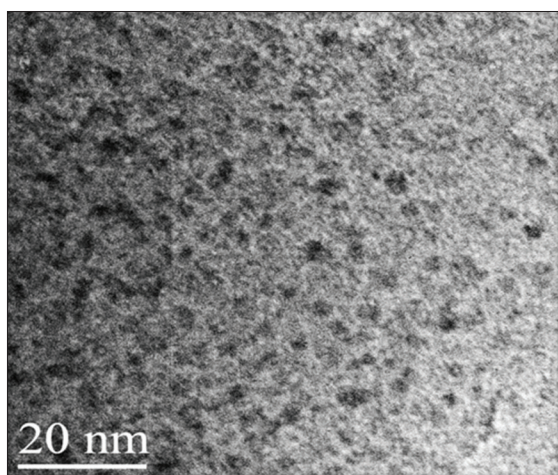


Fig. 2: Transmission electron microscopy images of the gel entrapped Makaradhwaja

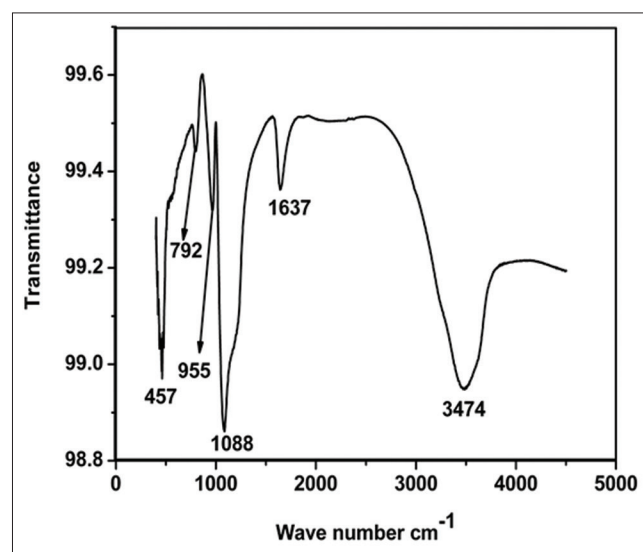


Fig. 4: Fourier transform infrared spectrum of the silica gel entrapped Makaradhwaja

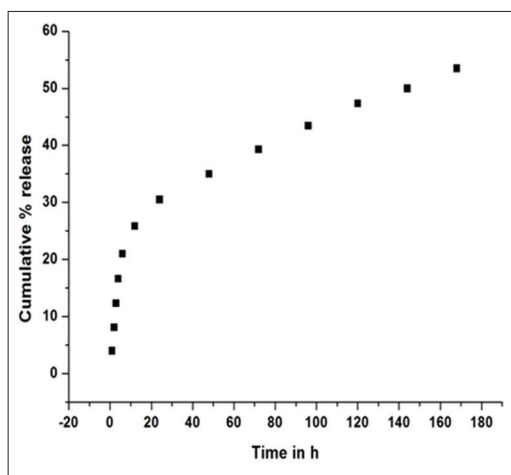


Fig. 3: The percentage of release of Makaradhwaja from silica gel matrix with time

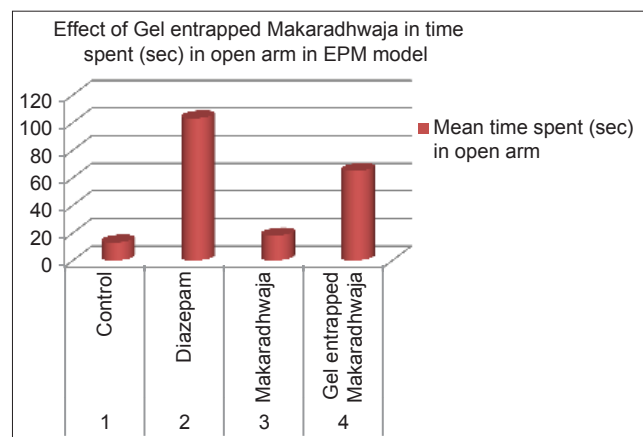


Fig. 5: The comparison in time spent in the open arm in elevated plus maze model among different treatment group

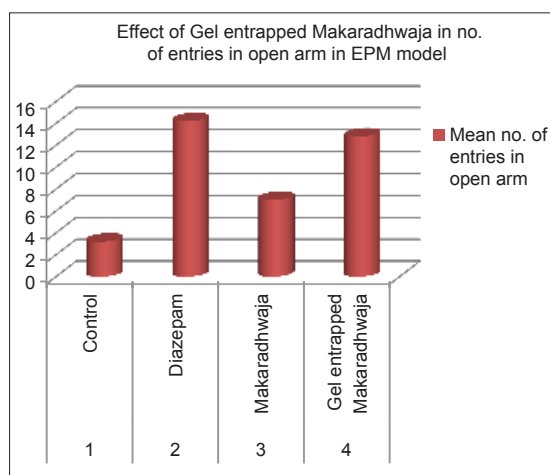
**Locomotor activity**

Table 2 shows the basal activity score (mean  $\pm$  standard error) in four groups recorded for 10 minutes. Locomotor activity is considered as an index of alertness, and the spontaneous decrease in basal activity score implicates the reduction of anxiety. Such types of effect can be found in the case of sedatives [12,16]. There is a significant decrease in the locomotor score in case of animal treated with the gel entrapped Makaradhwaja which is comparable to the effect of standard drug Diazepam. But in case of the animals treated with powder Makaradhwaja, minimal changes have been observed in comparison to gel entrapped Makaradhwaja. Fig. 7 shows the comparison of the

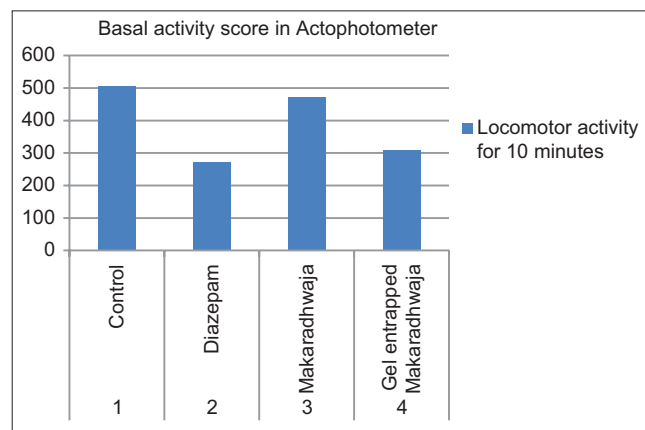
**Table 2: Effect of gel encapsulated Makaradhwaja and Makaradhwaja in laboratory animals in actophotometer model**

Group	Treatment	Locomotor activity for 10 minutes (mean $\pm$ SE)
1	Control	504.8 $\pm$ 2.1
2	Diazepam	271.5 $\pm$ 3.0***
3	Makaradhwaja	471.5 $\pm$ 2.5***
4	Gel entrapped Makaradhwaja	308.7 $\pm$ 5.4***

SE: Standard error; SEM: Standard error of the mean. Values are expressed as mean $\pm$ SEM; n=6; \*\*\*p<0.001 - very significant



**Fig. 6: The comparison in a number of entries in the open arm in elevated plus maze model among different treatment group**



**Fig. 7: The comparison in effects locomotor score among different treatment group in actophotometer model**

locomotor score among powder Makaradhwaja, silica gel entrapped Makaradhwaja and standard drug diazepam.

**CONCLUSION**

The gel encapsulated Makaradhwaja has shown significant anti-anxiety activity in both the animal models i.e. EPM and Acto-photometer. From the results of the study, it can be concluded that Makaradhwaja entrapped into the silica gel matrix exhibited more potent anti-anxiety activity than the crude drug (Makaradhwaja). The studies are under progress to evaluate other pharmacological actions of test drug.

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**REFERENCES**

- Eisenberg DM, Davis RB, Ettner SL, Appel S, Wilkey S, Van Rompay M, *et al.* Trends in alternative medicine use in the United States, 1990-1997: Results of a follow-up national survey. *JAMA* 1998;280(18):1569-75.
- Kulkarni SK, Reddy DS. Animal behavioural models for testing anti-anxiety agents. *Methods Find Exp Clin Pharmacol* 1996;18(3):219-30.
- Rabbani M, Sajjadi SE, Mohammadi A. Evaluation of the anxiolytic effect of *Nepeta persica* Boiss. in mice. *Evid Based Complement Alternat Med* 2008;5(2):181-6.
- Ramchandra Reddy K. Text Book of Rasa Shastra. 1<sup>st</sup> ed., Vol. 5. Varanasi: Chaukhamba Sanskrit Bhawan; 2007. p. 231-4.
- Debnath PK. Molecules of metals and minerals in Ayurveda: The interior science for health and diseases. In: Gupta B, editor. *Papers on the Ayurvedic Studies*. 1<sup>st</sup> ed. Kolkata: The Asiatic Society; 2006. p. 124-40.
- Dhundhukanath, Chintamani R, Mishra SN. Hindi Commentary. 1<sup>st</sup> ed., Vol. 8. Varanasi: Chaukhamba Orientalia; 2006. p. 112-3.
- Prajapati PK, Joshi D, Dube GP. An experimental study on Makaradhwaja. *Anc Sci Life* 1997;16(4):302-6.
- Bhattacharyya L, Debnath PK, Hajra J, Upadhyay SN, Mitra MK. Development of controlled drug delivery system for a traditional medicine and study of its antimicrobial property. *Asian J Pharm Technol Innov* 2014;02(08):65-73.
- Shula R, Paul D. Nanostructural control of implantable xerogels for the controlled release of biomolecules. *University of Pennsylvania. Sch Commons* 2003;171:59-74.
- Hans ML, Lowman AM. Biodegradable nanoparticles for drug delivery and targeting. *Curr Opin Solid State Mater Sci* 2002;6:319-27.
- Goyal S, Kumar S. Anti-anxiety activity studies of various extracts of *Pulsatilla nigricans* stoeck. *Int J Pharm Sci Drug Res* 2010;2(4):291-3.
- Chakraborty A, Amudha P, Geetha M, Singh SN. Evaluation of anxiolytic activity of methanolic extract of *Sapindus mukorossi gaertn.* in mice. *Int J Pharma Bio Sci* 2010;1(3):1-8.
- Sinyorita S, Ghosh CK, Chakraborty A, Auddy B, Ghosh R, Debnath PK. Effect of Ayurvedic mercury preparation Makaradhwaja on geriatric canine – A preliminary study. *Indian J Exp Biol* 2011;49(7):534-9.
- Sundaraganapathy R, Niraimathi V, Thangadurai A, Kamalakannan D, Narasimhan B, Deep A. Anti-anxiety activity of *Gloriosa superba* linn. *Hygeia J Drugs Med* 2013;5(1):148-51.
- Kulkarni SK, Dandiya PC. Hand book of experimental pharmacology. *Indian J Med Res* 1975;63:462-8.
- Hafeen SS, Srinath Reddy T, Arafath S, Nagarjuna S, Padmanabha Reddy Y. Evaluation of anti-anxiety and antidepressant activity of *Cassia occidentalis* leaves. *Asian J Pharm Clin Res* 2012;5(3):47-50.
- Harsha SN, Anilakumar KR. Anxiolytic property of hydro-alcohol extract of *Lactuca sativa* and its effect on behavioral activities of mice. *J Biomed Res* 2013;27(1):37-42.