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Research Article
Ameliorative Potential of *Lagenaria sicereria* Extract as Anti-Anxiety Drug in Various Models of Anxiety in Rats

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Abstract

Background and Objective: Anxiety is an emotional state with imbalance of neurotransmitters like Gamma Amino Butyric Acid, serotonin and leads to feeling of discomfort, concern or fears to define or undefined objects/situation. The aim of present study was to evaluate the potential effect of *Lagenaria sicereria* extracts in various models of anxiety in rats. **Materials and Methods:** Three well established models (Elevated Plus maze, Light Dark and social interaction in rats) were selected for evaluation of anxiety in rats. The protocol was designed by giving drugs for seven days and various behavioral parameters like Time spent in the open and closed arm, No. of entries in each arm, Latency, Time spent in light and dark compartment, No. of crossings, Immobility, Sniffing, Crawling and Aggressive behaviors were evaluated on 1st, 3rd and 7th day of study. The rats were treated at two doses (250 and 500 mg kg^{-1}, p.o.) of methanolic extract of *Lagenaria sicereria* (MELS) leaf. The dose selections were based on acute toxicity study in mice. The standard drug, Fluoxetine (10 mg kg^{-1}, p.o.) were also given to compare its beneficial effects in anxiety. At the end of an experiment, all animals were subjected to dissection and serotonin and GABA levels were determined in brain tissues. **Results:** MELS was found to possess a therapeutic effect against anxiety disorder. The Rats treated with 500 mg kg^{-1}, p.o. showed significant changes in behavioral and mobility in all three models during experiment. **Conclusion:** From the present findings, it was concluded that MELS extracts poss significant anxiolytic effects in rats and its due to modulation of GABA and serotonin level in brain tissues of rats.

Key words: Anxiety, elevated plus maze, light dark and social interaction in rats

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Anxiety is a complex progressive behavioral and physiological alteration of the organism which ultimately leads to a wide variety of CNS disorders if remain untreated. Traditional sources like most of the herbals are in great demand in both establish and developing countries so why not the natural and synthetic herbal molecules preferred to treat such disease with fewer side effects and less cost[1]. The only problem faced during their commercialization of herbals is standardization and purity of compounds. These herbals also preferred due to fewer withdrawal effects to the patients during treatment[2]. The biological screening/ drug discovery involves the preclinical findings to establish its therapeutic category[3]. The plant *Lagenaria siceraria* (Molina) standley (LS) (Family: Cucurbitaceae) is an annual herbaceous climbing plant with a long history of traditional medicinal uses in many countries, especially in tropical and subtropical regions. Since ancient times, the climber has been known for its curative properties and has been utilized for treatment of various ailments, including jaundice, diabetes, ulcer, piles, colitis, insanity, hypertension, congestive cardiac failure (CCF) and skin diseases. Its fruit pulp is used both as an emetic and purgative and for its cooling, diuretic, antibilious and pectoral properties. Plant fruit pulp is used to treat rheumatism and insomnia. A wide range of chemical compounds was reported in this species like sterols, terpenoids, flavonoids and saponins[4-7]. This plant was reported for various pharmacological activities like Analgesic, Antinflammatory[8], Antihyperlepedemic[9], Diuretic[10], Antithelmentic[11], anti Hepatic[12], Immunomodulatory, Antimicrobial[13] and Antioxidant[14, 15]. Previous Studies indicate its potential use in neurological disorders[14,15]. So, The current study designed using three animal models of anxiety to evaluate the anti-anxiety effect of *Lagenaria sicereria* extracts. This study will explore the traditional use of this plant with molecular mechanism to affect anxiety.

MATERIALS AND METHODS

Drugs and chemicals: All the chemicals like Fluoxetine, Dihydrogen Orthophosphate, etc. used for the study were of analytical grade, procured from Himedia Laboratories (Mumbai).

Plant Material: Standardized methanolic extract of *Lagenaria sicereria* leaves was procured from Herbo Nutra, New Delhi, India. The extract dose was prepared using 2% Carboxy methyl cellulose (CMC) as suspending agent prior to oral administration.

Animals: Wistar rats and albino mice of either sex weighing 180-200 g, 25-30 g, respectively, were procured from registered breeder and the environmental conditions were maintained. They were housed separately in a group of 6 animals each in the propylene cage with standard pellet chow and water. The experimental protocols were approved by as per guidelines provided by the Institutional Animal Ethics Committee and through governing body.

Acute toxicity studies: LD_{50} was determined according to the guidelines of Organization for Economic Operation and Development (OECD) following the up and down method (OECD guideline No. 423) and fixed dose method (OECD guideline No. 420). Based on these guidelines a limit test was to categories the toxicity class LD_{50} of the compound. The limit test was performed at 2500 mg kg^{-1}, p.o. A dose range of 250 and 500 mg kg^{-1}, p.o. was selected for the pharmacol.

Phytochemical screening: Phytochemical screening of plant extracts was done by the methods described by Kokate[17].

Total phenol quantification: Determination of total phenolic content of freeze-dried extracts of *Lagenaria sicereria* was performed by the Folin-Ciocalteu method[16]. The absorbance was recorded at 725 nm against the reagent blank with a double beam UV/Visible spectrophotometer (EI model No. 5512, Japan). The amount of total phenols was calculated as pyrogallol equivalents from the calibration curve by linear regression.

Estimation of total flavonoid: The Aluminum chloride colorimetric technique was used for estimation of flavonoids[17]. The Methanolic fraction (0.5 mL each) was taken (100 mg mLG1 of ethanol) in test tube and mixed with 1.5 mL of methanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M potassium acetate and 2.8 mL of distilled water. The reaction proceeded at room temperature for 30 min and the absorbance was subsequently measured at 415 nm. The calibration curve was plotted by preparing quercetin standard solution across a range of 10-70 ppm in methanol. The amount of flavonoids was calculated from the standard quercetin graph.

Experimental models: Grouping of animals:

Group 1: Control/saline treated
Group 2: Standard/Fluoxetine
Group 3: MELS (250 mg kg^{-1}, p.o.)
Group 4: MELS (500 mg kg^{-1}, p.o.)

Six animals were used for each dosage. Each group was treated for seven days before stress induced.

Elevated plus maze model: The test procedure and scoring methodology for elevated plus-maze test has been described by Kumar *et al.*[18]. In brief, the experiment was conducted between 9:00 and 16:00 h. to facilitate adaption to the new surroundings. Rats were transported to the laboratory at least 1 h prior to testing. The trial was started by placing an animal on the central platform of the maze facing an open arm. Standard 5 min test duration was used for subjects, the maze was thoroughly cleaned. Rats were randomly allocated to the following groups: vehicle control, positive control; Fluoxetine (10 mg kg^{-1}, p.o.) and test drugs (250 and 500 mg kg^{-1}, p.o.). The experiments were performed with an observer aware of the treatment of the rats inside the room. The following parameters were classically measured during this test: Frequency and duration (s) of arm visits, separately for open and closed arms.

The percentage of entries into open arms, closed arms (open or closed arm entries/total arm entries×100; % open or closed arm entries).

The percentage of time spent in open or closed arms (open or closed arm time/total arm time×100; % open or closed arm time) were used as traditional indices of the anxiety. In addition, the latency time (time spent in the center of the maze), was also recorded[19-21].

Light dark model: The apparatus used for this study, is an open-top wooden box with two chambers, a black chamber (20×30×35 cm) illuminated with dimmed red light and a bright chamber (30×30×35 cm) 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 of the center of the partition. Each animal was placed in the bright and dark arena paradigm. Study was carried out, 60 min. after the drug administration [fluoxetine (10 mg kg^{-1}, p.o.), test drugs (250 and 500 mg kg^{-1}, p.o.)] and vehicle administration, the animal was placed in the center of the brightly lit arena in the light and dark box. 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. Hand operated counters and stop watches were used to score the behavior of animals and experiments were performed with an observer inside the room[18-21].

Social interaction in rats: Male wistar rats were housed in groups of six animals. The apparatus used for the detection of changes in social behavior and exploratory behavior 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 (fluoxetine 10 mg kg^{-1}, p.o.) and test drug (250 and 500 mg kg^{-1}, p.o.). They were placed inside the box (with 60 W bright illumination 17 cm above) and their behavior is observed over a 10 min of the period and two test conditions were performed: high light, unfamiliar arena (HU) and high light, familiar arena (HF). Two types of behavior 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 behavior. Five pairs are used at each dose[22].

Tissue extraction: The tissue (1.55 mg) was homogenized in 0.1 mL HCl-butanol (O-85 mL 37% HCl in 1 L n-butanol for spectroscopy) for 1 min in a glass homogenizer made from a small centrifuge tube (vol. 1.5 mL). The total volume was considered to give O-105 mL, taking account of the tissue volume (1 mg = 0001 mL). The sample was then centrifuged for 10 min at 2000 g. An aliquot of the supernatant phase (0.08 mL) was removed and added to an Eppendorf reagent tube (vol. 1.5 mL) containing 0.2 mL heptanes (for spectroscopy) and 0.025 mL HCl 0.1 M. After 10 min 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 (0.02 mL) was then taken either for 5-HT assay. All steps were carried out at 0°C[23].

Serotonin assay: As mentioned earlier, some modifications in reagent concentration became necessary, together with changes in the proportions of the solvents, in order to obtain a good fluorescence yield with the reduced volumes. For 5-HT determination, the o-phthaldialdehyde (OPT) method was employed. From the OPT reagent (20 mg (%) in cont. HCl) 0025 mL was added to 0.02 mL of the HCl extract. The fluorophore was developed by heating to 100° for 10 min. After the samples reached equilibrium with the ambient temperature, excitation/emission spectra or intensity readings at 360-470 nm were taken in the micro cuvette described above[24].

Gamma amino butyric acid (GABA) estimation: it was determined from whole brain and was isolated immediately to

be transferred to homogenization tube containing 5 mL of 0.01 M hydrochloric acid. Brain homogenate was transferred to bottle containing 8 mL of ice cold absolute alcohol and kept for 1 h at 0°C. The content was centrifuged for 10 min at 16,000 rpm, supernatant was collected in a Petridis. Precipitate was washed with 5 mL of 75% alcohol for three times and washes were combined with supernatant. Next, samples were evaporated to dryness at 70°C on water bath. To the dry mass 1 mL water and 2 mL chloroform were added and centrifuged at 2000 rpm. Upper phase containing GABA (2.0 mL) was separated and 10 mL of it was applied as spot on Whatman paper (N°41). The mobile phase consisted of n-butanol (50 mL) acetic acid (12 mL) and water (60 mL). The paper chromatogram was developed with ascending technique. The paper was dried in hot air and then spread with 0.5% ninhydrin solution in 95% ethanol. The paper was dried for 1 h at 90°C. Blue color spot developed on paper was cut and heated with 2 mL of ninhydrin solution on water bath for 5 min. Water (5.0 mL) was added to solution and kept for 1 h. Supernatant (2.0 mL) was decanted and absorbance was measured at 570 nm by using spectrophotometry[25]. GABA is used as standard to extrapolate absorbance of the samples[24,26].

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't'-test (*p<0.05, **p<0.01, ***p<0.001). The p values lower than 0.05 were considered statistically significant.

RESULTS

Phytochemical screening: Phytochemical tests of MELS, revealed the presence of proteins, alkaloids, flavonoids, terpenoids, tannins, glycosides and saponins (Table 1). The contents of phenolic compounds were found in *Lagenaria sicereria* extract were (2.14±0.21 pyrogallol equiv L^{-1}) and the total flavonoid contents were (0.94±0.14 quercetin equiv L^{-1}).

Acute toxicity: The limit test was performed at 2500 mg kg^{-1}, p.o. A dose range of 250 and 500 mg kg^{-1}, p.o. was selected for the pharmacological activity.

Effects of MELS in latency of animals in EPM model: In the EPM model, the standard group showed a significant increase in the time spent, in the open arm as compared to control rats. While the rats treated with MELS at the dose of 250 mg kg^{-1} showed a non-significant increase on 1st day but significant increase (p<0.05) on 3rd and 7th day. In another group, rats

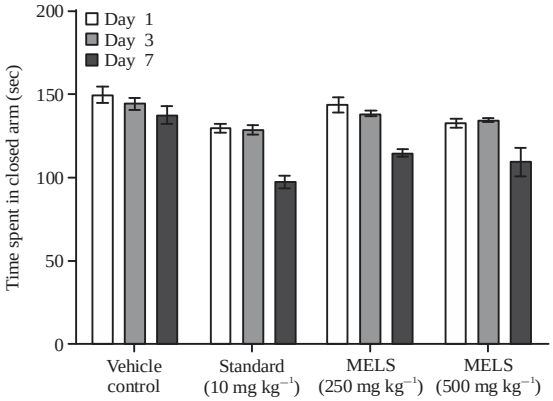

Fig. 1: Effect of MELS on time spent in closed arm on animals in EPM model

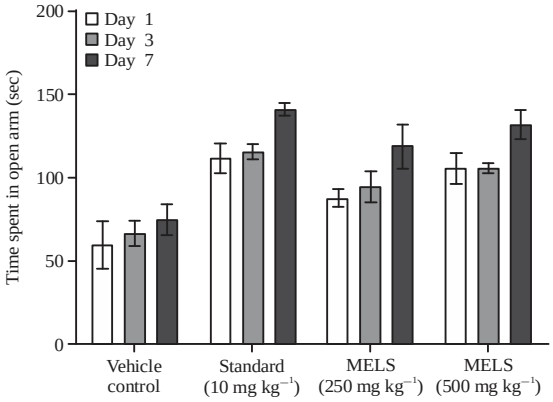

Fig. 2: Effect of MELS on time spent in open arm on animals in EPM model

Table 1: Phytochemical screening result

Extract constituents	Present (+) /Absent (-)
Alkaloids	+
Volatile oils	+
Carbohydrates	+
Phenolic/Tannins	+
Proteins	+
Glycosides	+
Flavonoids	+
Saponins	+

treated with MELS at the dose of 500 mg kg^{-1} showed a significant increase (p<0.01, p<0.05) in time spent in the open arm as compared to control group (Fig. 1). So the results were found opposite in the closed arm such as MELS at the dose of 500 mg kg^{-1} (1st, 3rd and 7th day) showed a significant decrease (p<0.05, p<0.01) in time spent in the closed arm as compared to control group. This reveals the reduction in anxiety (Fig. 2).

In the EPM model, the standard group showed significant decrease (p<0.01) in duration of latency as compared to

control. The duration of immobility was found reduced in rats treated with MELS at the dose of 500 mg kg^{-1} (1st, 3rd and 7th day) which showed the significant decrease ($p<0.05$, $p<0.01$) in duration of latency as compared to control group. This revealed the reduction in anxiety (Fig. 3).

In open arm, the rats treated with Fluoxetine showed a significant increase ($p<0.01$, $p<0.05$) in no. of entries in the open arm as compared to control group. MELS treated rats showed fewer entries in the open arm at a dose of 250 mg kg^{-1} which was found increased when treated with a higher dose (500 mg kg^{-1}) of MELS (Fig. 4). Where, In closed arm, the rats treated with Fluoxetine showed significant decrease ($p<0.01$) in No. of entries in the closed arm as compared to control group. MELS treated rats initially showed more entries in the closed arm at a lower dose (250 mg kg^{-1}) (1st, 3rd, 7th day) which was significantly decreased at higher dose (500 mg kg^{-1}) ($p<0.05$) on (1st, 3rd, 7th day) as compared to a control group (Fig. 5).

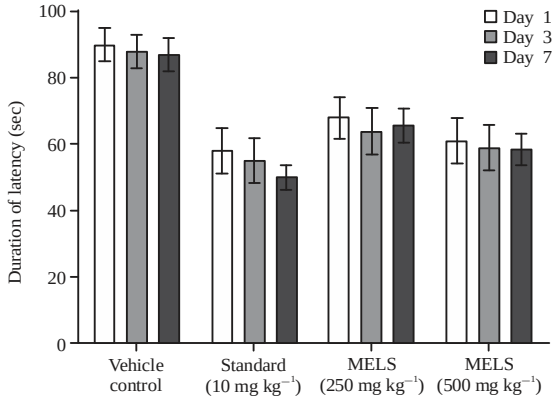

Fig. 3: Effect of MELS on Duration of latency in animals in EPM model

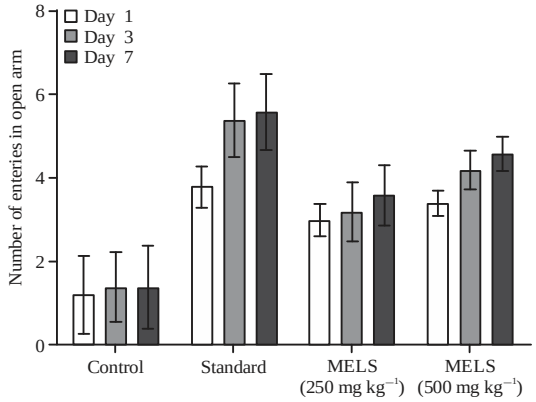

Fig. 4: Effect of MELS on number of entries in open arm on animals in EPM model

Effect of MELS on time spent in each compartment and duration of immobility on animals in light/dark model: The rats treated with standard drug (Fluoxetine) spent more time in light compartment when compared to control rats. The rats treated with MELS, 250 mg kg^{-1} showed non significant changes in time spent on 3rd day but found significant at later stage (7th day). The rats treated at a dose of 500 mg kg^{-1} of MELS showed a significant increase in time spent in light compartment as compared to control group. The standard treated rats showed less immobility as compared with control rats, which indicate less anxiety in rats. The MELS rats showed a gradual decrease in immobility in respect to increase in the dose of MELS when compared with controlled rats (Fig. 6-8).

Effect of MELS on no. of crossings on animals in a light/dark model: The standard group treated rats showed increased no. of crossings when compared with a control group. The MELS (250 mg kg^{-1}, p.o.) group of rats showed changed in no. of crossing on (1st, 3rd, 7th day). The MELS (500 mg kg^{-1}, p.o.) group showed a significant increase in a number of crossings as compared with a control group (Table 2, Fig. 9).

Effect of MELS on social interaction in rats: In this model, standard drug treated rats showed an increase in social

Table 2: Effect of MELS on no. of crossings on animals in light/dark model

	Number of crossings days		
Groups	1st	3rd	7th
Control vehicle	4.0±0.4944	6.2±1.068	8.2±1.993
Standard (10 mg kg^{-1}, p.o.)	7.0±0.7071**	11.0±0.8760**	21.6±0.9100**
MELS (250 mg kg^{-1}, p.o.)	4.2±0.5020^{ns}	8.0±0.9800^{ns}	12.6±0.0971^{ns}
MELS (500 mg kg^{-1}, p.o.)	5.0±0.7071*	10.0±0.4140*	16.2±1.994**

n = 6 (No. of animals), *p<0.05, **p<0.01, ***p<0.001 (one way ANOVA followed by Dunnett's 't' test). Values are expressed as Mean±SEM

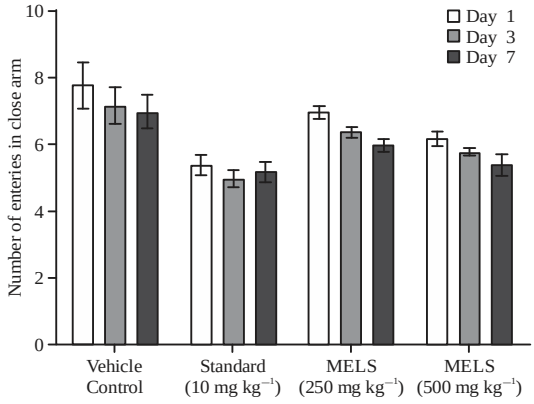

Fig. 5: Effect of MELS on number of entries in closed arm on animals in EPM model

Table 3: Effect of MELS on Social Interaction in Rats in High Familiar and Unfamiliar Light

	Groups			
Time spent (sec)				
Social interaction parameters	Control	Standard (10 mg kg^{-1}, p.o.)	MELS (250 mg kg^{-1}, p.o.)	MELS (500 mg kg^{-1}, p.o.)
Aggressive behavior	320±9.006	273±5.244**	299±3.860^{ns}	290±3.493*
Climbing and crawling	203±3.536	230±3.808**	218±3.464*	221±3.808**
Sniffing	77±2.983	97±3.450**	83±3.507^{ns}	89±2.243**

n = 6 (No. of animals), *p<0.05, **p<0.01, ***p<0.001 (one way ANOVA followed by Dunnett's 't' test). Values are expressed as Mean±SEM

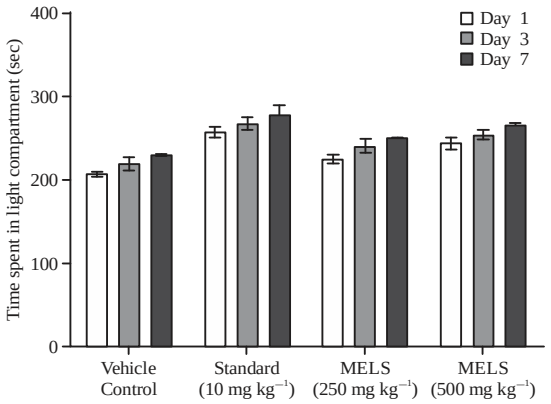

Fig. 6: Effect of MELS on time spent in light compartment on animals in L/D model

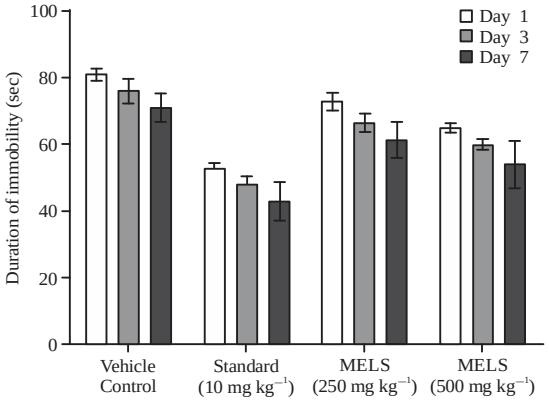

Fig. 8: Effect of MELS on duration of immobility on animals in L/D model

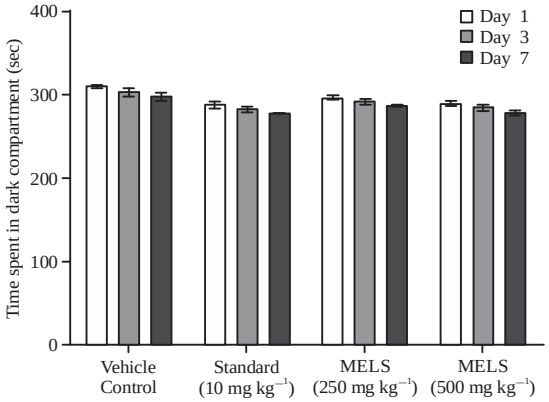

Fig. 7: Effect of MELS on time spent in dark compartment on animals in L/D model

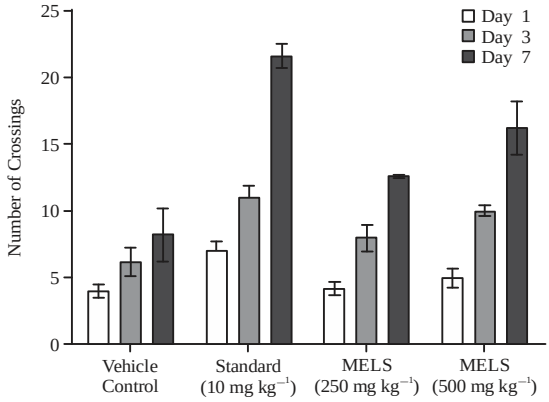

Fig. 9: Effect of MELS on Number of crossings of animals in L/D model

interaction (**p<0.01). Further analysis confirmed that both 250 and 500 mg kg^{-1} p.o. doses of MELS and fluoxetine significantly, increased the social interaction time in comparison to the control group and also markedly enhanced active social interaction (**p<0.01). 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<0.01 or *p<0.05) (Table 3 and 4).

Effect of MELS on the serotonin level in brain tissue: The controlled rats showed less concentration of serotonin level in brain tissues of rat, which was slightly increased in fluoxetine treated rats. The ALES group showed a significant increase in serotonin level in all three models when compared with controlled rats (Table 5).

Effect of MELS on GABA level in brain tissue: The negative controlled rats showed less concentration of GABA level in brain tissues of rat, which was slightly increased in fluoxetine treated rats. The ALES group showed a significant increase in GABA level in all three models when compared with controlled rats (Table 6).

Table 4: Time spent in social interaction in high unfamiliar light

Time spent (sec)	Groups			
Social interaction parameters	Control	Standard (10 mg kg^{-1}, p.o.)	MELS (250 mg kg^{-1}, p.o.)	MELS (500 mg kg^{-1}, p.o.)
Aggressive behavior	325±4.146	278±5.321**	301±6.920*	292±5.700**
Climbing and crawling	208±5.096	236±4.660**	219±2.972^{ns}	223±2.620*
Sniffing	67±1.676	86±3.650**	80±2.834*	85±4.674**

n = 6 (No. of animals), *p<0.05, **p<0.01, ***p<0.001 (one way ANOVA followed by Dunnett's 't' test). Values are expressed as Mean±SEM

Table 5: Effect of MELS on serotonin level in brain tissue of rats

	Group: Serotonin level (ng/mg Protein)		
Treatment	Elevated plus maze model	Light dark model	Social interaction test
Control	17.5±7.2	15.54±3.1	19.54±1.23
Standard (10 mg kg^{-1}, p.o.)	24.5±2.56**	21.87±1.27*	22.45±2.3*
MELS (250 mg kg^{-1}, p.o.)	20.5±8.5*	23.56±2.87**	17.54±1.25*
MELS (500 mg kg^{-1}, p.o.)	23.5±0.52**	26.74±1.56***	21.85±1.45**

n = 6 (No. of animals), *p<0.05, **p<0.01, ***p<0.001 (one way ANOVA followed by Dunnett's 't' test). Values are expressed as Mean±SEM. Fluorescence readings for these samples were below the limit sensitivity of the method defined as signal to tissue blank ratio of 2: 1

Table 6: Effect of MELS on GABA level in brain tissue of rats

	Group: GABA (ng/gm of Brain tissue)		
Treatment	Elevated plus maze model	Light dark model	Social interaction test
Control	25.5±1.8	25.14±4.1	21.58±1.53
Standard (10 mg kg^{-1}, p.o.)	44.5±1.76**	41.57±1.27*	42.45±2.3*
MELS (250 mg kg^{-1}, p.o.)	29.5±1.5*	33.56±0.87**	27.54±0.25*
MELS (500 mg kg^{-1}, p.o.)	53.5±0.52**	56.74±1.56**	61.85±1.45***

n = 6 (No. of animals), *p<0.05, **p<0.01, ***p<0.001 (one way ANOVA followed by Dunnett's 't' test). Values are expressed as Mean±SEM. Fluorescence readings for these samples were below the limit sensitivity of the method defined as signal to tissue blank ratio of 2: 1

DISCUSSION

The present study demonstrated the protective effect of methanolic extract of *Lagenaria sicereria* in various animal models of anxiety in rat's i.e. Elevated Plus Maze, Light Dark Model and Social Interaction in rats[26]. All these models are well established animal model and remained first choice to evaluate behavioral changes in animals. It is based on the natural conflict between the drive to explore a new environment and the tendency to avoid potentially dangerous areas[27]. In the present study, it was found that in rats, MELS (250 and 500 mg kg^{-1}) and Fluoxetine (10 mg kg^{-1}) increased both the percentage of time spent and entries into the open arms of the maze and decreases the time spent and entries in the closed arm which also supportive as per previous findings of proved drugs in anxiety[15]. The other model used for this study was a light/dark test where rats spent more time in the light box and increase in the number of entries between the two boxes, which indicated an anxiolytic activity. In this study, The rat treated with MELS (250 and 500 mg kg^{-1}) and Fluoxetine (10 mg kg^{-1}) treated groups shows increase in time spent in light chamber and no. of entries were also increased as compared to the control group and decrease in the time spent in dark chamber and decrease in the immobility of the rat.

The social interaction test of anxiety was developed to provide an ethologically based test that was sensitive to both anxiolytic and anxiogenic effects. In brief, the social interaction test is an extremely useful animal model for evaluating anxiolytic compounds, which are prescribed for treating social phobia, social failure/impairments and emotional immaturity. The findings suggest the anxiolytic effect of MELS, whereas a specific decrease in social interaction indicates an anxiogenic effect on the rat brain. The etiology of anxiety disorder is not fully understood, but various studies have shown that there is the involvement of GABA ergic, serotonergic neurotransmission in the etiology, expression and treatment of anxiety[1,27]. This study also showed the variation of GABA and Serotonin releases the synapse in rats. The adrenergic and dopaminergic systems have also been shown to play a role in anxiety[28]. The proposed hypothesis for MELS effect, it may due to *Lagenaria sicereria* contains protein's prolamins which contains the tryptophan, the precursor of serotonin, which is the main neurotransmitter within the brain, involved in various mechanisms in the anxiety. It promotes the availability of post synaptic serotonin by bindi receptors, -HT_{1A} receptors which helps in reduction of anxiety in rats[29]. Present study supports the previous findings of methanolic extract of *Lagenaria sicereria* in anxiety done by Kokate *et al.*[15] but this study was

limited to observational study only and present study was done with methanolic extract with determination of key biomarkers like GABA and Serotonin, which showed a better molecular approach for scientist to explore its potential for future studies.

CONCLUSION

Methanolic extracts of leaves of *Lagenaria sicereria* (MELS) 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. 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 for further research, must be carried out to explore the golden use of plant in neurological disorders.

Future findings: On the basis of results obtained in the present study, the following future findings may be summarized:

- The extract showed a potential anxiolytic effect, in all three models of anxiety and this will prove its anti anxiety effects in preclinical models which is to be further investigated in clinical models
- The present study will help researchers to explore the main constituents present in *Lagenaria sicereria* and their interaction with neurotransmitters like GABA, Serotonin in various neurodegenerative diseases

SIGNIFICANCE STATEMENT

This research discovers the anxiolytic effect of MELS on rats that can be beneficial for researchers to explore its chemical constituents responsible for this activity. This research also helps researchers to uncover the potential use of MELS as nutraceuticals supplement in clinical models to support its beneficial effects in various neurological disorders.

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