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Neuropharmacological Evaluation of Methanolic Extract of Lagenaria Siceraria Standley Fruits

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Abstract

Context: Lagenaria siceraria (Molina) Standley (LS), commonly known as 'bottle gourd' (English), possesses several medicinal properties; little is known about its action as nerve tonic.

Objective: The purpose of the study was to evaluate the neuropharmacological activities of methanolic extract of Lagenaria siceraria (Molina) Standley fruits (MLSF) using several experimental (animal behavioral) models.

Materials and Methods: Adult Wistar albino rats were subjected to behavior despair and elevated plus maze (EPM) tests. Thiopental induced sedation and rotarod tests were conducted on Swiss albino mice.

Results and Discussion: The effects of MLSF on anxiety, depression, thiopental induced sleeping time, and rotarod performance were evaluated. The anxiolytic activity of MLSF (100, 200 and 400 mg/kg) per os (p.o.) was characterized by increased time spent and number of entries in open arms in the EPM paradigm as compared to control group ($p < 0.001$). The MLSF (50,100, and 200mg/kg, p.o.) showed dose dependent significant reduction in duration of immobility ($p < 0.01$) in behavior despair test. The MLSF at the doses 50 and 100 mg/kg, i.p. was found to produce a significant reduction in motor coordination ($p<0.001$) and prolongation of thiopental induced sleeping time ($p<0.001$). The phytochemical screening revealed the presence of flavonoids, saponins and sterols in fruits.

Conclusion: The results of the study for the first time show that the plant possesses anxiolytic, anti-depressant and sedative like activities confirming the traditional claims. Future research should focus on the isolation and identification of the phytoconstituents responsible for activities of LS.

Keywords: Anxiety; Behavior despair test; Lagenaria siceraria; Depression; Elevated plus maze; Motor coordination; Neuropharmacology.

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INTRODUCTION

In modern era of globalization people are very much susceptible to various neurological (CNS) disorders such as anxiety, depression, stress, phobia etc. As per a report of WHO, such type of neurological disorders will become the second leading cause of death worldwide by the year 2020 (WHO, 1999).[1] Approximately 60% of the anxious

or depressed patients respond to the currently available allopathic treatments but the magnitude of improvement is still disappointing (Mora *et al.*, 2006).[2] Although there are many effective anxiolytic and antidepressants medicines available today, the therapy is still in adequate with unsatisfactory results in almost one-third of all pateints treated. Therefore it is necessary to develop some newer, safer and more effective antidepressants from traditional medicinal plants, whose psychotherapeutic potential has been assessed in a variety of animal models (Zhang, 2004).[3]

Lagenariasiceraria (Molina) Standley (LS) syn. *L. leucantha Rusby*; (Family: Cucurbitaceae) is commonly known as 'Bottle gourd', an outstanding fruit in the nature, as it has composition of all the essential constituents that are required for normal and good health.[4] LS fruits are traditionally used for its cardio protective, diuretic, general tonic, aphrodisiac and acts as alternate purgative.[5,6] It also cures pain, ulcers, fever, and used for pectoral cough, asthma and other bronchial disorders.[5] The fruits are edible and considered as good source of vitamin C, β-carotene, vitamin B-complex, pectin and also contain highest choline level-a lipotropic factor.[7,8] Modern phytochemical screening methods showed the presence of triterpenoidcucurbitacins, fucosterol, campesterol and flavone C-glycosides.[5,9-12] With this background the present study was intended to evaluate neuropharmacological profile of MLSF by using various animal experimental models.

MATERIALS AND METHODS

Collection and Authentication of plant materials

Fresh fruits of LS were purchased from local market of Rajkot, Gujarat. The plant was identified and authenticated by Prof. P.J. Parmar, Botanical Survey of India, Jodhpur. A specimen voucher (SU/ DPS/Herb/05) of the plant has been deposited at Department of Pharmaceutical Sciences, Saurashtra University, Rajkot for future reference.

Preparation of Plant extract

LS fruits were appropriately cleaned and cut in thin round slices and dried. The dried plant material was then subjected to coarse powder. The coarsely powdered dried fruits of LS were extracted with methanol by Soxhlet extraction method for 4h. After completion of extraction the solvent was removed by distillation and concentrated invaccuo and stored at freeze temperature. The test extracts wereprepared freshly in sodium carboxy methyl cellulose (SCMC) solution just prior to experiments. Moreover the MLSF was also subjected to phytochemical investigations for the detection of various plant constituents (Khandelwal, 2006).[13]

Experimental Animals

Male Wistar albino rats (250-300g) (n=6) were subjected to elevated plus maze (EPM). All the animals were housed in groups in polypropylene cages and placed in climate controlled central animal house having temperature 22±2°C, relative humidity 60±5%, and a 12 h light/dark cycle (lights on at 08:00 h and off at 20:00 h). The animals were fed standard pellet diet (Amrut, Pranav Agro Industries Ltd, India) and water ad libitum. All the protocols were approved (approval no-SU/ DPS/IAEC/9001) by Institutional Animal Ethics Committee (IAEC) of the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Government of India.

Drugs and Chemicals

Imipramine was obtained as a gift sample from Torrent Research Centre, India. Diazepam and thiopental were purchased from Sigma (USA). The solvents used in study were of analytical grade.

Administration of Drugs

Imipramine and thiopental were dissolved in distilled water, while diazepam and MLSF were prepared as suspension in distilled water using 0.5% SCMC as the suspending agent. Animals were assigned to different treatment groups (n=5). The control group received the vehicle (0.5% SCMC, 1 ml/kg) per os (p.o.), whereas different treatment groups received MLSF, imipramine or diazepam. All drugs and MLSF were prepared just before experimentation. All the doses of MLSF were administered orally, whereas standard drugs were administered intraperitoneally (i.p.).

Acute Toxicity Study

As per the OECD guidelines 420, the chloroform

and acetone fractions were administered orally at doses of 5, 50, 300 and 2000 mg/kg and the animals were examined for toxicity symptoms. There was no death or any toxicity symptom, observed. So as per annexure 4, the fractions were classified in Category 5 in GHS. Moreover, the study was also performed at doses 3000 and 4000 mg/kg, which were exceeding than 2000 mg/kg. Thus the anticipated maximum safe dose was determined as 4000 mg/kg and so 10%, 20% and 40% of the safe dose were selected to perform pharmacological study (Anonymous, 2000).[14]

Elevated Plus Maze Test

The method of Pellow *et al*. (1985) was followed. Briefly, the EPM apparatus comprised of two open arms (50 cm × 10 cm) and two closed arms (50 cm × 10 cm × 50 cm) that extended from a common central platform (10 cm × 10 cm). The floor and the walls of each arm were wooden and painted black. The entire maze was elevated to a height of 25 cm above floor level. Testing was conducted in a quiet room that was illuminated only by a dim light. Rats were individually placed on the centre of the maze facing an open arm. The number of entries and the time spent in open arms were recorded over a period of 5 min. Arm entries were recorded when rat enters all its four paws into an arm. The percent open arm entries (100 × open/total entries) were calculated for each animal. The experimental animals were pretreated with diazepam (5 mg/ kg, i.p.) or MLSF (100, 200, and 400 mg/kg, p.o.), 30 and 60 min, respectively, whereas the control group received 0.5% SCMC orally. Diazepam (5 mg/kg, i.p.) was used as standard anxiolytic drug. Between each trial, the maze was wiped and clean with a camp sponge and dried with paper towels to ensure uniform results (Emamghoreishi *et al*., 2005).[15]

Behavior Despair Test

The procedure described by Porsolt *et al*. (1978a)[16] was followed with slight modification of deep water level suggested by Detke *et al*. (1995)[17] to ensure that rats could not support themselves by touching the bottom with their feet. Male rats were used for this test (Padovan & Guimaraes, 2004; Calil & Marcondes, 2006).[18,19] Swimming sessions were conducted by placing rats in individual glass cylinder (35 cm x 25 cm) containing water (25 ± 1°C) having 27 cm depth. Two swimming sessions were conducted between 9:00-16:00 h. All the rats were subjected to an initial 15 min pretest followed 25h later by a 5 min test. Drugs were administered three times during the period between these two sessions, first immediately after pretest session and then, after 6 and 23 h of the first dose. Following both swimming sessions, the rats were removed from the cylinder, dried with paper towels, placed in the cages under a heating source (15 min), and returned to their home cages. The immobility period in seconds was measured in each test session of 5 min. The water in the cylinder was changed after every other trial. Imipramine (12.5 mg/kg, i.p.) served as standard drug in this model (Bhattamisra *et al*., 2008).[20]

Thiopental induced Sleeping Time

The procedure of Sukma *et al.* (2002) was followed. Briefly, 30 min after the administration of MLSF (50 and 100 mg/kg, i.p.) or SCMC (0.5%, i.p.), mice received thiopental (50 mg/kg, i.p.). The time elapsed from thiopental injection to loss of the righting reflex was taken as sleeping latency. The time elapsed between the loss and voluntary recovery from the righting reflex was considered as the total sleeping time.[21]

Test for Motor Coordination (Rotarod Performance)

The test was conducted as per the method described by Ibarrola *et al.* (2006).[22] Briefly, mice were placed in rotarod apparatus consisting of a horizontal rotating rod having 2.5 cm diameter with rotating speed 12 rpm and divided in six equal compartments. The animals remaining on the rod for two or more min in two successive trials were selected for the test. Animals were divided into four groups: control, diazepam (0.5 mg/kg, i.p.) treated, and MLSF (100 and 200 mg/kg, i.p.) treated groups. After 30 min of various treatments, they were placed on the spinning bar of the rotarod apparatus for 1 min. The time spent (in sec) on the rotating rod was recorded.

Statistical Analysis

All the data were expressed as mean ± SEM from five animals. The data obtained was analyzed using the one way ANOVA followed by Student Newman-Keuls test for determining the level of significance and $p < 0.05$ was considered statistically significant.

RESULTS

Acute toxicity studies

The acute toxicity studies showed that the LD50 of the MLSF in mice was 1000 mg/kg by i.p. route. So accordingly four dose levels 50, 100, 200 and 400 mg/kg, p.o. body weight were selected to perform tests, corresponding to 5, 10, 20 and 40% of LD50 value (1000 mg/kg, i.p.), respectively.

Preliminary Phytochemical Screening

Preliminary phytochemical screening (Table 1) of the methanolic extract of LS showed the presence of flavonoids, saponins, sterols, proteins, tannins and carbohydrates.

Table 1: Result of phytochemical screening of methanolic extract of Lagenaria siceraria fruits

Class of Compounds	Results
Alkaloids	-
Flavonoids	+
Saponins	+
Phytosterols	+
Carbohydrates	+
Tannins	+
Protein and Amino Acids	+
Fixed/Volatile Oils	-

Elevated Plus Maze

As shown in the Table 2, the control animals showed more preference for the closed (dark) arms and exhibited anxiety like symptoms characterized by immobility, freezing, and defecation on entering the open arms. As compared to the control group, the MLSF (100, 200, and 400 mg/kg, p.o.) treated animals showed significant increase in total number of entries ($p< 0.5$; $p < 0.001$) and the time spent in the open arms. Moreover, MLSF reduced anxiety like symptoms in dose dependent manner. Diazepam (5 mg/kg, i.p.), a standard drug, significantly increased the number of entriesas well as time spent in the open arms ($p < 0.001$), indicating anxiolytic activity. The higher dose of MLSF (400 mg/kg, p.o.) produced a peak anxiolytic effect that is well comparable to diazepam ($p < 0.001$).

Table 2: Effect of methanolic extract of *Lagenaria siceraria* fruits (MLSF) on the immobility period during behavior despair testa

Treatment	Dose (mg/kg, p.o.)	Immobility Period (Seconds)
Control	-	154.0 ± 17.264
Imipramine	12.5b	80.6 ± 17.154**
MLSF	50	109.6 ± 7.07*
MLSF	100	69.80 ± 10.59**
MLSF	200	64. 80 ± 12.26**

[a]Values are expressed as mean ± SEM (n=5). [b]intraperitoneal route

*p<0.05, **p<0.01, compared with control (one-way ANOVA followed by Student Newman-Keuls test).

Behavior Despair Test

Table 2 show the anti-depressant effect of MLSF and imipramine in the experimental animals. The control animals remained immobile for most of the time during the test session. MLSF (50, 100, and 200mg/kg, p.o.) induced a dose dependent significant reduction in the immobility time of rats ($p < 0.5$; $p< 0.01$) as compared to the control group. In the same experimental conditions, the antidepressant activity of the reference drug imipramine (12.5 mg/kg, i.p.) was clearly evident ($p< 0.01$). The anti-depressant effect produced by MLSF (100 and 200 mg/kg, p.o.) was comparable

Table 3: Effect of methanolic extract of *Lagenaria siceraria fruits* (MLSF) and diazepam on entries and time spent in both arms in the elevated plus maze (EPM) test in rats[a]

Treatment	Dose (mg/kg, p.o.)	Time spent (seconds)		No. of entries	
		Open arm	Close arm	Open arm	Close arm
Control	–	5.0 ± 4.074	295.0 ± 4.074	0.4 ± 0.25	4.6 ± 0.25
Diazepam	5b	152.4 ± 23.86***	147.6 ± 23.86***	3.2 ± 0.20***	1.8 ± 0.20***
MLSF	100	40.20 ± 3.76	259..80 ± 3.76	1.6 ± 0.25*	3.4 ± 0.25*
MLSF	200	92.40 ± 13.11 ***	207.60 ± 13.11***	2.6 ± 0.25***	2.6 ± 0.25**
MLSF	400	127.40 ± 11.00***	172.60 ± 11.00***	3.0 ± 0.55**	2.0 ± 0.55**

[a]Values are expressed as mean ± SEM (n = 5). bintraperitoneal route

*p < 0.05, **p< 0.01, ***p< 0.001, compared with control (one-way ANOVA followed by Student Newman-Keuls test).

Table 4a: Effect of methanolic extract of Lagenaria siceraria fruits (MLSF) on thiopental induced sleeping time in micea

Treatment	Dose (mg/kg, i.p.)	Total Sleeping time (sec)
Control	-	780.400 ± 25.639
Thiopental	50b	2430.000 ± 176.678***
MLSF	50	969.400 ± 35.655
MLSF	100	1263.000 ± 58.034**
MLSF	200	1620.400 ± 98.411***

[a]Values are expressed as mean ± SEM (n=5). bintraperitoneal route

**p< 0.01, ***p< 0.001, compared with control (one-way ANOVA followed by Student Newman-Keuls test)

Table 4b: Effect of methanolic extract of *Lagenaria siceraria* fruits (MLSF) on thiopental induced sleeping latency (onset of sleeping) in micea

Treatment	Dose (mg/kg, i.p.)	Latency of sleep (sec)
Control	-	182.40 ± 7.36
Thiopental	50b	50.00 ± 2.470***
MLSF	50	126.40 ± 5.61***
MLSF	100	111.20 ± 5.66***
MLSF	200	98.80 ± 3.94***

[a]Values are expressed as mean ± SEM (n = 5). bintraperitoneal route

***p< 0.001, compared with control (one-way ANOVA followed by Student-Newman-Keuls test).

Table 5: Effect of methanolic extract of *Lagenaria siceraria* fruits (MLSF) on rod performance[a]

Treatment	Dose (mg/kg, i.p.)	Time of permanence (seconds)
Control	--	281.20 ± 12.064
Diazepam	0.5b	34.40 ± 4.456***
MLSF	100	245.80 ± 17.86
MLSF	200	112.20 ± 16.35***

[a]Values are expressed as mean ± SEM (n=5); bintraperitoneal route

***p< 0.001, compared with control (one-way ANOVA followed by Student-Newman-Keuls test).

to that of imipramine.

Thiopental induced sleeping time

As shown in ure 1, MLSF (50 and 100 mg/kg, i.p.) showed dose dependent prolongation of thiopental induced sleeping time as compared to the control group. Prior treatment of diazepam (1 mg/kg, i.p.) potentiated thiopental-provoked sleep.

Test for Motor Coordination (Rota Rod Performance)

As shown in Table 4, MLSF did not exhibit anyinfluence in motor coordination test. Test was conducted with reference standard drug diazepam (0.5 mg/kg, i.p.), which showed marked reduction in motor coordination.

DISCUSSION

LS is traditionally used for the treatment of anxiety, depression, and other CNS disorders. Scientific data on these properties of the plant are not available. Therefore, we investigated the effects of different doses of MLSF using several neuropharmacological models. EPM is one of the most widely used models of animal anxiety (Hogg, 1996; Rodgers, 1997).[23,24] The anxiety produced in this model is due to natural stimuli, i.e. the fear of a new, brightly-lit open space and balance on a relatively narrow raised platform (Dawson & Tricklebank, 1995).[25] The frequency and time spent in the open arms is the major index of the anxiety due to the fact that an open area is extremely aversive to rodents (Pellow & File, 1986; Emamghoreishi *et al*., 2005).[26,27] In the present study, MLSF significantly increased number of entries and time spent in the open arms in dose dependent manner indicating anxiolytic activity. It has been shown that GAB Aergic neurotransmission plays an important role in stress and anxiety associated with elevated plus maze test (Zwanzger & Rupprecht, 2005).[28] It is likely that MLSF may have modulated the benzodiazepine or other sites of GABA receptors to produce the anxiolytic effect. Several pharmacological studies report that flavonoids isolated from *Passifloracoerulea* (Wolfman *et al*., 1994)[29] and *Tiliaamericana* (Herrera-Ruiz *et al*., 2008)[30] possesses anxiolytic activity. According to study of Kavvadias, flavonoidse.g., apigenin (Kavvadias *et al*., 2003) selectively binds with higher affinity to the central BDZ site of GABA receptor, possesses important anxiolytic (Paladini *et al*., 1999).[31] In consistent with these studies, the flavonoids were found to be present in L. siceraria fruits during phytochemical investigation, likely to exhibit the anxiolytic effect.

The behavior despair test has been validated as a suitable tool to evaluate drugs with putative antidepressant effects (Porsolt *et al*., 1978a; Anisman & Matheson, 2005; Matthews *et al*., 2005).[32,33,34] In this model, when rodents are forced to swim in a confined space, they tend to become immobile after vigorous activity (struggling). This inescapable stressful situation leads to depression (Porsolt *et al*., 1978b).[32] In the present study, administration of MLSF

significantly reduced total immobility time and enhanced struggling behavior in dose dependent manner, suggesting antidepressant effect. It is reported that GABA, an inhibitory neurotransmitter is involved in the pathophysiology of depression. Moreover, neurochemical research has revealed that the monoamines (5-HT, NA, and dopamine) have a crucial role in the development of the depression syndrome (Naughton *et al.*, 2000).[35] The antidepressant effect of the MLSF may be attributed to modulate one or more of these neurotransmitters. It has been found that flavonoids isolated from plant species such as *Hypericum perforatum* (Butterweck *et al*., 2000) showed antidepressant activity.[36] Thus, it is likely that flavonoids present in MLSF may be responsible for the observed antidepressant effect. The anxiolytic and antidepressant effects shown by MLSF suggest etiological similarity in the development of anxiety and depression. Several hypothesis have been proposed to explain this aspect. The serotonergic theory postulates excessive functioning of the serotonergic neurotransmission as the cause of depression and anxiety (Deakin, 1983).[37] Another theory proposes involvement of GAB Aergic neurotransmission, which forms the basis of action of anxiolytic activity of many drugs, may also be involved in the antidepressant activity (Lloyd *et al.*, 1989).[38] It can be hypothesised that MLSF may have acted by modulating one or more of the above mentioned neurotransmitters. In the thiopental induced sleep test, MLSF potentiated the effect of thiopental. The prolongation of thiopental induced sleeping time may be attributed to an inhibition of thiopental metabolism or to an action on the central mechanism involved in the regulation of sleep (N'Gouemo *et al.*, 1994).[39] Thus, suggesting MLSF as a mild neurosedative drug (Capasso *et al.*, 1996).[40] Phytochemical screening revealed presence of flavonoids, saponins, sterols, proteins, tannins and carbohydrates. Moreover triterpepenoids (steroidal compounds) are present in the fruits (Baranowska & Cisowski, 1994),[9] which are able to cross Blood brain barrier (BBB) due to their lipophilic nature and so it can be assumed that steroidal compounds might be responsible to elicit the neuropharmacological activities at molecular level in CNS (brain) (Librowaski, 2000).[41]

CONCLUSIONS

The present study for the first time provides evidence for the neuropharmacological activity of MLSF in experimental animals. The presence of flavonoids, saponins and sterols in MLSF could be responsible for these activities. The need of the hour is to identify and isolate the phytoconstituents responsible for the observed central effects in animals and to understand their molecular mechanisms.

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DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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