Anti-Depressant Activity Of Methanolic Extract Of Leaves Of *Lagenaria Siceraria* Linn

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**ABSTRACT**

**Introduction:** *Lagenaria siceraria* (Mol.) Standl. (Bottle gourd), of the family Cucurbitaceae, is a climbing perennial plant widely cultivated as a vegetable crop in tropical countries, such as India, Japan and Thailand. Depression is the most prevalent mental disorder and depression is recognized to be symptomatically, psychologically and biologically, heterogeneous. The disorder was characterized by apathy, loss of energy, retardation of thinking and activity, as well as profound feeling of gloominess despair and suicidal tendency. Patient with major depression have symptoms that reflect changes in brain monoamine neurotransmitter, specially norepinephrine, serotonin and dopamine.

**Methods:** *Lagenaria siceraria* leaf was dried and size reduction was done using grinder then the powder was sieved and extracted using different solvent namely petroleum ether, chloroform, methanol, ethanol and water were cold macerated methanolic extract shows the highest extraction yield in comparison to other solvent. Treatment with *Lagenaria siceraria* extract the dose 400mg/kg was compared to standard imipramine 15 mg/kg for 30 days, after inducing depression by using models forced swim test, tail suspension test. To check the immobility time of rats.

**Result:** The *Lagenaria siceraria* (100, 200, and 400 mg/kg, p.o.) showed dose-dependent significant reduction in duration of immobility in behavior despair test. The phytochemical screening revealed the presence of phytoconstituents, such as flavonoids, saponins in the leaf.

**Conclusion:** The *Lagenaria Siceraria* leaf extract at the dose of 400 mg/kg has a significant anti-depressant effect over in diazepam-induced depressant rats.

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INTRODUCTION
Mental depression is a chronic illness that affects a person’s mood, thoughts, behavior and physical health. Depression is a complex disorder of unknown etiology, which is manifested by low mood, anhedonia, low energy levels, pessimism, guilty feeling guilty. It may range from a very mild condition, bordering on normality, to severe depression—sometimes called ‘psychotic depression’ accompanied by hallucinations and delusions. Patients with major depression have symptoms that reflect changes in brain monoamine neurotransmitters, specifically nor-epinephrine, serotonin and dopamine [1]. Depression is a mood disorder occur due to deficiency of amines in brain. When a person suffered from depression, it needs emergency treatment because the major drawback of depression is suicidal tendency. Because of this it increases mortality rate in patients suffering from depression [2]. depressant is a type of serous neurological disorder [3]. Lagenaria siceraria (Cucurbitaceae) commonly known as bottle gourd/ghiya/lauki is reported to possess several medicinal properties such as, anti-oxidant activity, analgesic activity [4]. Lauki contains several pharmacologically active photochemical such as amino acids, vitamins, fucosterol [5]. bottle gourd contains high amount of neurotransmitters such as serotonin, dopamine, adrenaline and nor-adrenaline [6]. However, there is no scientific evidence for the therapeutic potential of ghiya (lauki) in neuropsychiatric disorders. Since serotonin and nor-adrenaline levels fall considerably in depression, we were interested to investigate the usefulness of ghiya (lauki) in depression [7]. Antidepressant drugs such as selective serotonin reuptake inhibitors (SSRIs), tricyclic antidepressants (TCAs), monoamine oxidase inhibitors (MAOIs), benzodiazepines (BZDs), serotonin-norepinephrine reuptake inhibitors (SNRIs), and noradrenergic and specific serotonergic drugs are used extensively in clinical practice to cure these disorders [2].

MATERIALS AND METHODS
Drugs and chemicals
The present study was undertaken to explore the anti-depressant potential of ghiya (Lagenaria siceraria leaves) using forced swim test and tail suspension test. To cause depression in the animals and Imipramine was used as standard drug for comparison with test drug. Methanolic extract of leaves of LS was used as test drug.

Sources of plant material and preparation of plant extracts
The leaves of LS were collected and the specimen was certified by Dr. G.P. Sinha, Scientist, Botanical Survey of India, Central Regional Center, 10 Chatham Lines, Prayagraj, Uttar Pradesh. Specimen with Voucher number SIP/2019/007 has been deposited at the Botanical Survey of India. The leaves of LSL were shade dried and then powdered by mechanical grinder. The coarse powder was passed through a 40-mesh sieve. The successive solvent hot extraction method used to obtain various extracts including petroleum ether, chloroform, methanol, ethanol and aqueous extract. The solvent was removed from the extracts under reduced pressure by using a rotary vacuum evaporator. The % of yield of extract was noted. The greenish extract was obtained and is dissolved in their respective solvents for pharmacological studies.

Qualitative phytochemical Screening
The preliminary qualitative phytochemical screening of LS was conducted for the presence or absence of amino acid, alkaloids, glycosides, flavonoids, tannins, saponins, triterpenes using standard laboratory procedure.

Determination of total phenolic content
Male albino wistar rats weighing 150-200gm were procured from Indian Veterinary Research Institute, Izatnagar, Bareilly, India. The animals were placed in the propylene cages with paddy husk bedding at the temperature of 24±3 ºC and relative humidity 30-70%, maintained under standard condition 12 h light; and 12 h dark
cycle. The experimental study protocol was authenticated by Institutional Animal Ethical Committee (IAEC) with Registration no. SIP-IAEC/005/09/18.

**Acute oral Toxicity**

The acute toxicity study was performed as per the method described by Litchfield and Wilcoxon and LD$_{50}$ was calculated accordingly [8]. Acute oral toxicity testing was carried out as per the guidelines of OECD (423), revised from CPCSEA, Ministry of social justice and empowerment, Government of India (OECD, 2005). The animals were randomly selected and marked for individual identification and keep them in cage for at least 5 days prior to dosing to allow for acclimatization to the laboratory conditions. The test substance is administered in single dose by using oral gavage or incubation canula [9].

**Animal grouping and treatments**

Overnight fasted animals were selected randomly on the day of experiment for administration of vehicle, standard drug and study drug. The animals were acclimatized one hour before for behavioral tests. Thirty minutes and 1 h time interval between drug administration and behavioral tests were maintained in case of intraperitoneal and oral administrations respectively.

The animals were divided into five groups of six animals each as follows:

1. Group-I (n=6): Control, received distilled water, orally
2. Group-II (n=6): Standard, Imipramine 15mg/kg, i.p
3. Group-III (n=6): LSL 100 mg/kg, p.o
4. Group-IV (n=6): LSL 200 mg/kg, p.o
5. Group-V (n=6): LSL 400 mg/kg, p.o

**Forced swim test (FST)**

Forced swim test in glass jar was performed as described by Porsolt et al. with few modification [10]. This test consists of two parts, an initial training period of 15 min followed 24 h later by actual test for 5 min duration [11]. was followed with slight modification of deep water level suggested by Detke et al to ensure that rats could not support themselves by touching the bottom with their feet [12]. Male rats were used for this test [13]. Swimming sessions were conducted by placing rats in individual glass cylinder (35 cm × 25 cm) containing water (25± 1 °C) having 27 cm depth. Two swimming sessions were conducted between 9:00 and 16:00 h. All the rats were subjected to an initial 15 min pretest followed 25 h 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 [14].

**Tail suspension test (TST):**

Tail suspension test commonly employed behavioral model for screening antidepressant-like activity in mice, was first given by Steru et al [15]. Depression was produced by suspending the animal from the edge of a table 50 cm above the floorby an adhesive tape placed approx. 1cm from the tip of the tail. Immobility time was recorded during a 6 min. period. Changes in the immobility duration were studied after administering drugs in separate groups of animals. The antidepressant activity was expressed as reduction in the immobility duration between the control, standard and animals treated with test drug [16].

**Spontaneous Locomotor activity**

The forced swim test, spontaneous locomotor activity was measured using actophotometer. Actophotometer registers the number of times infrared photo beams of light were broken as the rat moved around inside the cage. Each rat was placed in the center of the metal cage of actophotometer and its ambulatory activity was measured for at 5 min interval for the next 15 min [17].
Histopathological examination
Brain was isolated from rats of all groups and kept in 10% formalin solution for histopathological study. The brain was placed in plastic container and immersed in neutral buffered formalin for 24hrs. The fixed brain was embedded in paraffin, cut into 4µm thick and stained with hematoxylin and eosin. The extent of brain damage was evaluated by assessing the morphological changes in the brain section (Akther Nayeem et al.).

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

Behavior despair (forced swim behavior) test
Table 2 shows the antidepressant effect of methanolic extract Lagenaria siceraria leaves (MELSL) and imipramine in the experimental animals. The control animals remained immobile for most of the time during the test session. MELSL (100, 200, and 400mg/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 (15 mg/kg, i.p.) was clearly evident (p<0.01). The antidepressant effect produced by MELSL (100 and 400 mg/kg, p.o.) was comparable to that of imipramine.

Tail suspension
In this test animals treated with three doses of Methanolic extract Lagenaria siceraria leaves (MELSL) (100, 200 and 400 mg/kg, i.p) showed decreases in their immobility times, which was significant (135.33±3.19, 113.17±2.81 and 96.17±2.45 respectively; p<0.001) when compared with control (160.17±3.62). Similarly, animals treated with Imipramine as expected, showed a significant decrease in the immobility time (73.33±2.11; p<0.001).

Spontaneous Locomotor Activity (Actophotometer)
The locomotor activity count measured in 15 min of the test was significantly decreased by diazepam (5 mg/kg). Locomotor activity was not changed in animals pretreated with Methanolic extract Lagenaria siceraria (MELSL) (100 mg/kg) compared with that in the control group. However, at 200 and 400 mg/kg, MELSL slightly inhibited locomotor activity, but at lesser extent than diazepam.

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

<table>
<thead>
<tr>
<th>S.NO.</th>
<th>TESTS</th>
<th>RESULTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Protein (a)Biuret test</td>
<td>+</td>
</tr>
<tr>
<td>2.</td>
<td>Amino acid</td>
<td>+</td>
</tr>
<tr>
<td>3.</td>
<td>Carbohydrates</td>
<td>+</td>
</tr>
<tr>
<td>4.</td>
<td>Alkaloids (a)Wagner’s test</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>(b)Hager’s test</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>Steroids</td>
<td>+</td>
</tr>
<tr>
<td>6.</td>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>7.</td>
<td>Glycoside</td>
<td>+</td>
</tr>
</tbody>
</table>
Table 2: Effect of methanolic extract of Lagenaria siceraria leaf on the immobility period during forced swimming test

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg,p.o)</th>
<th>Immobility period (seconds)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>158±15.64</td>
</tr>
<tr>
<td>Imipramine</td>
<td>15</td>
<td>90.1±18.54</td>
</tr>
<tr>
<td>LSL 100</td>
<td>100</td>
<td>110±14.34</td>
</tr>
<tr>
<td>LSL 200</td>
<td>200</td>
<td>70.25±8.34</td>
</tr>
<tr>
<td>LSL 400</td>
<td>400</td>
<td>65.25±11.34</td>
</tr>
</tbody>
</table>

(Fig. 1: Forced swimming test)

Table 3: Effect of methanolic extract of Lagenaria siceraria leaf on the immobility period during behavior tail suspension test

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg,p.o)</th>
<th>Immobility period (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>160±3.61</td>
</tr>
<tr>
<td>Imipramine</td>
<td>15</td>
<td>73.32±3.18</td>
</tr>
<tr>
<td>LSL 100</td>
<td>100</td>
<td>135.32±3.18</td>
</tr>
<tr>
<td>LSL 200</td>
<td>200</td>
<td>133.16±2.80</td>
</tr>
<tr>
<td>LSL 400</td>
<td>400</td>
<td>96.16±2.45</td>
</tr>
</tbody>
</table>

(Fig. 2: Tail Suspension test)
**Histopathology observation:**

Brain was isolated from rats of all group and examine histopathology of brain .in this, we observe condition of brain. During normal condition of rats observe immobility period 158±15.64 second, but when we caused depression then immobility period goes to 165±18.36. To treat Ant-depressant effect gives an Imipramine 15mg/kg (standard drug) and immobility period 90.1±18.54.

Another group was treated by *Lagenaria siceraria* (LS) (100mg/kg, 200mg/kg,400mg/kg) and observed immobility period 110±14.34, 70.25±8.34, 65.25±11.34 sec respectively and compared it, with standard drug Imipramine. At last we observe that LS 400mg/kg more effective than standard drug and also fig. 8 support the data.

(Fig. 3: Normal group)

(Fig 4: Toxic group)

(Fig 5: Standard Imipramine (15mg/kg))
DISCUSSION:

*Lagenaria siceraria* (LS) is traditionally used for the treatment of depression, and other CNS disorders. Scientific data on this property of the plant are not available. Therefore, we investigated the antidepressant effect of different doses of Methanolic extract *Lagenaria siceraria* leaves (MELSL) using forced swimming model (behavior despair test). The behavior despair test has been validated as a suitable tool to evaluate drugs with putative antidepressant effects. In this model, a crucial role in the development of the depression, flavonoids present in MLSF may be responsible for the observed antidepressant effect. The serotonergic theory postulates excessive functioning of the serotonergic neurotransmission for the prevention of the
cause of depression. It can be hypothesized that MLSF may have acted by modulating one or more of the above-mentioned neurotransmitters. Moreover, cholinergic transmission also plays the promising role in CNS. Based on its irregular distribution within the CNS and the observation that peripheral cholinergic drugs could produce marked behavioral effects after central administration. Therefore, it can be predicted that the higher level of choline in LS fruits may be responsible to act on cholinergic transmission in CNS and may be helpful to prevent depression. Phytochemical screening of MLSF revealed presence of flavonoids, saponins, sterols, proteins, tannins and carbohydrates. Moreover, triterpenoids (steroidal compounds) are present in the fruits, those are able to cross blood brain barrier (BBB) due to their lipophilic nature and so it can be assumed that such steroidal compounds might also be responsible to elicit antidepressant and other neuropharmacological activities at molecular level in CNS (brain).

CONCLUSION

The present study for the first time provides evidence for the antidepressant activity of Methanolic extract Lagenaria siceraria (MELSL) in the experimental animals. The ghiya leaves extract produced powerful and consistent anti-depressant effects in both the experimental models viz tail suspension test and forced swim test in the present study. The present study for the first time provides evidence for the antidepressant activity of MELSL in experimental animals. The presence of amino acids, saponins and sterols in MELSL could be responsible for this activity. The need of the hour is to identify and isolate the phytocomponents responsible for the observed antidepressant effect in animals and to understand their molecular mechanisms.

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