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RESEARCH ARTICLE
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ANTIDEPRESSANT ACTIVITY OF *LAGENARIA SICERARIA L.* SEEDS USING FORCED SWIM AND TAIL SUSPENSION TESTS

Ishrat Younus[1]*, Faiza Anjum[1], Rafia Sadaf[2], Nimra Mujeeb[1], Noor ul Ain[3], Talha bin Fayyaz[4] Zubia Begum[5], Marium Mumtaz[1]

[1]Faculty of Pharmacy, Hamdard University, Karachi, Pakistan

[2]Jinnah College of Pharmacy, Sohail University, Karachi, Pakistan

[3]Institute of Pharmaceutical Sciences, Jinnah Sindh Medical University, Karachi, Pakistan

[4]Faculty of Pharmacy, Ziauddin University, Karachi, Pakistan

[5]Faculty of Pharmacy, Jinnah University for Women, Karachi, Pakistan

***Corresponding author:** Ishrat Younus
*email: ishratyounas@gmail.com

ABSTRACT
Traditionally *Lagenaria siceraria* was used for the treatment of various ailments both in males and females and evidences a great therapy for cures pain, ulcers, and fever, and it is used for pectoral cough, asthma, and other bronchial disorders. This study is planned to estimate the antidepressant action of ethanol extract of *Lagenaria siceraria* seeds at doses of 100 and 500 mg/kg by using two models i.e., a tail suspension test and a forced swim test. Significant antidepressant results were noted as compared to the control. The study results indicate that plant extracts are effective as antidepressants.

Keywords: *Lagenaria siceraria*, anti-depressant, tail suspension, and forced swim test.

INTRODUCTION
There exists a plethora of knowledge and information and benefits of herbal drugs in our ancient literature of Ayurvedic and Unani medicine. One of the earliest treatises of Indian medicine, the Charaka Samhita (1000 B.C.) mentions the use of over 2000 herbs for medicinal purpose. According to the WHO survey 80% of the populations living in the developing countries rely almost exclusively on traditional medicine for their primary health care needs. Exploration of the chemical constituents of the plants and pharmacological screening may provide us the basis for developing the leads for development of novel agents. In addition, herbs have provided us some of the very important lifesaving drugs used in the armamentarium of modern medicine [1]. There is a worldwide belief that herbal remedies are safer and less damaging to the human body than synthetic drugs. Therefore, laboratories around the world are engaged in screening of plants for biological activities with therapeutics potential. One major criteria for the selection of plant for such a study is traditional healer's claim for its therapeutics usefulness. The traditional Indian medicinal system mentions herbal

remedies for the treatment of variety of diseases. The ayurveda has emphasized importance of food in the management of diseases. Even practitioner of modern system has realized the significance of dietary items, in the form of nutraceutical elements, in the treatment of chronic diseases [2]. *Lagenaria siceraria* (Mol.) Standley, commonly known as bottle-gourd (in English), belongs to the Cucurbitaceae family an excellent fruit in nature having a composition of all the essential constituents that are required for the normal and good health of humans. It cures pain, ulcers, fever, asthma, and other bronchial disorders [3]. It also cures pain, ulcers, and fever, and is used for pectoral cough, asthma, and other bronchial disorders [4]. *L. siceraria* fruit is traditionally used for its cardioprotective, cardiotonic, general tonic, and aphrodisiac and acts as an alternate purgative, diuretic [5,6]. Cardiovascular disorder is claimed to be relieved following regular intake of bottled gourd juice for about 4-6 months [7]. The seeds (weight of 100 seeds, 15 gm) are edible. In China, they are boiled in salt water and eaten as an appetizer. The seed oil is applied in headache [8]. A decoction of *L. siceraria* is employed in the treatment of anasarca, ascites, and beriberi [9]. Lagenin-a novel ribosome-inactivating protein has been isolated from the lyophilized water extract of seeds which is known to possess immunosuppressive, antitumor, antiviral, antiproliferative, and anti-HIV activities [8, 10]. Seeds are reported to contain saponin. Analysis of seed kernels (68% of seed wt.) gives the following values: moisture, 2.47; protein,30.72; oil,52.54; carbohydrates,8.3; fiber, 1.58; ash,4.43; CaO,0.11; and P_2O_3, 2.46%. The oil obtained from seed kernels is clear and pale yellow. Kernels from ripe seeds gave 45% of oil with the following characteristics: n^{40}, 1.4711; sap equiv., 301.6; iodine value, 126.5; free fatty acids, 0.54%; and un-saponified matter, 0.67%. The components of free fatty acids are: linoleic acids 64.0; oleic, 18.2; and saturated fatty acids, 17.8% [2]. Seeds are reported to contain Lagenin [10]

MATERIAL AND METHODS:

Collection of plant material:

The Seeds of *Lagenaria siceraria* will be used. It was collected from Karachi Plants Nursery.

Extraction of plant material:

Soxhlet apparatus was used for the extraction of Seeds of *Lagenaria siceraria*. Seeds of *Lagenaria siceraria* were continuously extracted for 48 h with ethanol in a soxhlet apparatus. The extract will be filtered and concentrated in a rotary evaporator at 30–40°C to obtain semi-solid material. The extract will be dissolved in a suitable solvent for administration to the animals at different dose levels [11].

Drugs and chemicals:

The drugs, chemicals, and solvents were obtained from Hamdard University, Karachi.

Animals:

Swiss albino male mice were used in the study. Animals were kept in a noise-free area in a plastic cage maintained on a temperature of 25 degree and 12 /12-hour standard light / dark cycle and supplied with standard food and water.

Grouping and Dosing Protocol

Total five groups were made, each group comprised of n= 6 animals, the details are mentioned in table 1.

Table 1: Grouping and dosing protocol of behavioral screening

Groups	Control	Standard	Treated group I	Treated group II
No. of animals	n=6	n=6	n=6	n=6
Treatment	Normal saline	Fluoxetine	LSE I	LSE II
Dose	10 ml/kg	20 mg/kg	100 mg/kg	500 mg/kg

LSE: *Lagenaria siceraria Extract*

Paradigms for evaluation of Antidepressant activity
The following two models were used for antidepressant activity:

1. Forced Swim Paradigm
Forced swim paradigm, conducted as per the method proposed by [12]. The apparatus consists of a transparent plastic cylinder filled with water (maintaining temperature at 25 ^{0}C) which helps to induce stress. Antidepressant agents decrease immobility time. Randomly grouped mice received the drug treatment and control as per the above protocol. Initially, a pretest of two swim sessions for an initial 15 min was conducted, and at least 24 h later mice were placed again in the cylinder for 5 min in which the duration of immobility time was noted, and before returning to the home cage all mice were dried properly.

2. Tail Suspension Paradigm
Tail suspension paradigm, conducted as per the method proposed by Steru et al. 1985. All the mice were kept for 1 h before the behavioral procedure in the experimental room to acclimatize. The experimental model was performed 45 min after administration of EEPN, fluoxetine, and vehicle to respective groups as described above in the protocol, mice of all groups were suspended one by one from a horizontal surface via tail by using tape placed 1 cm, from the tip of the tail. After 1 min of acclimatization, immobility time was noted for 5 min. animals were only considered immobile when they were static and hung inactively. Figure 1 represents tail suspension test.

Figure 1: Tail Suspension Test

STATISTICAL ANALYSIS
The results were presented as mean + S.E.M. The standard and treated groups were assessed with a control group by applying a one-way ANOVA analysis. The results were assumed significant at $P<0.001$.

RESULTS
Anti-Depressant Activity of Ethanolic Seed Extract of *Lagenaria siceraria*
Table 2 and figure 2 illustrate the effects of LSE. In which the treated groups and standard group produces significant decreases in the meantime of immobility as compared control group. Moreover, it was also observed that a treated dose of 500 mg/kg of LSE produced greater antidepressant activity as compared to 100 mg/kg.

Table 2: Anti-depressant activity by Force swim test in mice

Treatment	DAY 1	DAY 7	DAY 14
Control	190.66±20.30	176.50±8.54	179.66±18.90
Standard	189.83±20.30	137.50±11.61**	88.0±7.9**
Treated group I (LSE 100 mg/kg)	187.66±17.09	166.0±4.69*	131.16±11.23*
Treated group II (LSE 500 mg/kg)	184.83±14.79	130.83±17.56**	89.16±6.73**

Values are Mean ± SEM, n=6, *indicates significant and **highly significant p<0.05 as compared to control

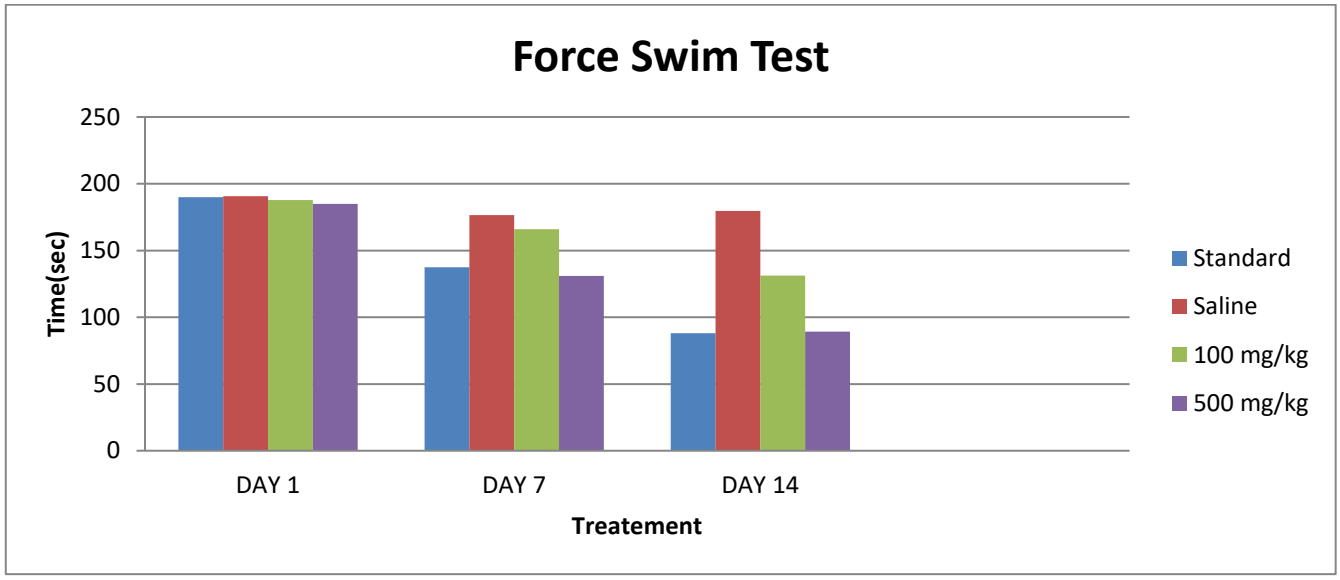

Figure 2: Comparative Graphical representation of antidepressant activity by Force swim test
Values are mean + SEM (n=6) P < 0.05 significant as compared to control.

Table 3 and figure 3 illustrates the Effects of Ethanolic seed extract of *Lagenaria siceraria* on Tail suspension test treatment at the x-axis while time interval at the y-axis (sec). each point represents the mean ±S.E.M. for 06 animals. Standard and treated groups have significant (*p<0.05) results as compared with the control group.

Table 3: Effects of Ethanolic seed extract of *Lagenaria siceraria* on Tail suspension test

Treatment	DAY 1	DAY 7	DAY 14
Control	190.50±8.50	189.16±10.47	179.50±7.81
Standard	187.50±5.68	142.33±5.24**	86.16±4.07**
Treated group I (LSE 100 mg/kg)	189.16±7.41	161.66±5.39*	132.50±5.31**
Treated group II (LSE 500 mg/kg)	190.16±7.67	137.16±6.79**	86.83±4.02**

Values are Mean ± SEM, n=6, *indicates significant and **highly significant p<0.05 as compared to control

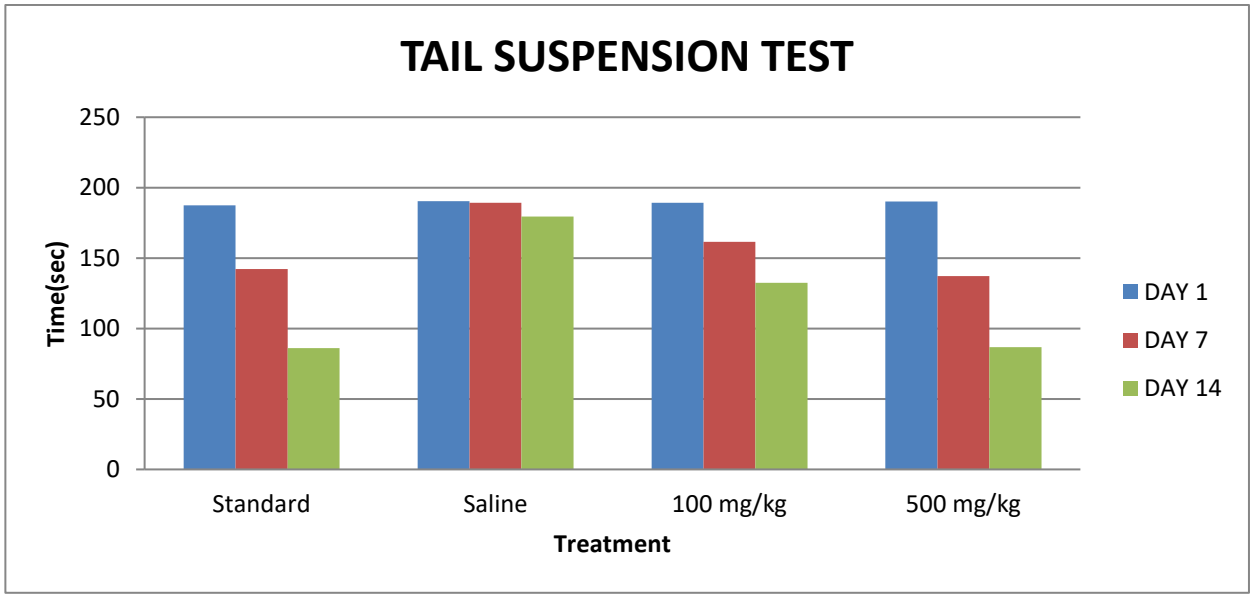

Figure 3: Comparative Graphical representation of anti-depressant activity by Tail suspension test

Values are mean + SEM (n=6) $P < 0.05$ significant as compared to control.

DISCUSSION

In the current study *Lagenaria siceraria* extract was evaluated for its antidepressant activity. It is evident from our study that the plant extract exhibited dose dependent anti-depressant effects in animal models. To estimate antidepressant activity in the current investigation, fluoxetine was used as standard. It is SSRIs that selectively increase serotonin levels in the brain [13] Physicians commonly use classical antidepressant agents, i.e. 5HT, TCAs, and MAOIs, but some of these agents produce toxicity due to which SSRIs are most frequently used [14].

In this study, it was noticed that LSE 100 and 500 mg/kg exhibited significant anti-depressant effects. In the forced swim test and tail suspension test, LSE and fluoxetine considerably decreased immobility time as compared to control. Immobility is the state of hopelessness when rats are allowed to swim in a narrowed or limited area or suspended by the tail due to which they cannot run away easily. This proposes disillusionment to continue in escape after persistent stress or develop passive behavior to cope with a stressful environment [15]. A decrease in immobility time signifies that the state of depression is also decreased [16, 17].

Earlier several theories have been proposed to explain this feature. In serotonergic theory, it was proposed that biochemical significance and acute and chronic behavior of antidepressant agents can reduce serotonergic neurotransmission which results in anxiety and depression due to unnecessary functioning of the serotonergic punishment system [18] There are also several reports relating serotonergic agents as antidepressants and anxiolytics [19, 20, 21].

Another hypothesis proposes that GABAergic, which possesses anxiolytic activity may also produce antidepressant activity. It has been also reported that several GABA-mimetic drugs are also effective as antidepressants [22]. All these studies reveal that anxiety and depression may have some mutual etiological factors additionally, those drugs which exhibit both antidepressant activities should be studied further for their beneficial uses. The current study uses behavioral paradigms to assess the antidepressant activity of plant extract which confirm the potential antidepressant effects however there is need to further explore the mechanistic action by which it exhibited the antidepressant potential in animal models. Furthermore, it is also recommended to explore and isolate the biologically active constituents which are responsible for such activity. It has been reported that *Lagenaria siceraria* contains flavonoids, alkaloids, tannins [23, 24]. The observed antidepressant activity could be due to the presence of such biologically active constituents.

The research need to be extended on large number of animals along with screening of active biological

constituents responsible for observed antidepressant effects of the plant. Furthermore studies on mechanistic evaluation of antidepressant activity should also be conducted.

CONCLUSION

In the current study, *Lagenaria siceraria* indicated an antidepressant effect which was evaluated on two different models i.e. forced swim and tail suspension. This study provides scientific knowledge of the antidepressant effect in mice models moreover further studies are required to evaluate the mechanism of action of the plant**.**

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Competing Interest declaration

All authors declare that they have no competing interests.

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