Original Article

Screening of antidepressant activity of *nelumbo nucifera* flower extract in mice

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ABSTRACT

Objective: To evaluate the anti-depressant activity of *"Nelumbo nucifera"* in experimental mice. **Methods:** The acute toxicity studies were conducted on fresh *"Nelumbo nucifera"* flower extract. The extract (100–200 mg/kg) was able to induce the mice's immobility duration in the Forced swimming and Tail suspension tests in a dose-dependent manner; the effects are comparable to those of popular drugs, such as Imipramine (10 mg/kg). **Result:** Both the lower dose (100mg/kg) and higher dose (200mg/kg) showed a dose-dependent significant decrease in depression. In line with Imipramine, our study found that the ethanolic extract of *"Nelumbo nucifera"* significantly ($p>0.005$) decreased immobility in both the tail suspension test and the forced swimming model of depression. These findings showed that *"Nelumbo nucifera"* exhibited in vivo effects that were selectively anti-depressant**. Conclusion:** The results of this study, in summary, revealed that *"Nelumbo nucifera"* ethanolic extract may have anti-depressant properties that make them potentially useful for treating patients with depressive disorders. However, more research is required to comprehend the mechanism of action and to pinpoint the key ingredient that produces anti-depressant-like activity.

Keywords: Anti-Depressant, *Nelumbo nucifera*, Imipramine, Tail Suspension Test, Force Swimming Test

Around 280 million individuals worldwide suffer from depression. Depression is distinct from common mood swings and fleeting emotional reactions to problems in daily life. Every year, around 700,000 people die by suicide a depressive episode lasts at least two weeks and is characterised by a depressed mood (sad, irritated, or empty feelings) or a loss of enjoyment or interest in activities for the majority of each day. Depending on the frequency and severity of symptoms, the influence on the person's functioning, and the length of the episode, a depressive episode can be classified as mild, moderate, or severe. Due to the negative side effects of synthetic pharmaceuticals, drugs of plant origin are becoming more and more popular. For the treatment of a variety of illnesses, Ayurveda uses plants, plant derivatives, and the active compounds found in plants. Despite the lack of scientific evidence supporting the antidepressant properties of *"Nelumbo nucifera,"* this study was chosen to examine the antidepressant effect of *"Nelumbo nucifera"* in mice. The flower is chosen for the study as a result [1].

The current study's objective was to assess the "*Nelumbo nucifera"* flower extract's anti-depressant effects. Millions of people worldwide suffer from depression, a serious mental health problem, and the standard medications used to treat it have several side effects. Therefore, there is a need for alternative therapies that are both safer and more potent. "*Nelumbo nucifera"*, which has the potential to be an anti-depressant, has been widely utilised in traditional medicine for a variety of reasons in this regard [2]. Two commonly utilised animal models, Tail suspension test and the Forced swimming test, were used to assess extract's anti- depressant potential.

The findings demonstrated that the extract significantly reduced depressive symptoms in both tests.Alkaloids, flavonoids, inositol, proteins, and saponins were all found in the "*Nelumbo nucifera"* flower extract, according to the results of the phytochemical screening. It is known that these phytoconstituents have a range of pharmacological properties, including antidepressant effects. These substances may be a factor in the extract's apparent antidepressant action [3, 4]. Although more research is required to pinpoint the precise mechanisms at play, the anti-depressant effects of *"Nelumbo nucifera"* flower extract may be caused by the presence of these phytoconstituents. However, "*Nelumbo nucifera"* may be used as an alternative treatment for depression, as shown by the study's findings.

MATERIALS AND METHODS

The nectar of the *"Nelumbo nucifera"* flower, which was verified by taxonomist Mrs Aparna Upadhyaya, will be obtained from a pond close to Mangalore. The Department of Pharmacology at Srinivas College of Pharmacy carried out the current investigation.

Animal: From the Indian Institute of Sciences, adult Swiss albino rats (weighing 22–25g) of either sex were obtained. They will be kept in a standard environment with the following parameters: temperature 22°C, relative humidity 50%, and a 12-hour light/dark cycle. All investigations were approved by the institutional animal ethics committee.

Plant extract: After being chopped, the fresh lotus flowers were dried for 72 hours at 50°C in a hot air oven. For seven days, dried flowers were macerated in 95% ethanol. The extract was turned into a powder by being evaporated in a rotary evaporator and dried in a freeze-dryer. It was then kept at -20°C until it was needed.

Preliminary phytochemical screening: The ethanolic extract of *"Nelumbo nucifera"* was screened for the presence of various phytoconstituents like Alkaloids, Flavonoids, inositol, proteins and saponins.

Acute toxicity: *"Nelumbo nucifera"* flower ethanolic extract was tested for acute toxicity by the 2002 revision of OECD guideline No. 423. Animals were monitored for fourteen days for any changes in behaviour, and 24 hours a day showed no toxicity in mice when administered in doses up to 2000 mg/kg orally, 100 and 200 mg/kg doses of the extract were utilised in subsequent investigations.

Experimental design

Tail suspension test: A total of 24 mice were divided into 4 groups of six**.**

GROUP 1: CONTROL (SALINE)
GROUP 2: STANDARD (IMPIRAMINE 10mg/kg)
GROUP 3: NNFE (LOW DOSE – 100mg/kg)
GROUP 4: NNFE (HIGH DOSE – 200mg/kg)

As a control group received a saline solution, Group 2 received Imipramine and Groups 3& 4 received a low and high dose of "*Nelumbo nucifera"* ethanolic extract, respectively. The medications were administered orally one hour before the trial. Each mouse was held 150 cm off the ground, about 1 cm from the tail tip, using adhesive tape. The testing will take place in a silent, well-lit environment. Six minutes in total were logged during the immobility time. The animal initially moved wildly in an attempt to escape, but when they were unable to do so, they became immobile. Following that, the mice received medication for 21 days. Following that, the immobility time was monitored for a total of 6 minutes.

Forced swim test: A total of 24 mice were divided into 4 groups of six**.**

GROUP 1: CONTROL (SALINE)
GROUP 2: STANDARD (IMPIRAMINE 10mg/kg)
GROUP 3: NNFE (LOW DOSE – 100mg/kg)
GROUP 4: NNFE (HIGH DOSE – 200mg/kg)

As a control group received a saline solution, Group 2 received Imipramine and Groups 3& 4 received a low and high dose of "*Nelumbo nucifera"* ethanolic extract, respectively. The medications were administered orally one hour before the trial. The animal was inserted into the model to begin the test. Individual mice were made to swim for 15 minutes in an 11-centimetre-diameter glass beaker that was kept at a constant temperature of 27°C and filled with fresh water to a height of 6 centimetres. Here the "Per test" session was over. 24 hours later, each mouse was required to swim for 6 minutes in the same habitat once more as part of a "Test session." The test session was conducted both before and after the medication therapy. A mouse is considered static if it floats stationary or moves just enough to keep its head above the water's surface. On average, the final four minutes of the six-minute exam were spent motionless.

Tail suspension test

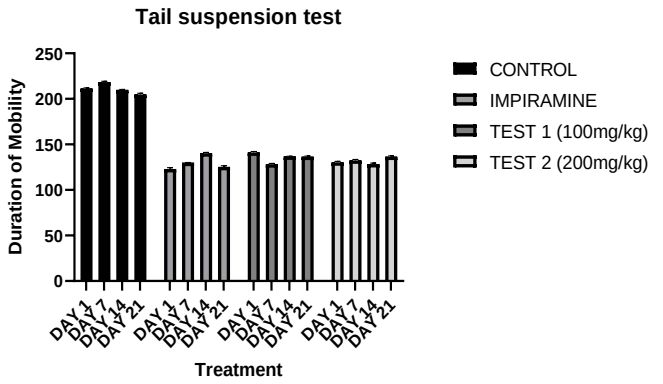
Table 1: Effect of "*Nelumbo nucifera*" flower extract in tail suspension test

DOSE	DURATION OF MOBILITY IN TST (IN SECONDS)			
	DAY 1	DAY 7	DAY 14	DAY 21
CONTROL (Saline)	211.3±0.7350	218.4±0.855	209.9±0.4900	204.8±1.370
STANDARD (Imipramine)	123±1.555***	129.8±0.2200***	140.3±0.8500***	125.4±1.060***
TEST 1 (*Nelumbo nucifera* 100mg/kg)	141.1±1.055**	128.3±0.8200**	136.8±0.2050**	136.5±0.700**
TEST 2 (*Nelumbo nucifera* 200mg/kg)	130.4±0.9250***	132.3±1.145***	128.5±1.080***	136.5±0.9700***

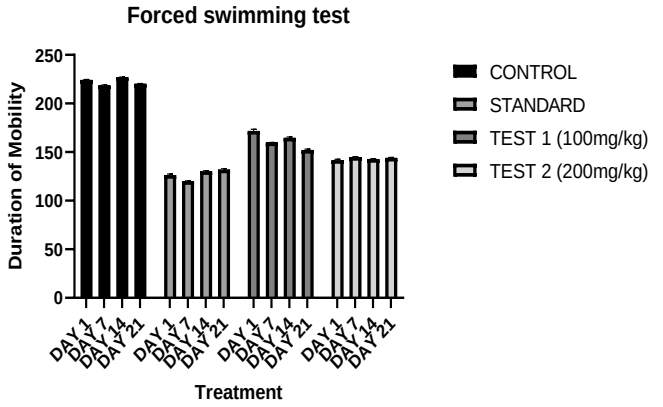
Forced swimming test

Table 2: Effect of *NELUMBO NUCIFERA* flower extract in Forced swim test on mice

DOSE	DURATION OF MOBILITY IN FST (IN SECONDS)			
	DAY 1	DAY 7	DAY 14	DAY 21
CONTROL (Saline)	224.3±0.400	218.7±0.4000	227.1±0.0500	220.3±0.2500
STANDARD (Imipramine)	126.3±1.000***	120.0±0.3500****	130.3±0.4000***	132.0±0.7500***
TEST 1 (*Nelumbo nucifera* 100mg/kg)	171.7±1.800**	159.8±0.0500**	164.4±0.8500**	152.1±0.900**
TEST 2 (*Nelumbo nucifera* 200mg/kg)	141.7±0.8400***	144.9±0.3350***	142.9±0.3300***	144.0±0.4900***



Graph 1: Effect of "*Nelumbo nucifera*" flower extract in tail suspension test



Graph 2: Effect of *NELUMBO NUCIFERA* flower extract in Forced swim test on mice

RESULTS

On preliminary phytochemical analysis of NNFE showed the presence of phytoconstituents like Alkaloids, Carbohydrates, Flavonoids, Glycosides, Saponins, Steroids, Tannins, Protein, Inositol. Acute toxicity of NNFE showed no behavioural changes nor mortality at a dose of 2000mg/kg. By examining the variations in the period of inactivity in the two models, the anti-depressant effects of the ethanolic extract of *"Nelumbo nucifera"* (100mg/kg and 200mg/kg) and standard medications Imipramine were investigated.

DISCUSSION

Animal models like the forced swimming test (FST) and the tail suspension test (TST) are frequently used to examine the effectiveness of possible antidepressant medications. A tiny rodent (such as a mouse or rat) is used in the FST, and the duration of time the animal remains motionless is tracked. The duration of immobility is used to gauge depressive-like behaviour. The animals are given antidepressant medication before the test, and the difference in immobility time is used to determine the effectiveness of the medication. Similar to this, the TST involves suspending a small rat by its tail and timing how long it remains still. The immobility time, like the FST, is used as an indicator of depressive-like behaviour, and the change in immobility time following the administration of antidepressant medications is used to assess the efficacy of those medications.

The FST and TST have both been extensively utilised in preclinical investigations and have been proven to be accurate measures of a drug's antidepressant effectiveness. These models have significant drawbacks as well, though. For instance, some have claimed that the immobility duration in the FST and TST might not truly reflect animal behaviour that resembles depression because the immobility could be caused by other things, such as exhaustion or hopelessness.

The FST and TST are nevertheless often employed in preclinical research despite these critiques because of their simplicity of use and the close relationship between the outcomes of these tests and the clinical effectiveness of medications in humans. The FST and TST are still useful methods for researching the effectiveness of possible antidepressant medication as a result. The Forced swimming test and the Tail suspension test were used in the study to evaluate the "*Nelumbo nucifera*" flower extract's anti-depressant potential. "*Nelumbo nucifera*" may be used as an alternative treatment for depression because the results demonstrated considerable anti-depressant effects.Alkaloids, flavonoids, inositol, proteins, and saponins were found in the extract, indicating the possibility that these phytoconstituents contributed to the anti-depressant effect that was reported.

The study did, however, have certain drawbacks, including a small sample size, a lack of human trials, and a limited understanding of the mechanism of action. It will take more investigation with larger sample sizes and human studies to validate these findings and pinpoint the precise mechanisms at play [5-10]. Hence the present study showed "*Nelumbo nucifera*" might be useful in depression, as it increases dopamine levels in the brain increase decrease monoamine oxidase. Our results confirm the traditional use of plants as antidepressants.

CONCLUSION

In summary, this study's findings suggest that "*Nelumbo nucifera"* flower extract may have promise as an antidepressant. Because of the considerable anti-depressant action seen in both animal models, there is reason to believe it could be used as an alternative to traditional depression treatments. More investigation is needed to identify the precise mechanisms at play and assess their safety and effectiveness in human studies. However, these findings add to the expanding body of research that shows traditional medicine is effective in treating mental health illnesses like depression.

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