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Antidepressant Activity of Different Extracts of *Nelumbo Nucifera* Leaves in Laboratory Animals

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A B S T R A C T

In the present study, the anti-depressant activity of *Nelumbo nucifera* Gaertn. leaves (Nymphaeceae) was studied in acute model forced swimming test (FST) and tail suspension test (TST) in mice, which serve as two predictive models of depression. NN has been widely used in medicine for the treatment of different central nervous system (CNS) disorders. Nevertheless, the available scientific information about this species is scarce and there are no reports related to its possible effects on the CNS. In this work, the effects of methanolic, hydroalcoholic and aqueous extracts of leaves of *Nelumbo nucifera* were evaluated in mice at the doses of 50, 100 and 200 mg/kg showed significant (*p<0.05) decrease in immobility time when compared with the control, which was received 2% tween 80. But among all these extracts, only the hydroalcoholic extract of NN showed a significant (#p<0.05) reduction in the duration of immobility time of mice at the dose of 200 mg/kg when subjected to both tail suspension and forced swim tests and the effects are comparable to that of standard drug i.e., fluoxetine (20mg/kg). These results demonstrated that NN showed antidepressant effects *in vivo*. Phytochemical screening showed presence of alkaloids and flavonoids in the extract of NN, which might affect certain mediators of brain to reduce immobility time in mice. The results of the present study indicate the potential for the use of NN as an adjuvant in the treatment of depression. In conclusion, the present study suggested that NN extracts possess potential antidepressant effects which could be of therapeutic interest for using in the treatment of patients with depressive disorders.

Keywords: *Nelumbo nucifera* leaves, Antidepressant, Forced swimming test (FST) and Tail suspension test (TST)

A R T I C L E I N F O

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1. Introduction

Depression is a heterogeneous disorder that affects a person's mood and behaviour. Patients with major depression have symptoms that reflect changes in brain monoamine neurotransmitters, specifically nor-epinephrine, serotonin and dopamine. Reserpine and other antihypertensive drugs that deplete neuronal storage granules of norepinephrine, serotonin and dopamine, causes clinically significant depression in 15% or more of patients [1]. Depression is considered as an affective disorder characterized by change in mood, lack of interest in the surroundings, psychomotor retardation and melancholia [2]. In spite of the availability of antidepressant drugs like tricyclic anti-depressants, selective reversible inhibitors of monoamine oxidase-A (MAO-A), selective serotonin reuptake inhibitors (SSRIs) and selective nor-adrenaline reuptake inhibitors (SNRIs), depression continue to be a major medical problem. Basic neuroscience offers the promise of improving our understanding of disease pathophysiology, identifying novel mechanisms that can be targeted by more effective pharmacotherapies and screening of herbal sources of drugs. These considerations implicate the search for new antidepressant agents that have a fast onset of action, with less side effects and a wider safety margin. Various plants are being used in complementary and alternative medicines for management of mood disorders [3].

Ayurveda, the Indian traditional system of medicine, mentions a number of single and compound drug formulations of plant origin that are used in the treatment of psychiatric disorders. On one hand these agents have a less adverse effect profile and on the other hand they have been shown to be comparable in efficacy to their synthetic counterparts[2]. On the basis of the above information, one of these can be the use of *Nelumbo nucifera* Gaertn. (Family: Nymphaeaceae) also known as Tamara (lotus). *Nelumbo nucifera* (NN) is widely distributed in South-East Asia. In India, it occurs from Kashmir in north to Kanyakumari in south [4]. The leaves of *Nelumbo nucifera* (NN) Gaertn, (Nymphaeceae) was selected for evaluating antidepressant activity. The leaves are large and orbicular; the young leaves are eaten as vegetables and used in traditional medicine. The major components present are alkaloids such as nuciferine, roemerine and benzyl isoquinoline [5]. In Ayurveda this plant is used as a diuretic and anti-helmintic and also in the treatment of vomiting, leprosy, skin diseases and nervous exhaustion [6]. Chemical constituents in NN include alkaloids, flavonoids, phenolic compounds and tannins. Therefore, the present study has been undertaken to investigate the effect of methanolic, hydroalcoholic and aqueous extracts of the leaves of *Nelumbo nucifera* on depression in mice using different experimental models on CNS.

2. Materials and Methods

2.1. Collection of Plant material

The *Nelumbo nucifera* Gaertn. (Nymphaeaceae) leaves were collected from the local regions and authenticated by taxonomists. These were made free from the adherent foreign material, air-dried, cut into small pieces and coarsely powdered mechanically.

2.2. Extraction of Herb

The dried and powdered leaves were defatted with petroleum ether (60-80°C). Methanolic extract and Hydroalcoholic extract (70% ethanol) were obtained by Soxhlet extraction method and aqueous extract by decoction method [7]. The Methanolic, Hydroalcoholic and Aqueous extracts of *Nelumbo nucifera* are termed as MENN, HENN and AQNN respectively.

2.3. Preliminary Photochemical Investigation

The extracts of *Nelumbo nucifera* were screened for the presence of various Phytochemical constituents like alkaloids, flavonoids, Carbohydrates, Glycosides, Phytosterols, Saponins, phenolic compounds and tannins [7-10].

2.4. Animals

Healthy Swiss albino mice, either sex weighing 20-30gram were procured from the Teena Bio-labs Pvt. Ltd. (Reg. No. 177/1999 CPCSEA), Hyderbad, Telangana. Animals were housed at CPCSEA approved animal house (1553/PO/a/11 /CPCSEA). The animals were stabilized for 1 week; they were maintained under standard conditions at temperature of 25 ± 1°C, 60 ± 5 % relative humidity and 12–hours light dark cycle. They had been given standard pellet diet supplied by Hindustan Lever Co., Bombay and water ad libitum throughout the course of study. All the study protocols were reviewed and approved by Institutional Animal Ethical Committee (IAEC). The studies were strictly followed Ethical norms during all experimental procedures.

2.5. Source of Drugs, chemicals and Equipments

Diazepam (Natco Pharma Ltd), chemicals- Diethyl ether and tween-80 (Merck, Mumbai), Equipment-Elevated Plus maze and Light-dark apparatus.

2.6. Acute Toxicity Study

The Acute Toxicity Studies were performed using Swiss albino mice as per OECD Guideline No.423 [11]. The median lethal dose of the pet-ether alcohol and aqueous were determined by orally administering the extracts in increasing dose levels of 0.1,0.2,0.5, 1, 1.5 and 2 g/kg body weight to healthy mice of either sex. The animals will be observed continuously for 2 h and later 24 hr intervals for a period of 48 hrs, at the end of this period, if any mortality in different dose groups were noted.

Nelumbo nucifera (NN) was found to be safe till a dose of 2000 mg/kg since no mortality and abnormal toxicity was observed at this dose. According to OECD guidelines, maximal safe dose was selected for this study. Hence, three doses of NN [MENN, HENN and AQENN] were selected for the study. The doses are 50, 100 and 200 mg/kg. All extracts are given by Oral administration (p.o.).

2.7. Study Design

For the following activities the animals were divided into eleven groups, each group containing six animals.

Group I Served as a control and received 2% Tween 80 (10ml/kg).

Group II, III and IV received Methanolic extracts (ME) of 50, 100 and 200 mg/kg respectively.

Group V, VI and VII received Hydroalcoholic extracts (HE) of 50, 100 and 200 mg/kg respectively.

Group VIII, **IX and X** received Aqueous extract (AQ) of 50, 100 and 200 mg/kg respectively.

Group XI Served as a standard and received Diazepam (2 mg/kg) a standard Drug. The test groups were from **II** to **X** group. The extracts of *Nelumbo nucifera* (NN) termed as MENN, HENN, and AQNN respectively.

2.8. Evaluation of Antidepressant Activity (*In-vivo* methods)

2.8.1. Forced Swim Test (FST)

Depression was produced by forcing the animal to swim individually in a glass jar containing fresh water of 15cm height and maintained at 25^{o}C. This constituted pre-test session. Twenty-four hour later each animal was again forced to swim. After an initial 2 min period of vigorous activity, each animal assumed a typical immobile posture. The total duration of immobility was recorded in next 4 min of a total 6 min test. The change in the immobility period was calculated after administering drugs to the groups as mentioned in the above.

2.8.2. Tail Suspension Test (TST)

The total duration of immobility induced by tail suspension was measured. Depression was produced by suspending the animal from the edge of a table 50 cm above the floor by 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 test drug [12].

2.8.3 Statistical analysis

Values are expressed in Means ± S.E.M. for six animals in each group and statistically assessed by one-way analysis of variance (ANOVA) and subjected to Dunnett's test. The $p<0.05$ was considered significant.

3. Results and discussions

3.1. Percentage of yield: The percentage yield of extraction of *Nelumbo nucifera* (NN) leaves were showed more in methanolic (MENN) and Hydroalcoholic (HENN) extracts when compared with Aqueous (AQNN) extract. (56.2%, 62.8% and 28.20% respectively)

3.2. Phytochemical Screening:

The methanolic, hydroalcoholic and aqueous extracts were showed positive results for Alkaloids, flavonoids, Carbohydrates, Glycosides and tannins but negative result for phytosterols.

3.3. Effect of *Nelumbo Nucifera* leaves extracts on immobility period in Forced swim test (anti depressant activity)

Depression was produced by forcing the animal to swim individually in a glass jar containing fresh water of 15cm height and maintained at 25^{o}C. The total duration of immobility was recorded in next 4 min of a total 6 min test. The change in the immobility period was calculated after administering drugs to the groups. The antidepressant activity was expressed as reduction in the immobility time between the control, standard and animals treated with test drug. The results are given in Table 4.18 and Figure 4.12 below. the treatment with the all extracts of NN (MENN, HENN and AQNN) showed moderately decreases in immobility time at dose of 50 and 100 mg/kg, but at 200 mg/kg dose of MENN and HENN there was a significant (#$p<0.05$) decrease in the immobility time and at these doses only changes in the swimming behavior was also observed when compared with control. Similarly, animals were treated with fluoxetine (20 mg/kg), as expected, showed significant decreases in the immobility time compared with control.

3.4. Effect of *Nelumbo nucifera* leaves extracts on immobility period in Tail suspension test (anti-depressant activity)

The total duration of immobility induced by tail suspension was measured immobility time was recorded during a 6 min. period. Changes in the immobility period were studied after administering. The antidepressant activity was expressed as reduction in duration of immobility time among the control, standard and animals treated with test drug. The results of this study are shown in Table 1 and in Figure 1 below. A significant (#$p<0.05$) decrease in the immobility period at 200 mg/kg dose of MENN and HENN when compared with that of fluoxetine a standard drug. But in the treatment of all extracts of NN (MENN, HENN and AQNN) showed moderately the decreases in immobility period at 50 and 100 mg/kg dose levels, when compared with control as well as fluoxetine, similarly, animals were treated with fluoxetine (20 mg/kg), as expected, showed a significant decreases in immobility period compared with control group.

Table 1: Effect of *Nelumbo nucifera* leaves extracts on immobility period in Forced swim test

Group No.	Treatment	Dose	Immobility period (Sec)		
			Pre-treatment	Post-treatment	
				7 days	14 days
1	2% Tween80	10 ml/kg	150.2±9.3	144.0±11.2	146.3±9.7
2	MENN	50 mg/kg	142.5±10.8	138.0±10.0*	132.8±16.5*
3		100 mg/kg	150.0±13.6	142.3±10.2*	130.3±12.0*
4		200 mg/kg	150.2±12.5	140.2±12.8*	120.0±10.1*#
5	HENN	50 mg/kg	158.1±12.8	150.2±12.9*	140.3±10.2*
6		100 mg/kg	159.3±10.2	147.6±12.3*	128.2±19.8*
7		200 mg/kg	160.2±16.5	133.0±11.0*	128.7±12.0*#
8		50 mg/kg	160.1±15.2	142.9±12.3*	133.8±12.8*

9	**AQNN**	**100 mg/kg**	150.5±10.6	140.2±10.3*	140.0±12.9*
10		**200 mg/kg**	160.7±17.3	153.2±12.3*	140.3±10.9*
11	**Fluoxetine**	**20 mg/kg**	169.1±10.1	132.3±9.3*	119.2±10.6*

Values are expressed as (Mean ± SEM), n= 6, All groups were compared with Normal control group *p<0.05 and standard group and significance shown by #p<0.05. Statistically analyzed by one- way analysis of variance (ANOVA) followed by Dunnett's test.

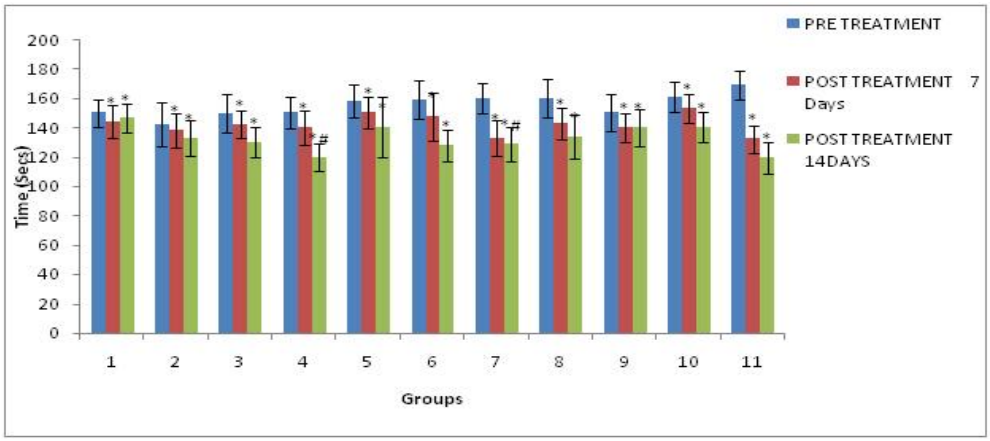

Figure 1: Effect of *Nelumbo nucifera* leaves extracts on immobility period in Forced swim test (Values expressed as Mean ± SEM)

Table 2: Effect of *Nelumbo nucifera* leaves extracts on immobility period in Tail suspension test

Group No	Treatment	Dose	Immobility period (sec)		
			Pre-treatment	Post treatment	
				7 days	14 days
1	**2%Tween80**	10 ml/kg	154.2±10.2	149.0±10.2	146.3±10.7
2	**MENN**	50 mg/kg	160.2±10.8	152.0±12.5*	148.6±14.0*
3		100 mg/kg	160.7±12.0	140.0±12.0*	130.0±12.2*
4		200 mg/kg	160.2±10.3	135.3±10.8*	125.2±12.5*#
5	**HENN**	50 mg/kg	158.3±16.6	154.5±15.4*	148.2±12.0*
6		100 mg/kg	156.1±15.0	140.2±12.0*	130.5±9.6*
7		200 mg/kg	164.3±13.2	140.8±12.3*	122.2±12.0*#
8	**AQNN**	50 mg/kg	158.0±15.0	147.7±12.4*	140.0±12.3*
9		100 mg/kg	159.3±12.0	150.3±12.8*	146.5±11.9*
10		200 mg/kg	158.0±12.9	146.0±11.1*	139.4±12.5*
11	**Fluoxetine**	20 mg/kg	163.1±10.4	122.3±10.2*	111.2±10.3*

Values are expressed as (Mean ± SEM), n= 6, All groups were compared with Normal control group *p<0.05 and standard group and significance shown by #p<0.05. Statistically analyzed by one- way analysis of variance (ANOVA) followed by Dunnett's test.

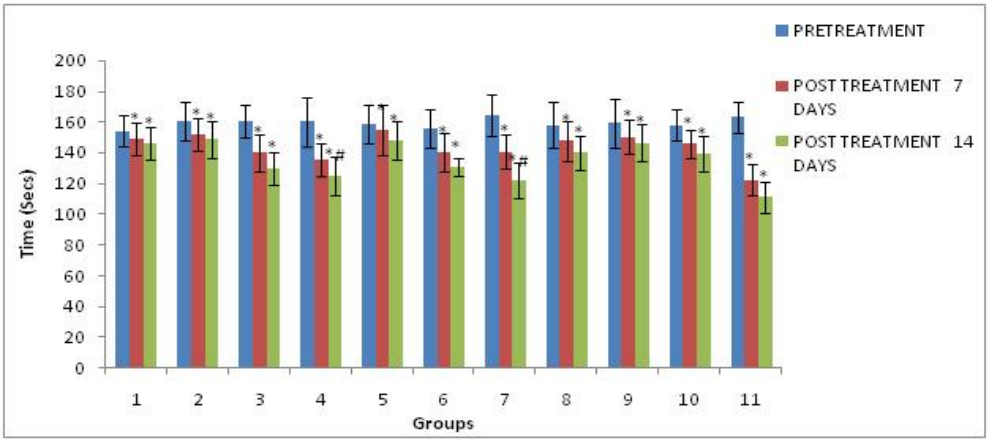

Figure 2: Effect of *Nelumbo nucifera* leaves extracts on immobility period in Tail suspension test (Values expressed as Mean ± SEM)

Discussion

The decrease in brain neurotransmitters; serotonin, norepinephrine and dopamine has been implicated in the incidence of depression. New theories suggest that the three neurotransmitters are interrelated in the treatment of depression [13]. On the basis of the clinical association of depressive episodes and stressful life events, many of the animal models used for the evaluation of antidepressant drug activity assess stress-precipitated behaviours. The two most widely used animal models for antidepressant screening are the forced swimming and tail suspension tests. These tests are quite sensitive and relatively specific to all major classes of antidepressants. In the FST, mice are forced to swim in restricted space from which they cannot escape. This induces a state of behavioral despair in animals, which is claimed to reproduce a condition similar to human depression. It has been seen that the TST is less stressful and has higher pharmacological sensitivity than FST [3].

In the present study we provide convincing evidence that the methanolic and hydroalcoholic extracts of NN and produce a specific antidepressant-like effect at doses of 100 and 200 mg/kg and showed a significant decrease in the immobility time in the forced swimming test and tail suspension test when compared to that of fiuoxetine. It has been established that the shortening of immobility time in both the tests depends mainly on the enhancement of central 5-HT and catecholamine neurotransmission [3] and is useful for the evaluation of the potential of antidepressant agents [14]. It is evident that the increase in adenylate cyclase activity and hence facilitation of serotonergic transmission by antidepressant drugs, is contributive to the management of depression[15].Based on the above studies the methanolic and hydroalcoholic extracts of leaves of *N nucifera* (200mg/kg) illustrated the significant maximum response antidepressant effect in mice. Similarly, in our study that NN, by acting through a similar mechanism, might increase the brain levels of monoamines like epinephrine and dopamine by inhibiting monoamine oxidase. These have an involvement in dopaminergic and serotonergic systems in its antidepressant like activity.

The alkaloids, flavonoids[3] and tannic acid[2] act as reversible monoamine oxidase inhibitors thereby increasing the levels of monoaminergic neurotransmitters in the brain by the reduction in metabolism of biogenic amines in the central nervous system [16]. Thus, our results suggest that the presence of alkaloids, flavonoids, phenolic compounds and tannins. The alkaloids and flavonoids are more present in methanolic and hydroalcoholic extracts of NN, which might shows mood elevation to its antidepressant activity. Fluoxetine is a selective serotonin reuptake inhibitor (SSRI) and raises the synaptic concentration of 5- HT and signal transduction for 5-HT pathway is operative through cAMP pathway, it seems that fluoxetine exerts augmented effect while acting in facilitated 5-HT pathway [15]. Based on above the studies, it can be suggested that the NN has able to decrease the immobility time in mice. Thus it may be concluded that NN has exhibited antidepressant activity in both TST and FST and their efficacies were found to be comparable to fluoxetine. These paradigms can exert their effect through a mechanism similar to that of the fluoxetine via the serotonin system. This effect seems most likely to be mediated through an interaction with adrenergic and dopaminergic systems. Thus extracts of NN may have potential therapeutic value for the management of depression disorder.

4. Conclusion

This study shows that *N. nucifera* in the doses of 100 mg/kg and 200 mg/kg in mice has antidepressant like action, similar to that of fluoxetine, the optimum effect was observed at a dose of 200 mg/kg which was significantly higher than the vehicle treated control group i.e it was concluded that the *Nelumbo nucifera* possess antidepressant activity which was evidenced by all the models as described above. The results have been obtained carefully from the controlled experimental models with laboratory animals. The statistical validity of the findings has been proven and they provide a scientific foundation for the use of the biologically active ingredients of *Nelumbo nucifera* in depression, which might affect certain mediators of brain, however, further research is required to gain closer insights into the exact mechanism of its action.

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