

**ANTIDEPRESSANT ACTIVITY OF ETHANOLIC EXTRACT OF
NYMPHAEA ALBA FLOWER IN ALBINO MICE****MADHAVI EERIKE*¹ AND UMA MAHESWARI .N²**

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ABSTRACT

The present study was undertaken to evaluate the anti-depressant activity of Nymphaea Alba Flower (NAF) in albino mice. Forty eight adult albino mice (Swiss strain) weighing between 25-35gr were chosen. 24 animals were assigned to forced swim test (FST) and 24 o tail suspension test (TST).In each model there were 4 groups. The control group received vehicle (10 ml /kg⁻¹, p.o.), the two test groups received ethanolic extract of Nymphaea Alba Flower 100, 200 mg kg⁻¹ and the standard, imipramine (10 mg /kg⁻¹, p.o.) p.o., respectively, 60 minutes prior to the acute study. In chronic study, the drugs were given orally once a day for 10 days and the last dose was given 60 minutes before the experiment. Duration of immobility was noted in FST and TST. Statistical analysis was performed using Mean±SEM and ANOVA followed by Dunnett's test. The p<0.05 was considered statistically significant. Ethanolic extract of Nymphaea Alba Flower produced significant antidepressant effect at both the doses (100, 200 mg kg⁻¹), as indicated by reduction in the duration of immobility compared to the control. The antidepressant effect was higher at 200mg than at 100 mg. In addition, the effect at 200 mg was greater than that of imipramine. Ethanolic extract of Nymphaea Alba Flower thus has shown significant antidepressant activity greater than imipramine in mice.

KEY WORDS: Anti-depressant activity, Tail suspension test , Forced swim test, Nymphaea Alba, Imipramine.

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INTRODUCTION

Depression is a common mental disorder that presents with depressed mood, loss of interest or pleasure, decreased energy, feelings of guilt or low self-worth, disturbed sleep or appetite, and poor concentration. Moreover, depression often comes with symptoms of anxiety. These problems can become chronic or recurrent and lead to substantial impairments in an individual's ability to take care of his or her everyday responsibilities. At its worst, depression can lead to suicide. Almost 1 million lives are lost yearly due to suicide, which translates to 3000 suicide deaths every day. For every person who completes a suicide, 20 or more may attempt to end his or her life. Depression is a significant contributor to the global burden of disease and affects people in all communities across the world. Today, depression is estimated to affect 350 million people. The World Mental Health Survey conducted in 17 countries found that on average about 1 in 20 people reported having an episode of depression in the previous year. Depressive disorders often start at a young age; they reduce people's functioning and often are recurring. For these reasons, depression is the leading cause of disability worldwide in terms of total years lost due to disability. The demand for curbing depression and other mental health conditions is on the rise globally¹. Suicide is the major consequence in most of the depressive illnesses. About 60% deaths are due to depression and related disorders². Chronic stress is one of the main triggers of inducing depression even though the mechanism of provoking depression is not clearly established³. Most of the synthetic drugs used in the treatment of depression have various adverse effects. Insomnia and loss of libido with selective serotonin (5 HT) reuptake inhibitors and tolerance and physical dependence with tricyclic antidepressants are very common; several drug-drug interactions may occur⁴. These limitations create a need for alternative treatment of depression such as medicinal plants and plant based antidepressant formulations. Among the medicinal plants *Nymphaea Alba* Flower is the most important one which is widely distributed

in Asian countries has more medicinal values. *Nymphaea alba*, also known as the European White Waterlily, White Lotus, or Nenuphar, is an aquatic flowering plant of the family Nymphaeaceae. It grows in water from 30-150 cm deep and likes large ponds and lakes. The leaves may be up to thirty centimeters in diameter and they take up a spread of 150 cm per plant. The flowers are white and they have many small stamens inside. It contains the active alkaloids nupharine and nymphaeine and is a sedative and an aphrodisiac/anaphrodisiac depending on sources. The root of the plant was used by monks and nuns for hundreds of years as an anaphrodisiac, being crushed and mixed with wine. It is rich in tannic acid, gallic acid, alkaloids, sterols, flavonoids, glycosides, hydrolyzable tannins and high-molecular-weight polyphenolic compounds⁵.

MATERIALS AND METHODS

i. Animals

The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of Chettinad Hospitals and Research Institute in its meeting dated on 10.01.13. The animals were kept in clean and dry plastic cages, with 12h: 12h light dark cycle at 24±2°C temperature and 45-55% relative humidity. The animals were fed with standard pellet diet and water was given *ad libitum*. The animals were used according to the CPCSEA guidelines for the use and care of experimental animals.

ii. Drugs and chemicals

The standard antidepressant drug imipramine (M/s. Alkem Ltd. Mumbai) was obtained from our institutional pharmacy. Standardized ethanolic extract of *Nymphaea Alba* Flower powder was purchased from M/s. Chemill Nutraceuticals Pvt. Ltd., Bangalore.

iii. Experimental design

On the day of the experiment, the animals were divided randomly into control and experimental groups (n = 6). Group 1 received the vehicle, normal saline (10 mL kg⁻¹) and served as the control, groups 2 and 3, the test

drug (NAF) in doses of 100 and 200 mg kg⁻¹ and group 4 received the standard drug imipramine (10 mg kg⁻¹) per orally. Single dose of Drugs/vehicle was administered to the animals 60 min prior to the evaluation in acute study. For chronic study, a new set of animals were used. They were grouped as in acute study and were administered the drug/vehicle orally once daily for a period of 10 days. Evaluation was carried out at 60 min post drug/vehicle administration on the 10th day. The antidepressant activity was evaluated by TST and FST.

iv. Tail suspension test (TST)

The method described by Steru et al. (1985) was used in our study. The animals were hung by the tail on a plastic string 75 cm above the surface with the help of an adhesive tape. Mice were considered immobile only when they hung passively and were completely motionless. The duration of immobility was observed for a period of 8 min and the last 6 min value was taken for calculation⁶.

v. Forced swim test (FST)

The method described by Porsolt et al. (1977) was used in our study. Each animal was placed individually in a 5 L glass beaker, filled with water up to a height of 15 cm and was observed for a duration of 6 min, last 4 min values were taken for calculation. The mouse was considered immobile when it floated motionless or made only those moments necessary to keep its head above the water surface. The water was changed after each test⁷.

vi. Statistical analysis

The Mean±SEM values were calculated for each group. The data were analysed using one-way ANOVA followed by Dunnett's multiple comparison test. The p<0.05 was considered to be significant.

RESULTS

Tail suspension test (TST)

A significant (p<0.01) decrease in the duration of immobility was seen with the standard drug imipramine and ethanolic extract of NAF in all the tested doses as compared to the control in acute study but in chronic study the dose of 100 mg kg⁻¹ produced a greater decrease in the duration of immobility as compared to 200 mg kg⁻¹ and the standard drug imipramine (Table 1).

Forced swim test (FST)

A significant decrease in the duration of immobility was seen with the standard drug imipramine and at both the doses of ethanolic extract of NAF as compared to the control. In acute study, ethanolic extract of NAF in a dose of 100 mg kg⁻¹ is more efficacious than imipramine in reducing the duration of immobility (Table 2). However, in chronic study, ethanolic extract of NAF in both the doses (100 and 200 mg kg⁻¹) was more efficacious than imipramine. The duration of immobility was however shorter for 100 than 200 mg.

Table-1
Effect of ethanolic extract of NAF on immobility time in the tail suspension test (TST) using mice

Group (Drug Treatment)	Duration of Immobility (sec)	
	Acute Study	Chronic Study
Group-1(Control)	231.16±10.33	209.66±15.44
Group-II (Test-100mg/kg)	137.16±11.88**	140.37±06.98**
Group-III (Test-200mg/kg)	163.33±05.55**	153.33±42.36**
Group-IV(Imipramine 10.0 mg/kg)	163.66±05.56**	153.00±05.50**

mean±S.E.M. (n=6), *P<0.05, **P<0.01 vs. control (group 1).

Table-2
Effect of ethanolic extract of NAF on immobility time in the forced swim test (FST) using mice

Group (Drug Treatment)	Duration of Immobility (sec)	
	Acute Study	Chronic Study
Group-1(Control)	119.17±4.34	122.83±06.61
Group-II (Test-100mg/kg)	41.89±6.53**	54.83±03.97**
Group-III (Test-200mg/kg)	67.50±5.03**	55.00±11.37**
Group-IV(Imipramine 10.0 mg/kg)	66.33±6.81**	56.33±03.59**

mean±S.E.M. (n=6), *P<0.05, **P<0.01 vs. control (group 1).

DISCUSSION

The prevention and management of stress disorders remains a major clinical problem. Depression is a widespread psychiatric disorder affecting approximately 5% of the world population⁸. In addition to the conventional anti depressant drugs, many plant products and ritual therapies have been used for thousands of years. Among the medicinal plants *Nymphaea Alba* all the parts of the plant have medicinal uses in traditional system of medicine. It is used as an aphrodisiac, anodyne, antiscrophulatic, astringent, cardiotoxic, demulcent, sedative and antiinflammatory. Further, it also produces calming and sedative effects upon the nervous system, and is useful in the treatment of insomnia, anxiety and similar disorders⁹⁻¹². Oxidative stress represents a loss of balance in oxidation-reduction reactions. It is characterized by the reduced ability of the antioxidant defense system to efficiently eliminate the excess of the oxygen-derived species production, eliciting the toxicity of oxygen and its detrimental effects. Increased oxidative stress is seen in patients suffering from depression^{13, 14}. Antioxidants such as N-acetylcysteine has been tried as a newer modality for the treatment for depression with encouraging results¹⁵. Antioxidant property of NAF could also contribute to its antidepressant

activity. In this work, it was demonstrated that the administration of different doses of the ethanolic extract of *NAF* in mice was able to induce antidepressant effects. In forced swimming test, the extract can decrease the immobility time in rats with mild sedative effect. It was found that *NAF* can produce antidepressant like activity at a dose of 100mg and 200mg/kg body weight in a dose dependent manner. The decrease in the immobility time is accompanied with the increase in swimming time. Previous demonstrated that many neurotransmitters were involved in the pathophysiology of depression. Numerous studies have demonstrated that antidepressant drugs such as Thioxetine, Imipramine stimulated the action of serotonin and act by inhibiting the reuptake of biogenic amines in CNS. These drugs were widely used as antidepressant drugs and agreed with studies in animal models, such as forced swimming test¹⁶.

CONCLUSION

In conclusion our present study confirmed that the ethanolic extract of *Nymphaea Alba* Flower has the antidepressant activity as it significantly reduces the immobility time. Further research is required to gain closer insights into the exact mechanism of its action.

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