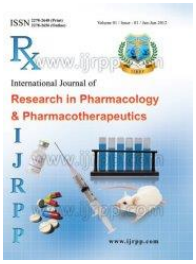
International Journal of Research in Pharmacology & Pharmacotherapeutics



ISSN Print: 2278-2648 ***IJRPP |Vol.7 | Issue 3 | July - Sep - 2018***
ISSN Online: 2278-2656 ***Journal Home page: www.ijrpp.com***

Research article ***Open Access***

Antidepressant activity of ethanolic extract of *neptunia natans* on swiss albino mice

[1]P. Seetharam, [2]Venkataiah G, [2]Ajay Karthik, [2]Humera Naaz

[1]KVK, College of Pharmacy, Hyderabad, Telangana, India
[2]Dhanvanthri College Pharmaceutical Sciences, Mahabubnagar, T.S. India.
***Corresponding author: P. Seetharam**

ABSTRACT

The aim of the study is to study the phytochemical screening and antidepressant activity of ethanolic extract of the *neptunia natans leaf extract* in *Swiss* albino mice. The Phytochemicals was extracted using ethanol as a solvent, The maximum tolerated doses study was performed with the dose of 2000mg/ kg of EENN leaf extract and 50 and 100 mg/kg body weight of EENN was selected as test-I and test-II Respectively and imipramine was used as standard drug performed by despair swim test, tail suspension test and locomotor activity on *Swiss* albino mice. The EENN showed the significant ((p<0.001) antidepressant effect at the dose of 100 mg/kg body weight against control treated group. It is concluded that that the oral administration ethanol extracts of *neptunia natans* leaf extract shown a significant antidepressant effect at 100mg/kg body weight may be attributed due to the presence phytochemicals such as phenols, flavonoids, tannins, triterpenoids, alkaloids. Hence, further study is needed to identify, isolate the specific phytochemical drug candidate showing antidepressant activity and therapeutic confirmation of EENN leaf extract on different animal models.

Keywords: *Neptunia natans*, Immobility, Climbing, Swimming, Antidepressant, Imipramine.

INTRODUCTION

Depression is a major contributor to the global burden of disease and affects people in all communities across the world and 450 million people suffer from some type of mental or behavioral disorder. [1] The lifetime prevalence for major depression is reported to be as high as 14-17% and the one-year prevalence is 4-8%. The lifetime prevalence rates of MDD among women are 10-25%, and for men 5-12%. [2, 3]

Almost 10% of the total burden of disease in sub-Saharan Africa is attributed to neuropsychiatric disorders. [4] The lifetime prevalence of the minor depressive disorder in Ethiopia was reported to be 2.2%[9]. Medicinal plants are a rich source of bioactive molecules which are used approximately 80% of the world population for their basic health needs. Around 500 species are known worldwide, 24 species of which are native to India. [5]

Neptunia natans is commonly known as water sensitive plant or water mimosa, belonging to the

family Mimosaceae, a synonym of *Neptunia oleracea* of aquatic herb. Stems creeping, often swollen and floating, rooting at the nodes. Leaves sensitive, bipinnate with 2-4 pairs. Leaflets in 7-22 pairs, oblong 5-20 mm long, mostly hairless. Flowers in sub spherical axillary heads, 1.5-2.5 cm in diameter, bright yellow, on very long peduncles. Pods shortly oblong, up to 1.2 × 3.5 cm, in umbel-like clusters, bent at an angle to a short basal stipe. Water mimosa has been used by some south-east Asian communities as a vegetable and is occasionally sold in local markets in the Brisbane area. Two collections of Water mimosa were made from farm dams in south-eastern Queensland in 2006, one from the Logan area and the other from the Boonah district. It has since been recorded at 15 sites in the Logan City area, but all of these known populations have been controlled. [6] The aim of this study is to screening phytochemicals, an antidepressant of ethanolic extract of *neptunia natans* leaves on albino mice.

MATERIALS AND METHODS

Materials

Chemicals and instruments

Imipramine (Abbott healthcare Pvt ltd), Ethanol (Merck specialties Pvt. Ltd), Tween -80(Vijay enterprise Pvt. Ltd), Normal saline-(Claris Pvt. Ltd), Reagents (Asian scientific instruments, Pvt. Ltd) and actophotometer (SISCO)

Animals

Swiss albino mice from Sainath agencies Hyderabad (Reg.No. 282/PO/Bt/S/2000/CPCSEA).

METHODS

Collection and preparation of Plant extract

The fresh leaves of *neptunia natans* were collected and authenticated by botanist Dr. Madhava chetty, Assistant Professor, Department of Botany in S.V. University, Tirupathi, and A voucher specimen number (1261) and the leaves were cleaned and dried under shade in clean dust free environment, ground and stored in an air-tight container. The total 200 g of course powder was extracted with 1 L of 90% ethanol in a Soxhlet apparatus at 60-75°C for 48 hrs. The extract was concentrated by evaporation. The yield was about 14.30% and stored at 4˚C for future use. The solid EELQ was dissolved by using 1 % v/v DMSO as a vehicle for oral administration. [7-9]

Phytochemical screening

Phytochemical screening of the EELQ was performed according to standard procedures. [9, 10]

Experimental animals

Swiss albino mice (20-30g) maintained under standard conditions (27 ± 2°C; relative humidity 60 ± 5 %, light-dark cycle of 12 hrs) and fed with standard pellet diet and water *ad libidum* were used for the present study. All the experimental protocols were duly approved by Institutional Animal Ethics Committee (Reg. No: 1477/PO/a/11/CPCSEA), Dhanvanthri College of Pharmaceutical Sciences, Mahabubnagar, Telangana, India.

Determination of maximum tolerated dose

Swiss albino mice are taken for the study of limit test. Three animals were kept for overnight fasting prior to drug administration and treated with 2000mg/kg as a single dose of *Neptunia natans leaf* extract. via oral route as per the limit test of OECD guide lines 425 and the food was withheld for further 3–4 hours and The rats were individually observed individually at least once during the first 30 minutes after dosing, periodically during the first 24 hours (with special attention given during the first 4 hours), and daily thereafter, for a total of 14 days, for behavioral, neurological, and autonomic profiles (skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous systems, and somatomotor activity and behavior pattern. Attention should be directed to observations of tremors, convulsions, salivation, diarrhea, lethargy, sleep, and coma) and for any lethality, morbidity & Mortality. [11]

Experimental design

The *Swiss albino mice* (either sex) of weighing around (25-30g) were divided into five groups each consisting of six animals is treated as described below.

Table 1: Treatment schedule of antidepressant activity by Behavior despair swim test

GROUP NO	GROUP NAME	NO. OF ANIMALS	TREATMENT
I	Normal Control	06	Normal saline 10ml/kg,b.wt p.o.)
III	Standard	06	Imipramine 15mg/kg, b.wt (p.o)
IV	Test 1	06	50mg/kg EENN (p.o)
V	Test 2	06	100mg/kg EENN (p.o)

Table 2: Treatment schedule of antidepressant activity by Tail suspension test

GROUP NO	GROUP NAME	NO. OF ANIMALS	TREATMENT
I	Normal Control	06	Normal saline 10ml/kg,b.wt p.o.)
III	Standard	06	Imipramine 15mg/kg, b.wt (p.o)
IV	Test 1	06	50mg/kg EENN (p.o)
V	Test 2	06	100mg/kg EENN (p.o)

Table 3: Treatment schedule of antidepressant activity by locomotor activity

GROUP NO	GROUP NAME	NO. OF ANIMALS	TREATMENT
I	Normal Control	06	Normal saline 10ml/kg,b.wt p.o.)
III	Standard	06	Imipramine 15mg/kg, b.wt (p.o)
IV	Test 1	06	50mg/kg EENN (p.o)
V	Test 2	06	100mg/kg EENN (p.o)

All the drugs are administered via the oral route with An 22 –gauge oral feeding needle, where the edge was made blunt; the needle was fixed to a 1ml tuberculin syringe. The mice were held firmly in left hand, the tubing was moistened with glycerin and inserted right into the esophagus and gently pressing plunger for drug administration, and this was followed by 0.2ml of distilled water to ensure administration of a correct dose of the drug.

Behavior despair swim test

The studies were carried out on mice according to the method of Porsolt (Porsolt et al., 1977a). Briefly, the mouse was individually forced to swim individually for 6 min, in glass cylinders (20 cm in height; 14 cm in diameter), containing fresh water up to a height of 10 cm at 25±1 ^{0}C. After 6 min, they were removed and dried with a towel. The duration of immobility was measured during the final 4 min interval of the test. [12-14]

Tail suspension test

The tail suspension test was based on the method of Steru (Steru et al., 1985). The mouse was individually suspended by the tail with clamp (1 cm distant from the end) for 6 min in a box (25 X 25 X 30 cm) with the head 5 cm to the bottom. Testing was carried out in a darkened room with minimal background noise. The duration of immobility was observed during the final 4 min interval of the test. [12-14]

Locomotor activity test

In order to determine the antidepressant-like action, we have to find out whether FAE has significant action on the central nervous system by measuring the spontaneous motor activity of mice. For measuring locomotor activity, mice were divided into five groups (n=8/group): control (0.9% normal saline), 15 mg/kg imipramine, 50 mg/kg NN, 100 mg/kg NN. All the drugs were given via the oral route. The test was conducted 60 min after the first acute treatment, in this test, the locomotor activity was assessed using an actophotometer which is operated on photoelectric cells which were connected in circuit with a counter. When the beam of light falling on the photocell was cut off by the animal, a count was recorded. These cutoffs were counted for a period of 10 min and the figure was taken as a measure of the locomotor activity of the animal. [12-14]

Statistical analysis

All the results were expressed as mean±SEM. Data were analyzed by two-way analysis of variance (ANOVA) followed by Dunnett's multiple

comparisons test using the software Prism, version 5.03. The level of statistical significance considered was $p < 0.05$, when compared with the control group.

Preliminary Phytochemical screening

Preliminary Phytochemical screening reveals the presence of Carbohydrates, Phenols, Flavonoids, Alkaloids, Steroids, Terpenoids, Tannins and Saponins

Maximum Tolerated Dose

The result of the toxicity study showed that the EELQ was not shown any signs of morbidity and mortality at a dose of 2000mg/kg body weight for 14 days of observation. Hence, the biological evaluation was carried out at doses of 50 and 100 mg/kg doses of extract.

Behavioral despair swim test

Table 4: Mean data of Immobility during Behavior despair swim test

Group	Treatment	Dose (Kg/p.o.)	BDA (Mean±SEM)	ADA (Mean±SEM)
Control	Distilled Water	10 ml	82.83±1.291	82.666±1.085
Standard	Imipramine	15 mg	84.33±1.453	69.167±2.455**
Test-I	EENN	50 mg	89.833±1.493	70.500±1.147
Test-II	EENN	100 mg	83.832±1.887	72.00±2.436*

Values are expressed as mean±SEM (n=6); by using two way ANOVA followed by Dunnett's multiple comparisons test, * $p<0.05$, ** $p<0.01$, *** $p<0.001$ compared to distilled water treated group; BDA= before drug administration, ADA= after drug administration, EENN=Ethanolic extract of *neptunia natans.*

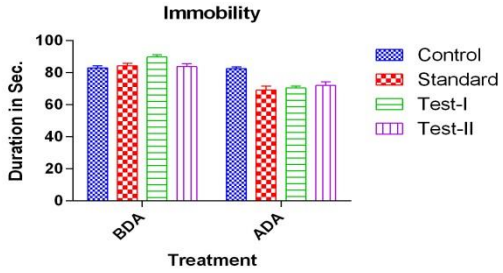


Figure 1: Effect of EENN on the immobility of Despair swim test.

Values are expressed as mean±SEM (n=6); Statistical Analysis of data was carried out by two way ANOVA followed by Dennett's multiple comparisons test.

Table 5: Mean data of Climbing during Behavior despair swim test

Group	Treatment	Dose (Kg/p.o.)	BDA (Mean±SEM)	ADA (Mean±SEM)
Control	Distilled Water	10 ml	3.50±.428	3.00±.365
Standard	Imipramine	15 mg	3.167±.477	5.557±.494*
Test-I	EENN	50 mg	2.667±.333	3.667±.332
Test-II	EENN	100 mg	2.833±.307	5.667±.333*

Values are expressed as mean±SEM (n=6); by two way ANOVA followed by Dunnett's multiple comparisons test, * p<0.05, **p<0.01, ***p<0.001 compared to distilled water treated group; BDA= before drug administration, ADA= after drug administration, EENN=Ethanolic extract of *neptunia natans.*

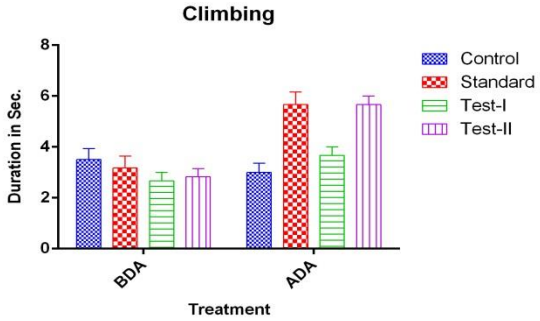


Figure 2: Effect of EENN on the immobility of Despair swim test.

Values are expressed as mean±SEM (n=6); Statistical Analysis of data was carried out by two way ANOVA followed by Dunnett's multiple comparisons test.

Tail suspension method

Table 6: Mean data of Immobility during tail suspension test

Group	Treatment	Dose (Kg/p.o.)	BDA (Mean±SEM)	ADA (Mean±SEM)
Control	Distilled Water	10 ml	128.333±4.492	129.00±4.494
Standard	Imipramine	15 mg	125.667±3.853	122.00±3.688*
Test-I	EENN	50 mg	131.333±2.261	113.833±3.049
Test-II	EENN	100 mg	134.00±2.000	105.667±3.148*

Values are expressed as mean±SEM (n=6); by two way ANOVA followed by Dunnett's multiple comparisons test, * p<0.05, **p<0.01, ***p<0.001 compared to distilled water treated group; BDA= before drug administration, ADA= after drug administration, EENN=Ethanolic extract of *neptunia natans.*

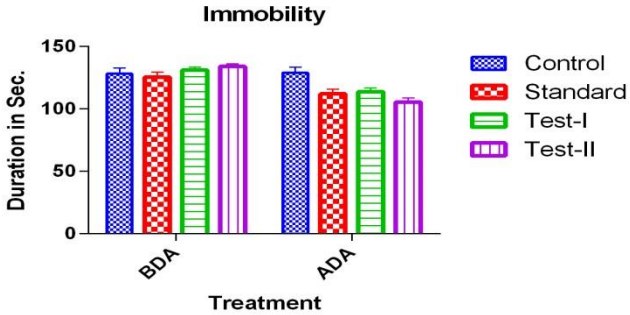


Figure 3: Effect of EENN on the immobility of tail suspension test.

Values are expressed as mean±SEM (n=6); Statistical Analysis of data was carried out by two way ANOVA followed by Dunnett's multiple comparisons test.

Locomotor activity

Table 7: Mean data of animals in locomotor activity

Group	Treatment	Dose (Kg/p.o.)	BDA (Mean±SEM)	ADA (Mean±SEM)
Control	Distilled Water	10 ml	247.667±12.601	247.500±12.082
Standard	Imipramine	15 mg	240.500±11.997	264.167±13.785
Test-I	EENN	50 mg	222.667±11.120	242.500±13.897
Test-II	EENN	100 mg	222.667±11.120	249.500±11.251

Values are expressed as mean±SEM (n=6); by two way ANOVA followed by Dunnett's multiple comparisons test, * p<0.05, **p<0.01, ***p<0.001 compared to distilled water treated group; BDA= before drug administration, ADA= after drug administration, EENN=Ethanolic extract of *neptunia natans.*

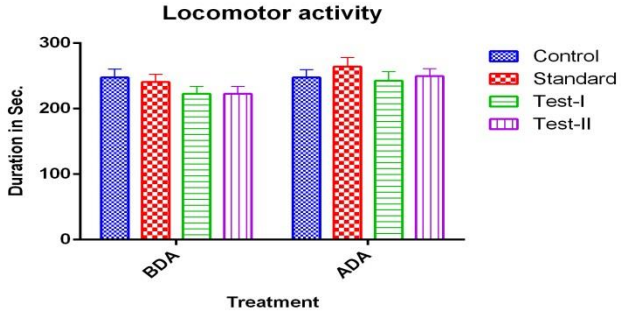


Figure 4: Effect of EENN on the immobility of locomotor activity.

Values are expressed as mean±SEM (n=6); Statistical Analysis of data was carried out by two way ANOVA followed by Dunnett's multiple comparisons test.

DISCUSSION AND CONCLUSION

Depression is a serious mood disorder that afflicts several millions of the world population. Furthermore, the World Health Organization revealed that depression is the fourth leading cause of disability worldwide, exceeded by lower respiratory infections, perinatal conditions and HIV/AIDS [4] Approximately, two third of depressed patients experience suicide thoughts and 10-15% of them attempt suicide. The main symptoms of depression are due to a functional deficiency in the levels of monoaminergic transmitter's noradrenalin, 5-hydroxytryptamine and dopamine in the brain (3). Drugs that increase the level of these neurotransmitters in the CNS show antidepressant activity[5] .The major antidepressant therapies aim for an enhancement in the transmitters levels in the neurons and thus normalize the neurotransmission. Many of the currently available antidepressant drugs have proven to be effective but they are burdened with some disadvantages such as various adverse effects, problematic interactions, and relatively low response [5].

The ethanolic extract of *neptunia natans* leaf extract was shown in the presence of phytochemicals such as phenols, flavonoids, Terpenoids, alkaloids, steroids, saponins, and carbohydrates. The result of the toxicity study showed that the EELQ was not shown any signs of morbidity and mortality at a dose of 2000mg/kg body weight for 14 days of observation. Hence the biological evaluation was carried out at doses of 50 and 100 mg/kg doses of extract and also shown the significant antidepressant activity on Swiss albino mice.

Ethanolic extract *neptunia natans* at 100mg/kg body weight was shown significant results in immobility, climbing and swimming activities in despair swim test and same dose shown the effective results in immobility test of tail immersion test shown in table 4, 5 and 6, and figure 1, 2 and 3 but not shown the significant results in locomotor activity

when compared to normal control groups shown in table 7 and figure 4. The extract was shown sign incant antidepressant activity when compared to standard drug.

Based on the above results we can say that the ethanolic extract of *neptunia natans* leaves is safe at dose of 2000mg/kg body weight of rodents and also having good therapeutic beneficial effect at the dose of 100mg/kg for depression on animal's studies but need to prove the same activity on other animal models and human beings using various models for conforming the antidepressant activity of the EENN.

Finally, we can **conclude** that the ethanolic extract of *neptunia natans* was not having any toxicological symptoms at the dose of 200mg/kg and shown significant antidepressant activity at 100mg/kg on Swiss albino mice. This results might be the presence of different phytochemical constituents. Further, need to identify the phytochemical constituents responsible for the activity and to be confirmed by studying on different animal models.

REFERENCES

[1]. Jones, W.P., Chin, Y.W. and Kinghorn, A.D. The Role of Pharmachology in Modern Medicine and Pharmacy. Current Drug Targets. 7, 2006, 247-264.
[2]. Murugan U, Suganthi J, kanmani S. Evaluation of antidepressant like activity of cucurbita pepo seed extracts in rats. Int J Curr Pharm Res. 3, 2011, 108-113.
[3]. World Health Organization Depression: A Global public health concern. Department of Mental Health and Substance Abuse, WHO 2012.
[4]. Garriock H Genetics of major depressive disorder in treatment resistance and tryptophan depletion. University of Arizona USA 2006.
[5]. Abdur Rauf, Ghias Uddin, Bina Shaheen Siddiqui, Haroon Khan. In vivo sedative and muscle relaxants activity of Diospyros lotus L. Asian Pac J Trop Biomed 5(4), 2015, 277-280.
[6]. Hyde, M.A., Wursten, B.T. and Ballings, P. (2002-2014). Neptunia oleracea Lour. Flora of Zimbabwe website. Accessed 2014 at pecies_id=126310. Tariq Ahmad Wani, Dhirendra Kumar et al., Analgesic activity of the ethanolic extract of Shorea robusta resin in experimental animals, Indian Journal of Pharmacology 44(4), 2012, 493.
[7]. Chandana Choudhury Barua, Jayanti Datta Roy et al., Analgesic and anti-nociceptive activity of hydroethanolic extract of Drymaria cordata Willd, Indian Journal of Pharmacology 43(1), 2011, 6-12.
[8]. Nripendra Nath Biswas, Subarna Saha, Mohammed Khadem Ali, Antioxidant, antimicrobial, cytotoxic and analgesic activities of ethanolic extract of Mentha arvensis L, Asian Pacific Journal of Tropical Biomedicine 4(10), 2014, 792-797.
[9]. Khandewal KR. Practical Pharmacognosy Technique and experiments, Nirali Publications, 9, 2000, 149-156.
[10]. Kokate CK, Purohit AP. Text Book of Pharmacognosy. Pune, Maharashtra, Nirali Prakashan 2004.
[11]. Oral acute toxicity study, OECD guidelines 425, 2008.
[12]. Z.F. Yu, L.D. Kong, Y. Chen. Antidepressant activity of aqueous extracts of Curcuma longa in mice. Journal of Ethnopharmacology. 83, 2002, 161-65.
[13]. Jayesh sanmukhani, ashish anovadiya and chandrabhanu b. tripathi. Evaluation of antidepressant like activity of curcumin and its combination with fluoxetine and imipramine: an acute and chronic study. Acta Poloniae Pharmaceutica ñ Drug Research, 68(5), 2011, 769-775.
[14]. Sldv Ramana Murty Kadali, Das M.C, Srinivasa Rao A.S.R, Karuna Sri G. Activity of Brahmi in Albino Mice. Journal of Clinical and Diagnostic Research. 8(3), 2014, 35-37.