Int J Pharm Bio Sci 2013 July; 4(3): (P) 866 - 872

Research Article **Pharmacology**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

PHARMACOLOGICAL EVALUATION FOR ANTIDEPRESSANT ACTIVITY OF *VANDA SPATHULATA* IN MICE

RAJANEEKAR DASARI*[1], D. SATHYAVATHI[2], SAMPATH KUMAR BELIDE[3] AND B.R.SOUMYA[4]

[1,3,4] Department of Pharmacology Mallareddy Institute of Pharmaceutical Sciences, Dhulapally, Maisammaguda, Quthbullapur Rangareddy Dist, Hyderabad, (AP). Jawaharlal Nehru Technological University Hyderabad.
[2] Department of Pharmacology Teegala Krishna Reddy College of Pharmacy (Principal) Medbowli, Meerpet, Saroonagar, Hyderabad.

ABSTRACT

The present study was designed to screen the antidepressant potential of *Vanda spathulata* using forced swim test and tail suspension test. Imipramine (30mg/kg) and Fluoxetine (20mg/kg) were used as standard drugs. The vehicle (10ml/kg, p.o), fluoxetine (20mg/kg, i.p.), imipramine (30mg/kg, i.p.) and *Vanda spathulata* (100mg/kg, 200mg/kg and 400mg/kg, p.o. respectively) were administered for 14 days. Duration of immobility was noted in both the models. In our study, both imipramine and *Vanda spathulata* significantly reduced the duration of immobility was obseved when compared to control group. The antidepressant activity of *Vanda spathulata* was comparable to that of standard drug impramine. Significant decrease in brain MAO-A and MAO-B activities levels were observed upon methanolic extract administration to mice as compared to control. Therefore, methanolic extract of *Vanda spathulata* showed significant antidepressant activity probably by inhibiting MAO-A and MAO-B. Hence, methanolic extract of *Vanda spathulata* may be explored further for the management of mental depression.

KEYWORDS: ***Vanda spathulata* , Antidepressant, MAO, Forced swim test, Tail suspension test.**

RAJANEEKAR DASARI
Department of Pharmacology Mallareddy Institute of Pharmaceutical Sciences,Dhulapally, Maisammaguda, Quthbullapur Rangareddy Dist, Hyderabad, (AP). Jawaharlal Nehru logical University Hyderabad.

Corresponding author

INTRODUCTION

According to world health report, about 450 million people suffer from a mental or behavioural disorder [1]. Depression is a heterogenous disorder that affects a person's mood, physical health and behavioural skills. Patient with major depression have symptoms that reflect changes in brain neurotransmitter, specifically norepinephrine (NE), Serotonin and Dopamine. An estimated 5.8% of men and 9.5% of women experience the depressive episodes in their life time. Suicidal tendency remains one of the common outcomes of depressive illness being responsible for 60% of the death [2-5]. Patients with depression have decreased social, occupational and educational functioning. An accurate diagnosis followed by an effective treatment can improve this outcome [6]. The main symptoms of depression are due to functional deficiency in the levels of mono aminergic transmitters: Noradrenalline, 5-hydroxy triptamine (5-HT) and Dopamine in the brain[7]. Drugs that increase the level of these neurotransmitters in the CNS show antidepressant activity [8]. The major antidepressant therapies aim for an enhancement in the transmitters levels in the neurons and thus normalize the neurotransmission [9]. 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 [10]. In addition, it is also reported that only two out of three patients respond to any given treatment and of these, one would probably have responded to placebo alone [11].

On the other hand, drugs obtained from natural sources have good efficacy, least risk and low side effects profile. Recently, the search for novel pharmacotherapy from medicinal plants for psychiatric illness has progressed significantly. Therefore, herbal therapies should be considered as alternative or complimentary medicines [12]. *Vanda spathulata Spreng.,* also known as Mara Vazha belongs to the family Orchidaceae. Maram means a tree and vazha is a plantain. Mara vazha means a tree-top plantain. It is a small perennial herb. It as an orchid that grows atop trees (epiphytic), deriving moisture and minerals through its aerial roots that holds on to the substratum (the tree). Roots are vermiform. Flowers are golden yellow in colour. *Vanda spathulata* (VS) is found in south India (A.P., Tamil Nadu, Kerala) and Srilanka. In Kerala (Malayalam) VS is known as Ponnam Ponmarva or ponnam pomaraiva [13,14]. Dried flowers powder are given for consumption, asthma, depression and maniac troubles, juice of the plant is given to temper the bile and abate frenzy and as a liver tonic [13,14]. Based upon the above literature, the present study was undertaken to investigate the effect of methanolic extract of *Vanda spathulata* on depression in mice employing forced swim test and tail suspension test and to also explore the possible underlying mechanisms of antidepressant activity of the extract.

MATERIALS AND METHODS

Plant Collection and Extraction

The flowers of *Vanda Spathulata* were collected and authenticated by ethnopharmacologist Dr. K. Madhava chetty, Sri Venkateswara University, Tirupathi, A.P. India. Flowers of *V.spathula* were dried under shade, powdered with a mechanical grinder and passed through sieve no. 40. The sieved powder was stored in airtight container and kept in room temperature. Dried plant material (500g) was extracted with Soxhlet apparatus using petroleum ether for about 48 hours. After defatting, the marc was dried in hot air oven at 50^0 C, packed in Soxhlet apparatus and further extracted with Alcohol (Methanol) exhaustively. The solvent was removed from the extract under reduced pressure by using a rotary vaccum evaporator. The yield of extract was 21%.

Drugs and chemicals

Fluoxetine hydrochloride, imipramine hydrochloride, baclofen (Sigma-Aldrich,

Mumbai); acetic acid, chloroform and tris hydrochloric acid, EDTA, serotonin, benzylamine (Hi media Laboratories, Mumbai, India); sucrose, disodium hydrogen phosphate, sodium hydroxide and 1% gum acacia were used in present study. The dosage range used here is found to be optimum from various pilot experiments. All other reagents used were of analytical grade.

Vehicles

Vanda spathulata suspension was made in 1% gum acacia, which was used as vehicle control. Fluoxetine hydrochloride and imipramine hydrochloride were dissolved in distilled water.

Animals

Swiss albino mice weighing 25-30 g, of either sex were procured They were housed in standard polypropylene cages and maintained under the standard conditions: room temperature (25±3) ^{o} C, pellet diet and water was allowed *ad libitum.* The animals were acclimatized to the laboratory humidity 45%-55%, 12/12 hr light/dark cycle. They were fed with commercially available mouse normal conditions atleast five days prior to the behavioral experiments. The animal handling was performed according to the Good Laboratory Practice (GLP) guidelines. Animals used in this study were treated and cared for in accordance with the guidelines recommended by the Institutional Animal Ethics Committee of College.(Reg. No. 1217/A/08/CPCSEA/MRCP/PHD/4)

Acute Oral Toxicity Study [15]

Acute toxicity study was performed as per OECD 423 guidelines in mice. For the LD50 dose determination, methanolic extract of *Vanda spathulata* flowers was administered upto dose 2000 mg/kg body weight. Animals were observed for signs of toxicity, continuously for 2 hr, and for mortality upto 48 hr, after oral administration of different doses of extract but extract did not produce any mortality, thus 1/5th, 1/10th of maximum dose tested were selected for the present study.

Experimental Design

Grouping and drug treatment

The animals were grouped into 12 different groups, each containing 6 animals, according to different tests of antidepressant activity as follows:

Group 1, 2 : 1% gum acacia 10 ml/kg (p.o.)
[Control for forced swimming test (FST) and tail suspension test (TST) respectively.]

Group 3, 4 : Fluoxetine 20 mg/kg (i.p.)
[Standard for FST and TST respectively.]

Group 5, 6 : Imipramine 30 mg/kg (i.p.)
[Standard for FST and TST respectively.]

Group 7, 8, 9 : Methanolic extract of *V.spathulata* 100, 200, 400 mg/kg (p.o.) respectively.

Group 10, 11, 12 : Methanolic extract of *V.spathulata* 100, 200, 400 mg/kg (p.o.) respectively.

Group 7-9 and 10-12 served as test group for FST and TST respectively.

Fluoxetine and imipramine (reference standard drugs) were dissolved in distilled water and administered via i.p. route half an hour before each test. Test groups were treated with methanolic extract p.o.one hour prior to the test. Animals were administered with the drugs/ vehicle for a period of 14 days. Behavioural evaluation was carried out 60 minutes post drug/ vehicle administration on 14th day.

Methods for Assessment of Antidepressant Activity

Forced swim test

Behaviour despair was proposed as a model to test antidepressant activity by Porsolt *et al* [16]. Mice were forced to swim individually in a glass jar (25×12×25 cm^3) containing fresh water of 15 cm height and maintained at 25^0C after an initial 2 minute period of vigorous activity, each animal assumed a typical immobile posture. A mouse was considered to be immobile when it remained floating in the water without struggling, making only minimum movements of its limb necessary to keep its head above water. The following behaviors were recorded during the last 4 min of a total 6 minute test:

1. Immobility: floating in water without swimming.
2. Swimming: active movements of extremities and circling in the container.
3. Climbing: active movements of forelimbs on the container wall.

The changes in the immobility duration were studied after 30 minutes of administration of the VS extract (100, 200, 400 mg/kg p.o.) in test group, fluoxetine and imipramine in the standard groups and vehicle in the control group.

Tail suspension test

The tail suspension test (TST) was performed according to the method described by Steru *et al* [17]. The principle of this test is that suspending mice upside down leads to characteristic behaviour immobility which resembles to human depression. After the administration of respective sample, mice were suspended on the edge of the table 50 cm above the floor by adhesive tape placed approximately 1 cm from the tip of the tail. Immobility duration was recorded for the last 4 min during 6 min period. Mice were considered immobile when they hanged passively and completely motionless.

Biochemical estimations

On 14^{th} day, mice were sacrificed after 6 min exposure to FST, and the brain samples were collected immediately on an ice plate. The collected brain samples were washed with cold 0.25M Sucrose- 0.1M Tris- 0.02M EDTA buffer (pH 7.4) and weighed. The whole procedure of brain isolation was completed within five minutes [18,19]. The collected brain samples were analyzed for MAO-A and MAO-B levels as described below:

Measurement of MAO-A and MAO-B activities

Mitochondrial fractions of mouse brain were prepared following the procedure of Schurr and Livne[18].The MAO activity was assessed spectro-photometically with slight modifications[19,20,21]. Briefly, the buffer washed brain samples were homogenized in 9 volumes of cold 0.25M Sucrose- 0.1M Tris- 0.02M EDTA buffer (Ph 7.4) and centrifuged twice at 800 rpm for 10 min at 4^0 C in cooling centrifuge (Remi instruments). The pellet was discarded. The supernatant was then centrifuged at 12000 rpm for 20 min at 4^0 C in cooling centrifuge. The precipitates were washed twice with about 100 ml of sucrose-tris-EDTA buffer suspended in 9 volumes of ice cold sodium phosphate buffer (10 mM, pH 7.4, containing 320 mM sucrose) and mixed well at 4^0 C for 20 min. The mixture was then centrifuged at 15000 rpm for 30 min at 0^0 C and the pellets were re-suspended in cold sodium phosphate buffer. The protein concentration was estimated by Lowry method using bovine serum albumin as standard [22]. The assay mixture contained 4 mM 5-HT and 0.1 M benzylamine in 1:1 proportion which acts as the specific substrate for MAO-A and MAO-B respectively,to which 150 µl solution of mitochondrial fraction and 2.5ml sodium phosphate buffer (100 mM, pH 7.4) were added For estimating MAO-B activity, 2.75 ml sodium phosphate buffer (100 mM, Ph 7.4) and 100 µl of 0.1 M benzylamine were mixed and take into a quartz cuvette which was then placed in double beam spectrophotometer. This was followed by the addition of 150 µl solution of mitochondrial fraction to initiate the enzymatic reaction and the change in absorbance was recorded at wavelength of 249.5 nm for 5 min against the blank containing sodium phosphate buffer and benzylamine. For estimating MAO-A activity, 2.75 ml sodium phosphate buffer (100 mM, pH 7.4) and 100 µl of 4 mM 5-HT were mixed in a quartz cuvette which was then placed in double

beam spectrophotometer. This was followed by the addition of 150 µl solution of mitochondrial fraction to initiate the enzymatic reaction and the change in absorbance was recorded at a wavelength of 280 nm for 5 min against the blank containing sodium phosphate buffer and 5-Hydroxy tryptamine.

Statistical analysis

All the results were expressed as Mean ± Standard Error (SEM). Data was analyzed using one-way ANOVA followed by Dunnett's t-test. The data for locomotor activity scores were subjected to paired t-test. In all the tests, the criterion for statistical significance was $p<0.05$.

RESULTS

Effect of methanolic extract of *Vanda spathulata* on immobility periods in TST and FST Significant decrease in the immobility periods was observed by administration of methanolic extract (100, 200 and 400 mg/kg p.o.) of *Vanda spathulata* to mice in a dose- dependent manner in both TST and FST, indicating significant antidepressant activity. A dose of 400 mg/kg p.o. of methanolic extract showed potent antidepressant effect in both TST and FST as indicated by decrease in immobility period (Table 1 and 2). Effect of methanolic extract of *Vanda spathulata* on brain Monoamine oxidase (MAO) activity Methanolic extract of *Vanda spathulata* (400 mg/kg p.o.) administered for 14 successive days to mice significantly decreased brain MAO-A and MAO-B as compared to control. MAO inhibition was comparable to imipramine (Tables 3 and 4).

Table-1
Effect of Methanolic extract of Vanda Spathulata on Immobility period in Tail Suspension Test

Group No.	Drug treatment for 14 days P.O.	Number of animals	Dose (kg^{-1})	Immobility time (sec) (mean ± SEM)
1	Control (Distilled water)	6	10 ml	154.6 ± 5.4
3	Fluoxetine	6	20 mg	78.4 ± 4.3*
5	Imipramine	6	30 mg	73.6 ± 7.9*
7	Methanolic Extract	6	100 mg	125.3 ± 8.4*
8	Methanolic Extract	6	200 mg	69.6 ± 6.2*
9	Methanolic Extract	6	400 mg	65.6 ± 6.5*

Statistical analysis of data was carried out by one-way ANOVA followed by Dunnett's t-test. *p < 0.05 as compared to control ; F (5, 28) = 26.79 (p<0.0001).

Table-2
Effect of Methanolic extract of Vanda Spathulata on Immobility period in Forced Swim Test

Group No.	Drug treatment for 14 days P.O.	Number of animals	Dose (kg^{-1})	Immobility time (sec) (mean ± SEM)
2	Control (Distilled water)	6	10 ml	124.6 ± 8.4
4	Fluoxetine	6	20 mg	58.4 ± 7.3*
6	Imipramine	6	30 mg	63.6 ± 6.9*
10	Methanolic Extract	6	100 mg	85.3 ± 5.7*
11	Methanolic Extract	6	200 mg	49.6 ± 4.3*
12	Methanolic Extract	6	400 mg	25.6 ± 2.5*

Statistical analysis of data was carried out by one-way ANOVA followed by Dunnett's t-test.*p < 0.05 as compared to control ; F (5, 29) = 22.99 (p<0.0001).

Table-3
Effect of methanolic extract of Vanda spathulata on brain Monoamine Oxidase-A (MAO-A) activity

Group No.	Drug treatment for 14 days P.O.	Dose (kg^{-1})	MAO-A activity (µ/g proteins) (mean ± SEM)
1	Control (Distilled water)	10 ml	35.87 ± 3.31
5	Imipramine	30 mg	17.37 ± 1.82*
9	Methanolic Extract	400 mg	20.54 ± 2.12*

Statistical analysis of data was carried out by one-way ANOVA followed by Dunnett's t-test. N =6 in each group, *p < 0.05 as compared to control F (2, 17) = 28.79 (p<0.0001).

Table-4
Effect of methanolic extract of Vanda spathulata on brain Monoamine Oxidase-B (MAO-B) activity

Group No.	Drug treatment for 14 days P.O.	Dose (kg^{-1})	MAO-B activity (µ/g proteins) (mean ± SEM)
2	Control (Distilled water)	10 ml	41.52 ± 2.25
6	Imipramine	30 mg	30.57 ± 3.54*
12	Methanolic Extract	400 mg	24.36 ± 5.44*

Statistical analysis of data was carried out by one-way ANOVA followed by Dunnett's t-test. N =6 in each group, *p < 0.05 as compared to control F (2, 14) = 18.76 (p<0.0001).

DISCUSSION

Depression is a wide spread psychiatric disorder affecting around 5% of the population.In the present study, methanolic extract (100, 200 and 400 mg/kg p.o) of *Vanda spathulata* administered for 14 successive days produced siginificant anti-depressant effect in mice employing TST and FST. The efficacies of the extract were found to be comparable to fluoxetine and imipramine. The methanolic extract (400 mg/kg p.o) administered for 14 successive days to mice significantly decreased brain MAO-A and MAO-B activity as compared to control. Hence, methanolic extract showed antidepressant activity probably by inhibiting MAO enzyme, thus increased brain levels of monoamines. MAO inhibitors (like phenelzine, moclobemide) are well known antidepressants. Traditionally *Vanda spathulata* has also been reported to be effective in depression[13,14]. for, methanolic extract of *Vanda spathulata* showed significant antidepressant activity probably by inhibiting MAO-A and MAO-B. Hence, methanolic extract of *Vanda spathulata* may be explored further for the management of mental depression.

REFERENCES

1. The world health report: Mental Health: new understanding new hope. WHO, Geneva, 2001.
2. Stahl SM. Essential Psychopharmmacology: Neuroscientific Basis and Practical: Applications. Cambridge University Press; 1998.
3. Rechelson E. Pharmacology of Antidepressants. Mayo Clin Proc 1990;65:1227-36.

4. Rechelson E. Pharmacology of Antidepressants. Mayo Clin Proc 2001;76:516-27.
5. Tripathi KD. Essential of Medical Pharmacology. 5th ed. New Delhi, India: Medical Publishers Pvt. Ltd; 2005. p. 168-72.
6. Akiskal H.S.: in Comprehensive Textbook of Psychiatry, Sadock B.J., Sadock V.A. Eds., p.1284, Williams & Wilkins, Baltimore 2000.
7. Moallem SA, Hosscinzadeh H, Ghoncheh F. Evaluation of antidepressant effect of aerial parts of *Echium vulgare* on mice. Iran J Basic Med Sci 2007; 10:189-196.
8. Meyers S. Monoaminergic supplements as natural antidepressants. Altern Med Rev 2000; 5:64-71.
9. Jithan A, Chinnalalaiah R. Synthesis and evaluation of antidepressant activity of some curcumin-like compounds. In Pharm Communique ?? 2009; 2:38-41.
10. Tamminga CA, Nemeroff CB, Blakely RD, Brady L, Carter CS, Davis KL, *et al.* Developing novel treatments for mood disorders: Accelerating discovery. Biol Psychiatry 2002; 52:589-609.
11. Shalam Md, Shantakumar SM, Narasu ML. Pharmacological and biochemical evidence for the antidepressant activity of the herbal preparation trans-01.Indian J Pharmacol 2007; 39:231-234.
12. Jintanaporn W, Prasert P, Kittisak S , Supaporn M, Bungorn S. Evaluation of the anxiolytic and antidepressant effects of alcoholic extract of *Kaempferia parviflora* in aged rats. Am J Agri Bio Sci 2007; 2:94-98.
13. T. Pullaiah Encyclopedia of world medicinal plants volume 4; 2006; p.2019
14. Rajaneekar dasari, D.Sathyavati and Sampath kumar Belide Evaluation of Antioxidant activity of two Important memory enhancing medicinal plants Celtis timorensis and Vanda Spathulata, Asian J Pharm Clin Res , Vol 6, Issue 2, 2013;p 153-155.
15. OECD guidelines for testing of chemicals. Acute Oral Toxicity- Acute Toxic Class Method 423.
16. Kulkarni SK. Handbook of experimental pharmacology. 3rd ed. New Delhi: Vallabh Prakashan; 2005.
17. Steru L, Chermat R, Thierry B, Simon P. The tail suspension test: a new method for screening antidepressants in mice. Psychopharmacology 1985; 85:367-370.
18. Schurr A, Livne A. Differential inhibition of mitochondrial monoamine oxidase from brain by Hashish components. Biochem Pharmacol 1976; 25: 1201-1203.
19. Pan Y, Kong L, Xia X, Zhang W, Xia W, Jiang F. Antidepressant-like effect of icariin and its possible mechanism in mice. Pharmacol Biochem Behav 2005; 82:686-694.
20. Charles M, McEwan J. Metabolism of amino acids and amines. Monoamine oxidase (Human Serum/ Plasma and rabbit serum). In: Taboor, H., Taboor, C.W. (Eds.), Methods in Enzymology. New York and London: Academic Press, Volume XVIIIB, 1971; pp. 686-698.
21. Yu ZF, Kong LD, Chen Y. Antidepressant activity of aqueous extracts of *Curcuma longa* in mice. J Ethnopharmocol 2002; 83 (1-2): 161-165.
22. Lowry OH, Rosebrough NJ, Farr AL, Randall R. Protein measurement with folin phenol reagent. J Biol Chem 1951; 193: 265-75.