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Evaluation of Nootropic Potential of *Vanda Spathulata* Extract Using Different Experimental Models in Mice

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ABSTRACT

Background: Dementia is a brain disorder characterized by decreased intellectual functioning interfering with the functions such as memory, language, perception, judgment and reasoning. Dementia is the main side effect in Alzheimer's disease patients mostly in older subjective.

Objective: Objective: The following study was designed to assess the potential of *Vanda spathulata* as a nootropic agent and also to study its influence on brain cholinergic system of mice.

Methods: The scopolamine and aluminum induced cognitive deficits models in mice were selected for the present study on elevated plus maze and Morris water maze test. A significant reversal effect was produced by *Vanda spathulata* extract measured the transfer latency on day 1 and day2 in scopolamine treated mice and it was also found that in *Vanda spathulata* extract on elevated plus maze decreased the cognitive deficits produced by sub-chronic administration of aluminum in mice. The animals showed a significant reduction in escape latency on days 2,3,4,5, 6, and 7 as compared with the control in Morris water maze.

Results and Discussion: The activity of acetylcholinestarase enzyme was significantly increased in both scopolamine and aluminum induced mice. Whereas, decrease in the impairment of memory consolidation and also reduction in the activity of acetyl cholinesterase was observed upon the administration flowers of *Vanda spathulata* methanolic extract simultaneously with aluminum and scopolamine. *Vanda spathulata* produced the levels of AChE were decreased significantly in the whole brain homogenate. Hence the flowers of *Vanda spathulata* methanolic extract could be useful in various cognitive disorders like dementia and Alzheimer's disease.

KEY WORDS: Acetylcholinesterase, Aluminium Chloride ($AlCl_3$), Nootropicactivity, Scopolamine, Transfer latency and Morris water maze.

1. INTRODUCTION

The free radicals like oxygen, oxidative metabolism byproducts which are harmful may be responsible for the Alzheimer's disease production, in elderly, and also it is a neurodegenerative disorder, which according to World Health Organization (WHO) affects 22 million people worldwide, out of which, over 3 millions are in India. Neuropathological examination of AD brain reveals extensive atrophy, accumulation of tangles of neurofibrillary intraneuronals and ß-amyloid (Aß) fibrillar deposits (Aß plaques) in vulnerable areas of brain (e.g. cortex, hippocampus). Based on intellectual performance, learning and memory the nootropics acts as a psychotropic agent which effects on central nervous system. Nootropic agents like aniracetam, donepezil and piracetam are used for learning and memory, behavior and mood improvements, but resulting side effects with these drugs have made their limited applicability6. The Indian medicine system suggests the changes in life style, neutracueticals and uses of different herbs for controlling neurodegenerative disorders that are related to age.

Vanda spathulata belongs to Orchidaceae family, it also known as Mara Vazha. It means a tree-top plantain. It is a perennial small herb. It is an orchid deriving minerals and moisture through its aerial roots stick to the tree and it also grows a tree tops.

Vanda spathulata is an origin of southern part of India mostly Tamil Nadu, Kerala and Andhra Pradesh and also part of SriLanka. It also found in Kerala called as Ponnam, Pomaraiva.

The roots are vermiform, flowers are yellow color and dried flowers are powered and also used in asthma treatment, manic disorders, depression and also used as liver toinc. The juice of Vanda plant used for temper bile and frenzy abate.

Based upon the above literature, the following study was undertaken to find out the effects of *Vanda spathulata* methanolic extract as a nootropic agent in mice using elevated plus maze model. To study the mechanism by which VS exerts nootropic action, its effect on brain acetyl cholinesterase levels was determined.

2. MATERIALS AND METHODS

Animals: For the present study either sex of Swiss albino mice weighing about 25-30 gr were used. The animals were placed in cages and maintain under the standard conditions like room temperature 25-30^{0}C, humidity 45%-55%, 12/12 hr light/dark cycle. The animals were fed with pellets which are available in market and regular water ad libitum.

The experimental mice were acclimatized to the animal lab conditions at least one week before start the behavioral animal experiments. The experimental animal handling was performed according to the Good Laboratory Practice (GLP) guidelines. The animals used for the present study were cared and treated with guidelines of Institutional ethical committee of college reg No: 1217/A/08/CPCSEA/MRCP/PHD/4).

Collection and Preparation of methanolic extract of Plant material: Collection and Preparation of *Vanda spathulata* methanolic leaf extract: The *Vanda spathulata* flowers were collected and authenticated by Dr. K. Madhava Shetty botanist, Sri Venkateswara University, Tirupathi, Andhra Pradesh, India. The collected flowers were washed thoroughly with water and air dried in shade at room temperature. The dried flowers were ground well to coarse powder (500 Gms). The dried flower powder was extracted successfully with methanol by Soxhlet apparatus by continuous hot percolation method. Then collect the extract and distilled the solvent. The collected extract was evaporated to dryness in vacuum and stored in a refrigerator. The yield of extract was 25.62%.

Drugs and chemicals: Scopolamine and Piracetam were procured from Sigma-Aldrich pvt.ltd, India and all other reagents and chemicals used for the experiments were of analytical grade, procured from SD Fine chemicals Ltd, India.

Vehicles: *Vanda spathulata* suspension was made in 1% gum acacia, which was used as vehicle control, whereas, scopolamine, aluminum chloride and piracetam were diluted in normal saline.

Acute Oral Toxicity Study: For acute toxicity the LD50 dose determination, *Vanda spathulata* methanolic extract was administered to animal's up to the dose of 2000mg/kg per body weight. Observed the animals continuously for 2 hrs and for mortality up to 48 hrs for signs and symptoms of acute toxicity after the oral administration of different doses of methanolic extract but did not produce any mortality in experimental animals , according to this $1/5^{th}$ and $1/10^{th}$ of maximum dose tested were selected for the following study.

Experimental design for scopolamine-induced cognitive deficits in mice on Elevated plus-maze: The experimental animals were distributed into 6 groups each containing 6 animals. Group I used as control and received only vehicle , Group II animals treated with scopolamine 0.3 mg/kg, group III received scopolamine 0.3 mg/kg along with Piracetam 50 mg/kg treated as standard and group IV, V and VI received *Vanda spathulata* extract 100 mg/kg, 200mg/kg and 400mg/kg per body weight respectively along with scopolamine. *Vanda spathulata* was administered orally while scopolamine and piracetam were given intraperitoneally.

The mice were placed individually at the end of one arm facing away from the centre of the maze and the time the mice took to move from the open arm to either of the enclosed arms (Transfer latency, TL) time was recorded. On the first day, the mice were allowed to explore the plus maze for 20sec after the measurement of TL. Then the used mice were returned to their home cages after the first trial.

After 24 hrs, mice were placed on the elevated plus maze apparatus individually as earlier and transfer latency time was recorded again. Transfer Latency time measured on 1^{st} and 2^{nd} day served as parameters for acquisition and retrieval respectively. All the drugs used for control and standard were administered 30 mins before start the experiments.

Experimental design for Aluminium-induced cognitive deficits in mice on Elevated plus-maze: Animals were randomly grouped into 6 each containing 6 animals.

Group I treated as control and received only vehicle, Group II treated with Aluminium Chloride (1000mg/10ml/kg), group III treated with Aluminium chloride along with Piracetam 50 mg/kg which served as standard and groups IV, V and VI received *Vanda spathulata* extract 100mg/kg, 200mg/kg and 400mg/kg per body weight respectively along with Aluminium chloride. Mice were administered Aluminium chloride dissolved in normal saline (1000mg/10ml/kg) once daily orally for a period of 40 days.From day 21 of aluminium treatment, to different groups of drugs were given once daily. *Vanda spathulata* methanolic extract was administered orally while piracetam was given intraperitoneally. After 40 days treatment mice were subjected to elevated plus maze task.

Morris water maze: Animals were randomly distributed into 5groups.

Group I treated as control and received only vehicle, Group –II treated with Piracetam 50mg/kg which used as standard and group III, IV and V received *Vanda spathulata* extract 100mg/kg, 200 mg/kg and 400mg/kg per body weight respectively. The test methanolic extract were administered for 30minutes before start the experiment for 7 days.

Morris water maze consists of large circular tank 1.0-1.2 m in diameter and 0.2-0.4 m height. The pool is filling up with water (25°C) and rendered opaque by the addition of a small quantity of titanium dioxide suspension. Four quadrants of the tank distributed equally along the perimeter treated as starting locations and an escape platform (5 cm width) was located in the center.

The experimental animals received per day 4 trails with 5mins inter-trial interval for 8 days until the performance was stable and the latency time to find the platform. The drugs used for experiment were administered 30 mins before the first trail every day. Escape latency time is the time to find the hidden platform in the tank.

The platform in the water maze was kept in the same place entire the test to find out the effects of test compounds on spatial reference memory. Escape latencies for each animal in all groups for seven days were noted.

Estimation of Brain Acetyl Cholinesterase (AChE) Enzyme activity: After end of the study the experimental animals sacrified with procedure of cervical dislocation and the whole brain were taken out carefully to avoid disturbance of other tissue. The whole brain AChE enzyme activity was measured using the Ellman method. The dissected out brains were then weighed and then suspended in phosphate buffer. Then they were homogenized using a tissue homogenizer. To this, 100µl of Ellman's reagent was added and then 20µl of the substrate (Acetyl thiocholine iodide) was added. The yellow colour observed as end point due to the reaction of thiocholine with dithio bis nitro benzoate ions. Using a spectrophotometer the amount of formation of thiocholine from acetyl choline iodide in the presence of tissue cholinesterase was measured. The optical density (OD) of the yellow colour compound formed in the reaction at 412nm every minute for a period of 3mins was measured. The protein estimation was done using Lowry's (Folin-phenol reagent) method.

AChE activity was calculated using the following formula:

$$R = \frac{\Delta\ O.D. \times Volume\ of\ Assay\ (3\ ml)}{E\ \times mg\ of\ protein}$$

Where R = Rate of enzyme activity in 'n' mole of acetylcholine iodide hydrolyzed/minute/mg protein. Δ O.D. = Change in absorbance/min and E = Extinction coefficient = 13600/M/cm.

Statistical Analysis: All the results were expressed as mean (Standard error. A probability level of $p<0.01$ was considered as significant. Acetyl cholinesterase enzyme activity in mice brain for different groups were analysed using ANOVA, followed by Dunnett's test.

3. RESULTS

Data was presented in Table.1. In the following study, on the 1st day the cholinergic muscarinic antagonist, scopolamine significantly increased the transfer latency when compared to control group animals but on the 2nd day, the transfer latency time was drastically decreased by scopolamine when compared to the 1st day. This indicates the learning behavior of animals on the 2nd day. However, a significant scopolamine –induced reversal deficits was shown by the nootropic agent, Piracetam. *Vanda spathulata* methanolic extract significantly and dose dependently decreased the transfer latency (TL) on the 1st day. The same degree of effect on TL on elevated plus maze was shown by the extract even on the 2nd day. On 2nd day, effect of extract with three different doses was almost comparable to that of the standard drug, Piracetam.

Data was presented in Table.2. The transfer latency time for Alcl3 significantly increased on the 1st day and still a slight increase in TL on the 2nd day was observed. This indicates that there was no learning behavior of animals on the 2nd day with Alcl3 induced cognitive deficits. However, a significant reversal of Alcl3 induced cognitive deficits was shown with the standard drug, Piracetam. *Vanda spathulata* methanolic extract significantly and dose dependently decreased the transfer latency (TL) on the 1st day. Effect of extract dose of 400mg/kg was comparable with the standard drug. Even on the second day, more degree of effect on TL on elevated plus maze apparatus was shown by the extract when compared to the first day effect.

Data was presented in Table.3. The *Vanda spathulata* methanolic extract treated animals showed significant reduction in escape latency when compared with control. From day1 to day7 there was a reduction in escape latency in dose depending manner.

Effect on Whole Brain Abbreviation Activity: Effect on Whole Brain Abbreviation Activity: A significant elevation in Acetyl cholinesterase enzyme activity was exhibited as compared to control and piracetam (200 mg/kg, i.p.). The *Vanda spathulata* methanolic extracts (100, 200 and 400 mg/kg, p.o.) significantly reduced AChE activity (Table-3).

Table.1. Nootropic activity of *Vanda spathulata* for scopolamine-induced cognitive deficits in mice on Elevated plus-maze summary

Groups	Treatment	Dose	Transfer Latency (in Sec)	
			1st day	2nd day
Control (Normal group)	Vehicle (Distilled Water)	10ml	31.16 ± 1.47**	26.16 ± 1.16**
Negative control	Scopolamine	0.3mg	99.16 ± 2.78	64.83 ± 2.48
Positive control (Standard group)	Scopolamine+Piracetam	0.3mg + 50mg	39.50 ± 1.87**	31.5 ± 1.87**
MEVS-100 (Test group)	Scopolamine + MEVS	0.3mg +100mg	66.83 ± 1.47**	52.33 ± 1.63**
MEVS-200 (Test group)	Scopolamine + MEVS	0.3mg +200mg	58.66 ± 1.63**	45.66 ± 1.36**
MEVS-400 (Test group)	Scopolamine + MEVS	0.3mg +400mg	34.33 ± 1.03**	35.50 ± 1.38**

Values are expressed as mean ± SEM; N = 6; groups were analysed using ANOVA, followed by Dunnett's 't' test.; **P < 0.01 compared with Negative control Group;* P < 0.05 compared with normal control group; MEVS-Methanolic extract of *Vanda spathulata.*

Table.2. Nootropic activity of *Vanda spathulata* for Aluminium-induced cognitive deficits in mice on Elevated plus-maze summary

Groups	Treatment	Dose	Transfer Latency (in Sec)	
			Before	After
Control (Normal group)	Vehicle (Distilled Water)	10ml	24.66 ± 1.21**	22.16 ± 1.47**
Negative control	$AlCl_3$	0.3mg	55.83 ± 1.47	57.50 ± 1.87
Positive control (Standard group)	$AlCl_3$ + Piracetam	0.3mg + 50mg	35.50 ± 1.04**	31.01 ± 0.996**
MEVS-100 (Test group)	$AlCl_3$ + MEVS	0.3mg +100mg	42.01 ± 1.79**	38.00 ± 1.41**
MEVS-200 (Test group)	$AlCl_3$ + MEVS	0.3mg +200mg	37.50 ± 1.04**	37.50 ± 1.04**
MEVS-400 (Test group)	$AlCl_3$ + MEVS	0.3mg +400mg	30.50 ± 1.04**	27.50 ± 1.87**

Values are expressed as mean ± SEM; N = 6; groups were analysed using ANOVA, followed by Dunnett's 't' test.; **P < 0.01 compared with Negative control Group;* P < 0.05 compared with normal control group; MEVS-Methanolic extract of *Vanda spathulata;* $AlCl_3$- Aluminium Chloride.

Table.3. Nootropic activity of *Vanda Spathulata* on Morris Water maze

Group	Treatment mg/kg	Escape Latency in seconds (MEAN ± SE Values)						
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
I	Control	42.5± 2.23	41.1± 2.123	38.8± 1.8	32.5± 1.2	22.3± 1.11	15.5± 1.58	10.26± 1.02
II	Piracetam (50mg/kg)	44.9± 1.832	52.6.4± 3.431	54.8± 3.987	58.4± 2.622 *	62.3± 3.965 **	68.9± 3.512 **	72.22± 3.320 **
III	MEVS (100mg/kg)	39.4± 1.806	28.0± 2.345 **	26.2± 3.826 **	18.0± 2.345 **	17.0± 2.345 **	12.6± 0.8713 **	8.4± 0.400 **
IV	MEVS (200mg/kg)	39.8± 4.66	28.4± 3.389	24.8± 3.023 **	23.4± 2.482 **	20.6± 2.088 **	14.6± 1.368 **	9.8± 1.855 **
V	MEVS (400mg/kg)	36.6± 3.945	27.6± 3.855	24.10± 4.561	17.4± 4.802 **	17.2± 3.813 **	13.0± 2.205 **	8.6± 1.939**

Values are expressed as mean ± SEM; N = 6; *p<0.05* and **p<0.01 control Vs treated groups using one way ANOVA followed by Dunnett's test; MEVS-Methanolic extract of *Vanda spathulata*

Table.4. Nootropic Activity of *Vanda spathulata*- Summary of Acetyl Cholinesterase enzyme activity in brain (Both Models)

Groups	Treatment	Acetycholinesterase Enzyme Activity in brain {Mean ±SEM} (In moles $X10^{-6}$/ min/ g of tissue)
Control (Normal group)	Vehicle (Distilled Water)	30.51 ± 0.763**
Negative control	Scopolamine	40.16 ± 0.601
	Aluminium chloride	43.16 ± 0.610
Positive control (Standard group)	Scopolamine + Piracetam	12.01 ± 0.577**
	Aluminium chloride + Piracetam	14.16 ± 0.60**
MEVS-100 (Test group)	Scopolamine + MEVS-100	17.01 ± 0.577**
	Aluminium chloride + MEVS-100	18.16 ± 0.792**
MEVS-200 (Test group)	Scopolamine + MEVS-200	16.16 ± 0.60**
	Aluminium chloride + MEVS-200	14.33 ± 0.499**
MEVS-400 (Test group)	Scopolamine + MEVS-400	12.50 ± 0.428**
	Aluminium chloride + MEVS-400	12.50 ± 0.42**

Values are expressed as mean ± SEM; N = 6; groups were analysed using ANOVA, followed by Dunnett's 't' test.; **P < 0.01 compared with Negative control Group;* P < 0.05 compared with normal control group; MEVS-Methanolic extract of *Vanda spathulata.*

4. CONCLUSION

Though several methods available, the scopolamine induced cognitive deficits has been proposed to have symptomalogical similarities with Alzheimer's disease and other amnesia related disorders.

The present study shows the nootropic effects of Vanda spathulata methanolic extract on Scopolamine and $AlCl_3$ induced cognitive deficit using elevated plus maze in mice.

From the obtained data in the study we can conclude that Vanda spathulata methanolic extract at a dose of 400mg/kg has significant neuroprotection and memory enhancement, which might also be useful in the treatment of elderly memory loss, hence Vanda spathulata can be used in the treatment of Alzheimer's disease and other cognitive disorders.

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