Research Article

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EVALUATION OF THE EFFECT OF *VIGNA RADIATA* LINN ON SCOPOLAMINE INDUCED WORKING AND MEMORY DEFICITS IN MICE BY USING DIFFERENT MODELS

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ABSTRACT

Memory is the ability of an individual to record the information and recall it whenever needed. Dementia is a mental disorder characterized by loss of intellectual ability (judgment or abstract thinking) which invariably involves impairment of memory. The most common cause of dementia is Alzheimer's disease which is a progressive neurodegenerative disorder associated with loss of neurons in distinct brain areas and cord. Stressful conditions are often associated with loss of memory and cognitive functions which may lead to threats of schizophrenia and Alzheimer's disease.

Traditionally herbal drugs have been used to enhance cognitive functions and to alleviate other functions associated with the Alzheimer's disease. The present study was undertaken to assess the potential of *Vigna radiata* Linn extract on learning and memory in mice. *Vigna radiata* Linn extract (Aqueous 700, 900 mg/kg and Ethanol 700, 900mg/kg) ameliorated the amnesic effect of scopolamine (0.4mg/kg) which induced memory deficit in mice. Elevated plus-maze and passive-avoidance paradigm served as an exteroceptive behavioural model. *Vigna radiata* Linn extract decreases transverse latencies and increases step down latencies, when it is compared with the scopolamine induced group. *Vigna radiata* Linn preparation could be beneficial in the treatment of cognitive disorders like Dementia and Alzheimer's disease.

Keywords: Alzheimer, Dementia, Learning, Memory, *Vigna radiata*, Scopolamine.

INTRODUCTION

Aging is a continual, universal, progressive, intrinsic but deleterious process that decreases an organism's ability to maintain homeostasis in the face of environmental stressors¹⁻³. Because of increases in life span, age-related disorders have become a major health hazard⁴. The brain undergoes many structural and functional changes during aging⁵. The most striking feature of aging brain is its shrinkage, weight loss, expansion of the ventricular volume, and degeneration of hippocampal circuits⁶. Levels of metabolites of neurotransmitters, viz., acetylcholine, dopamine, and norepinephrine, are reduced in the cerebral cortex because of aging⁷. Dementia is an age-related disorder that involves loss of neurons in distinct brain areas⁸. Alzheimer's disease is the leading cause of dementia in the elderly community⁹. Alzheimer's disease is associated with anomia, apraxia, and agnosia, with loss of memory being the cardinal symptom¹⁰. The main histological features of Alzheimer's disease include degeneration of cholinergic neurons¹¹, extracellular deposition of β -amyloid plaques, and appearance of intraneuronal neurofibrillary tangles¹². Inflammatory processes¹³ and the presence of injurious chemicals such as free radicals (superoxide and nitric oxide) and cytokines (interleukin-1 and tumor necrosis factor) also contribute to the neurodegeneration¹⁴.

Green gram [*Vigna radiata*] belongs to the family Fabaceae. It is a small herbaceous annual plant growing to a height of 30 to 120 centimeters with a slight tendency to twining in the upper branches. The crop is fully self fertile and self pollinated^{15,16}. It is a rich source of vitamin, mineral, proteins, iron, calcium and fiber, ascorbic acid, folic acid, alkaloid, flavonoids and tannin is also present¹⁷. Traditionally seeds were used as diuretic, in

rheumatism, as hypotensive, hypolipidemic, on the basis of literature survey it also acts as hepatoprotective¹⁸, antibacterial activity¹⁹, antifungal²⁰, nematocidal²¹ and antidiabetic²². However, the reports available in the literature are not sufficient enough to reflect the learning and memory enhancing properties of *Vigna radiata* Linn. Therefore, the present study was undertaken to examine whether the *Vigna radiata* Linn extract improves cognitive function in mice by comparing with the scopolamine group. The effect of *Vigna radiata* Linn aqueous and ethanolic extract were compared with the scopolamine induced memory deficit were investigated in the present study. Phytochemical investigation was also estimated in this study.

MATERIAL AND METHOD

Drugs

Drugs and chemicals were obtained from the following drug houses: scopolamine hydrobromide were obtained from the Sigma Aldrich chemical Co. St. Louis, Mo, USA. Merck Ltd and Piracetam injection were obtained from earth chemicals Mumbai.

Animals

Swiss albino mice weighing -18 g (younger ones, aged 8 weeks and 25 g (older ones, aged 28 weeks) were used in the present study. These mice were housed in standard laboratory conditions of temperature, relative humidity and under standard environmental conditions (12h light and 12h dark) with adequate food and water. The mice were transferred to the laboratory at least one hour before the start of the experiment and all experiments were carried out from 8.00-11:00 am. Institutional Animal Ethical Committee approved the protocol and care of animals was taken as per guidelines of CPCSEA,

Department of Animal Welfare, and Government of India²³.

Method of extraction

Selection of plant

The seeds of *Vigna radiata* ie mung beans were collected in the month of September 2010 from the mahabej bhavan washim District 444505, Maharashtra, India. The plant material were identified and authenticated by professor and head of department Dr. A. Chaturvedi; Rastrasant Tukdoji Maharaj Nagpur University, Nagpur, India. A voucher (No: 9593) was deposited at herbarium, Department of Botany, RTM Nagpur, Maharashtra, India.

Alcoholic extract of *Vigna radiata*

The seeds of *Vigna radiata* are collected then it will be washed thoroughly and dried under shade then seeds were grind to flour with the help of mechanical grinder. The powder was passed through the sieve No.40 and stored in an air tight container for extraction.

Continuous hot percolation method by using Soxhlet apparatus for this process.

Petroleum ether 60-80°C Alcohol 99%v/v, Distilled water was used as solvent.

Petroleum ether extract

The dried seeds of *Vigna radiata* Linn were extracted with petroleum ether 60-80 °C up to 72 hrs. After completion of extraction, the solvent was recovered and dark yellow colored oil was recovered.

Ethanol extract

The marc after petroleum ether extraction was dried and then extracted with ethanol 99% v/v (75-78), up to 72 hrs in soxhlet apparatus. Extract was evaporated under reduced pressure to obtain an average yield 35.11%. The solvent was then removed under reduced pressure which obtained a brownish-black color sticky residue and was used for Identifying its activity on learning and memory activity.

Aqueous extract of *Vigna radiata*

Aqueous extract of *Vigna radiata* was prepared by using maceration process by making a fine powder of seed then soaked in water with a little quantity of chloroform for a period of ten days and then filtered with muslin cloth and the extract was evaporated under reduce pressure collected into very fine powder by means of evaporation and then the powdered drug was use for activity.

Phytochemical screening

The various extract of the *Vigna radiata* Linn were subjected to preliminary phytochemical screening using the standard screening method. The molish's test and fehling's test were carried out for carbohydrate, foam test for saponins, Salkowski test & Libermann burchard test for phytosterol. Aqueous Sodium hydroxide test, concentrated sulphuric acid test and shinoda's test were carried out for flavonoids. Biuret test, Ninhydrin test and Million's test were carried for proteins & amino acid, libermann test, sulphur test for sterol and triterpenoids test²⁴.

Experimental Design

Mice were divided into seven groups; each consisting of six mice for each experiments.

Group-I Normal control group received only vehicle.

Group-II Positive control group received only vehicle against scopolamine induced amnesia.

Group-III Aqueous extract 700mg/kg p. o treated group which receive aqueous extract against scopolamine induced amnesia.

Group-IV Aqueous extract 1000mg/kg p. o treated group which receive aqueous extract against scopolamine induced amnesia.

Group-IV Ethanol extract 700mg/kg p. o treated group which receive ethanol extract against scopolamine induced amnesia.

Group-IV Ethanol extract 1000mg/kg p. o treated group which receive ethanol extract against scopolamine induced amnesia.

Dose selection

According to literature survey doses of *Vigna radiata* were use from 100 to 1300 mg/kg on this basis we select the 700, 1000mg/kg dose for the evaluation of learning and memory activity in mice.

Assessment of learning and memory

Passive avoidance paradigm

Passive avoidance behavior based on negative reinforcement was used to examine the long-term memory The apparatus consisted of a box (20 cm×20 cm×20 cm) having three walls of wood and one wall of Plexi glass, featuring a grid floor (made up of 3 mm stainless steel rods set 8 mm apart), with a wooden platform (10 cm×7 cm×1.7 cm) in the center of the grid floor. The box was illuminated with a 15Wbulb during the experimental period. Electric shock (20 V, A.C.) was delivered to the grid floor^{25, 26}.

Training (i.e. 10th day of drug treatment) was carried out in two similar sessions. Each mouse was gently placed on the wooden platform set in the center of the grid floor. When the mouse stepped-down, placing all its paws on the grid floor, shocks were delivered for 15 s and the step-down latency (SDL), which was defined as the time (in seconds) taken by the mouse to step down from the wooden platform to grid floor with all its paws on the grid floor, was recorded. Animals showing SDL in the range of 2–15 s during the first test were used for the second session and the retention test. The second-session was carried out 90 min after the first test. During second session, if the animals stepped down before 60 s, electric shocks were delivered once again for 15 s. During the second test, animals were removed from shock free zone, if they did not step down for a period of 60 s and were subjected to retention test. Retention (memory) was tested after 24 h (i.e. 11th day, 24 h after last dose) in a similar manner, except that the electric shocks were not applied to the grid floor observing an upper cut off time of 300s. Significant increase in SDL value indicated improvement in memory²⁷⁼²⁹.

Elevated Plus Maze

The elevated plus maze served as the exteroceptive behavioral model (wherein the stimulus existed outside the body) to evaluate learning and memory in mice. The apparatus consisted of two open arms (16 cm · 5 cm) and two covered arms (16 cm · 5 cm · 12 cm). The arms extended from a central platform (5 cm · 5 cm), and the maze was elevated to a height of 25 cm from the floor.

On the first day, each mouse was placed at the end of an open arm, facing away from the central platform. Transfer latency (TL) was taken as the time taken by the mouse to

move into any one of the covered arms with all its four legs. Transfer latency was recorded on the first day. If the mouse did not enter into one of the covered arms within 90s, it was gently pushed into one of the two covered arms and the Transfer latency was assigned as 90s. The mouse was allowed to explore the maze for 10 s and then was returned to its home cage. Memory retention was examined 24 h after the first day trial on the second day^{30, 31}.

Statistical Analysis

The data were statistically analyzed by Mean $\pm$ SD. The data were also analyzed by one way ANOVA followed by Dunnett's t-test and values * $p < 0.05$ were considered as significant and ** $p < 0.01$ were considered as more significant.

RESULT AND DISCUSSION

Step down Passive avoidance paradigm

Effect of Ethanol and Aqueous extract of *Vigna radiata* Linn on Acquisition and Retrieval memory using step down model in mice.

Table 1: Values are mean $\pm$ SD, n= 6, * $P < 0.05$, ** $p < 0.01$ when compared with scopolamine. One way ANOVA followed by Dunnett's multiple comparison tests

Treatment schedule mg/kg	Time taken in seconds	
	SDL on acquisition test (sec)	SDL on retention test (sec).
I Vehicle control	53 $\pm$ 9.20	113.16 $\pm$ 23.35
II scopolamine induced	49.5 $\pm$ 4.88	29.16 $\pm$ 12.93
III Test (Aqueous)	41.66 $\pm$ 14.82	81.5 $\pm$ 19.98*
IV Test (Aqueous)	46.33 $\pm$ 10.55	112 $\pm$ 50.99**
V Test (Ethanol extract)	48.33 $\pm$ 9.72	119.5 $\pm$ 33.47
VI Test (Ethanol)	53.83 $\pm$ 5.03	132.66 $\pm$ 31.99**
VII (Piracetam)	56 $\pm$ 4.56	144.83 $\pm$ 10.72**

Table 2: Effect of *Vigna radiata* Linn extract on Acquisition and Retrieval memory in elevated plus maze task in mice

Treatment mg/kg	Transverse latencies in seconds	
	Acquisition days	Retrieval days
I Normal control	16.16 $\pm$ 4.02	15.16 $\pm$ 3.31
II Scopolamine	22.83 $\pm$ 17.46	30.16 $\pm$ 7.02
III Test (Aqueous)	34 $\pm$ 12.043	19.83 $\pm$ 9.06*
IV Test (Aqueous)	32.66 $\pm$ 9.58	18.5 $\pm$ 2.88*
V Test (Ethanol)	31.66 $\pm$ 10.65	16.5 $\pm$ 7.89**
VI Test (Ethanol)	28.5 $\pm$ 10.15	14.5 $\pm$ 10.25 **
VII (Piracetam)	26.16 $\pm$ 16.96	11.33 $\pm$ 4.84 **

Passive avoidance paradigm

Figure 1: Effect of *Vigna radiata* Linn extract and Piracetam on the step down latency (sec) at retention test in mice using step down Passive avoidance response

Elevated plus maze model

Figure 2: Effect of *Vigna radiata* Linn extract and Piracetam on Acquisition and Memory Retention against Scopolamine Induced Amnesia Using Elevated plus Maze model in mice

DISCUSSION

In the present study *Vigna radiata* Linn improved cognitive performance in mice.

Step down passive avoidance model response has been used to examine long term memory based on step down latency. The increased in SDL is an indication of improved cognitive performance. Vr. Ethanol 1000, 700 mg/kg and aqueous extract 1000mg/kg in retention test significantly increased the Step down latency by $p < 0.01$ and ethanol, aqueous lower significantly increased the step down latencies by $p < 0.05$ and in case of elevated plus maze task aged mice shows higher transverse latency value on first day and on second day after 24 hrs as compared to young mice, indicating impairment in learning and memory (ie ageing induced amnesia).

Transfer latency (TL) was defined as the time (in seconds) taken by the animal to move from the open arm into one of the covered arms with all its four legs. Significant reduction in transfer latency value of retention indicated improvement in memory. *Vigna radiata* Linn (700, 1000 mg/kg, p.o.) showed dose-dependent reduction in transfer latency of 11th day in young and aged animals, when compared to respective scopolamine induced groups indicating ethanolic 700,1000mg/kg significantly reduces the time by $p < 0.01$. Scopolamine (0.4 mg/kg, S.c.) was injected before training significantly increased transfer latency of 11th day indicating impairment in memory but *Vigna radiata* Linn (700, 1000 mg/kg, p.o. for 10 days) successfully reversed memory deficits induced by scopolamine. Piracetam 400mg/kg i.p. used as a standard drug significantly reduces the time by $p < 0.01$ of both young and aged mice and reversed the amnesia induced by scopolamine^{33,34}. Then the aqueous 1000, 700mg/kg significantly reduces the time by $p < 0.05$ to enter in to the closed arm and in the significant ($P < 0.01$) improvement in discrimination index by the three doses (700 and 1000 mg/kg of ethanol and 1000 aqueous) of extract proved that *Vigna radiata* met major criteria for nootropic activity, improvement of memory in absence of cognitive deficit. In the aforementioned results, effectiveness of *Vigna radiata* towards the facilitation of learning and retention of learned task suggested the nootropic activity by the extract. However to know exact neurochemical's involved there is need of further investigations.

CONCLUSION

Alcoholic extract of 1000,700 and aqueous 1000mg/kg possess significant nootropic action. The present study indicates that chronic pretreatment of *Vigna radiata* possesses significant facilitator effect on aversively motivated learning and memory in mice on scopolamine induced working and memory deficits. Suggesting possible use of *Vigna radiata* extract to improve the learning and memory processes

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