



## Evaluation of Nootropic Activity of *Vigna trilobata* [L.] Verdc. on Experimental Animals

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### ABSTRACT

The present study aimed to evaluate the nootropic activity of *Vigna trilobata* [L.] Verdc., in rats. Ethanolic extract of *Vigna trilobata* [L.] Verdc., was used to evaluate nootropic activity, donepezil (2.5 mg/kg, p.o) was used as a standard, and scopolamine (2 mg/kg i.p) was used to induce amnesia. The effect of drugs on learning and memory in rats was evaluated by using the Y-maze task and open field test on scopolamine-induced amnesia models. Also, levels of acetylcholinesterase, including estimation of brain neuro transmitter, were assessed. Ethanolic extract of *Vigna trilobata* [L.] Verdc., showed increased alteration of the behavior response and percentage spontaneous alteration with the Y-maze task. In the open field test scopolamine-induced amnesia model, the data indicate that EEVT exhibits potential cognitive-enhancing effects, with its high dose demonstrating efficacy comparable to donepezil in reversing scopolamine-induced memory deficits. Ethanolic extract decreased the levels of acetylcholinesterase enzyme significantly. The results suggested that the administration of EEVT higher dose enhances learning and memory in different experimental models. The study indicates that the extract may be used in the treatment of Alzheimer's disease.

**Keywords:** Nootropic activity, Alzheimer's disease, scopolamine, *Vigna trilobata* [L.] Verdc.

### INTRODUCTION

According to the World Health Organization, approximately 450 million people suffer from a mental or behavioral disorder. Dementia (age-related mental disorder) is a characteristic symptom of Alzheimer's disease (AD). [1] AD is a progressive, neurodegenerative, and cerebrovascular disease. It destroys cells in the brain, causing problems with memory, unusual behavior, difficulty thinking, personality changes, and ultimately death. AD is characterized by the loss of neuronal cells and is primarily linked to neurofibrillary tangles and neuritic plaques. [2] The cholinergic system in the brain plays an important role in learning and memory, which involves acetylcholine (Ach). Dementia is produced due to reduction of Ach in the brains of patients with AD. In rodents and human beings, drugs like scopolamine impair learning and memory. [3, 4] Memory loss, amnesia, dementia, anxiety, schizophrenia, and AD may be produced due to certain conditions like age, stress, and emotion.

There are a few nootropic medicines used in the treatment of AD, called nootropic drugs, belonging to the class of psychotropic agents. The term nootropic was coined by Giurgea in 1972, from the Greek noon (mind) and tropos (turn). [5] Nootropics are also referred to as smart drugs, as they improve mental functions such as memory, increase blood circulation to the brain, and improve the oxygen supply to the brain. Synthetic medicines like tacrine, donepezil, aniracetam, piracetam, and rivastigmine are used for the treatment of cognitive dysfunction and memory loss associated with AD. However, these drugs pose some adverse effects and bioavailability issues. [6, 7]

After recognizing the harmful effects and limitations of synthetic medications, the world is turning back to traditional systems of medicine. [8] Plants have been used as therapeutic agents in both un-ionized (Unani, Ayurveda) and unstructured forms since ancient times. *Vigna trilobata* [L.] Verdc., a leguminous plant belonging to the family Fabaceae, is traditionally used in Indian folk medicine to treat a range of ailments, including inflammation, fever, and digestive disorders. [9] Phytochemical studies reveal that the plant contains a variety of bioactive compounds such as flavonoids, alkaloids, saponins, and phenolic acids, many of which are known to exert neuroprotective and antioxidant effects—two mechanisms that play a key role in nootropic activity. [10] While several members of the *Vigna* genus, including *Vigna mungo* and *Vigna aconitifolia*, have demonstrated cognitive-enhancing properties in experimental models, there is a significant lack of scientific data regarding the neuropharmacological effects of *Vigna trilobata*. This presents a valuable opportunity for investigation. [11] The present study seeks to determine whether the ethanolic extract of aerial parts of *Vigna trilobata* shows nootropic activity in an animal model.



## MATERIALS AND METHODS

**Plant Material:** The collection and authentication of plant materials of *Vigna* species involve a systematic approach to ensure the correct identification, purity, and documentation of the plant material for research or conservation purposes. Plant materials can be collected from cultivated fields. Authentication of the collected plant materials is a critical step that ensures their taxonomic and genetic identity. This begins with morphological characterization using standard descriptors for *Vigna* species, focusing on traits such as leaf shape, flower and pod characteristics, seed size and color, and growth habit. The leaves, fruits and stems were picked from the plant, cleansed with a dry cloth, and dried for 7 days in the shade as well as in a hot air oven set at 45°C. The dried leaves were ground into a fine powder. [12, 13]

**Thin Layer Chromatographic Profiling:** Commercial sheet pre coated with silica gel are available. Select a solvent by testing out the samples in various solvents. Dissolve a small quantity of ethanolic extract of *Vigna trilobata* aerials parts of the unknown in different flask containing solvents of different polarity. Place the TLC plates, the spotted side down in to the chamber so that the lower the pencil line about the solvents. Remove the plate from the development chamber and allowed to dry. Plate is placed under UV light, dark spots are observed. [14, 15]

$$R_f = \text{Distance moved by solute} / \text{Distance moved by solvent}$$

R<sub>f</sub> = retardation factor

## Pharmacological Activity

**Acute Toxicity Study:** In a research study, when a drug is introduced to a biological system, various interactions may occur. In most cases, these interactions are beneficial and intended, but numerous effects can be unfavorable or harmful. To evaluate the safety of a new drug, manufacturers conduct acute, subacute, and chronic toxicity assessments. Acute toxicity testing involves determining the LD<sub>50</sub>, which is the lethal dose that causes death in 50% of the test animal population. [16]

**Animals:** Male and female Wistar albino rats, each weighing between 150 and 200 grams, were sourced from a breeder accredited by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Prior to the commencement of the experimental procedures, the rats were accommodated in sanitized polypropylene cages, with a maximum of six animals per cage, for a duration of seven days. The environmental conditions were maintained at a standard temperature range of 25–30°C, relative humidity between 45–55%, and a 12-hour light/dark cycle. These conditions were consistent with the guidelines set forth by the Institutional Animal Ethics Committee (IAEC), which approved the study protocol. The animals were weighed at the outset, and an acclimatization period of five days was observed before initiating the experiments. During this period, they were provided with a commercial pelleted diet and access to potable water ad libitum. [17, 18]

**Preparation of samples:** A 0.1% (w/v) carboxymethyl cellulose (CMC) solution was utilized as the suspending agent for the ethanolic extract of *Vigna trilobata* (EEVT). CMC, a cellulose derivative, is commonly employed in pharmaceutical formulations due to its non-toxicity, hypoallergenic properties, and ability to enhance the viscosity of aqueous solutions, thereby facilitating the uniform dispersion of active compounds. For the control group, distilled water served as the vehicle, providing a baseline for comparison. Both vehicles were administered orally to the rats at a volume of 10 mL/kg body weight. [19]

**Experimental design:** Animals were divided into six groups of six rats in each group.

- Group I : Vehicle control ( 0.5 % w/v Na CMC)
- Group II : Scopolamine only (2 mg/kg i.p)
- Group III : Scopolamine+ donepezil (2.5 mg/kg, p.o)
- Group IV : Scopolamine + ethanolic extract (100 mg /kg)
- Group V : Scopolamine + ethanolic extract (200 mg /kg)
- Group VI : Scopolamine + ethanolic extract (400 mg /kg)



**Induction of Amnesia by Scopolamine:** The ethanolic extract of *Vigna trilobata* was administered orally to the experimental groups over a total duration of 10 days. Scopolamine was introduced intraperitoneally 30 minutes prior to the initiation of each behavioral paradigm across all representative groups. Behavioral evaluations were conducted within 24 hours following the final dose of treatment. [20, 21]

**Neurochemical Analysis:** Upon completion of the behavioral assessments, animals were euthanized using an appropriate anesthetic agent, such as ketamine. Subsequently, the brains were carefully excised and rinsed with ice-cold isotonic saline to remove residual blood and other contaminants. The brain tissue was then meticulously dissected into specific regions of interest, such as the hippocampus and cortex, for subsequent biochemical analyses. [22]

## Behavioral studies

### i) Morris Water Maze Test [23, 24]

Water maze consist of a circular tank with 100 cm of diameter and a wall 20 cm above the water level

Training take place on 3 consecutive days, with the rats receiving four consecutive trials per day with an inter-trial interval of 6-10 minutes

Each trial was started from one of four assigned polar position with different sequence each day

The latency to find the platform is measured as the of placement of the rat in the water to time it find the platform

On day 4 a probe test was performed the platform was removed and the time spend in the target quaderant was measured.

Increases the latency to find the platform in the training period

The rat which solve the escape immediately are considered to have retain memory

### ii) Open Field Apparatus [25]

- **Animal Placement:** The subject animal is gently removed from its home cage and placed into one corner of the open field arena.
- **Habituation:** Prior to the testing session, the animal is allowed to acclimate to the arena for a brief period to minimize stress and ensure natural behavior.
- **Testing Session:** The animal is observed for a duration of 10 minutes. During this period, various behaviors are recorded, including:
  - **Exploration:** Time spent in the inner  $6 \times 6$  squares of the arena, indicating active exploration away from the periphery.
  - **Overall Activity:** The total number of squares crossed by the animal, reflecting its general locomotor activity.
  - **Additional Parameters:** Other behaviors such as rearing (standing on hind legs), grooming, stretch attend postures, and latency to enter the center area may also be measured to provide a comprehensive assessment of the animal's behavior.
- **Post-Test Procedures:** After the testing session, the animal is returned to its home cage. To eliminate any residual olfactory cues that could influence subsequent tests, the arena is thoroughly cleaned with a 5% ethanol solution between trials.

**Acetyl cholinesterase Inhibition Assay:** The enzymatic activity of acetylcholinesterase (AChE) was quantified using a spectrophotometric method based on Ellman's assay, with modifications to optimize the reaction conditions. The final reaction mixture (3 mL) comprised 2.6 mL of 0.1 M phosphate buffer (pH 7.2), 100  $\mu$ L of the test sample or donepezil (concentrations ranging from 1.25 to 40  $\mu$ g/mL), 100  $\mu$ L of 75 mM acetylthiocholine iodide (ATCI) as the substrate, and 100  $\mu$ L of 10 mM 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) as the chromogenic reagent. The reaction was initiated by the addition of ATCI, and the final concentration of dimethyl sulfoxide (DMSO) in the reaction mixture was maintained below 1% to prevent solvent-induced interference. [26] AChE hydrolyzes ATCI to produce thiocholine, which then reacts with DTNB to form a yellow-colored product, 5-thio-2-nitrobenzoate, detectable at an absorbance of 412 nm. The increase in absorbance over time correlates with the enzymatic



activity of AChE. The assay was conducted in triplicate, and the mean absorbance values were used to calculate the enzyme activity. [27]

**Estimation of Brain Neurotransmitter:** At the conclusion of the experiment, rats were euthanized, and their brains were promptly removed and dissected. A 0.25 g portion of the brain tissue was accurately weighed and homogenized in 5 mL of ice-cold HCl–butanol solution using a motor-driven, Teflon-coated homogenizer for approximately 1 minute. The homogenate was then centrifuged at 2000 rpm for 10 minutes to separate the cellular debris. An aliquot of the supernatant (1 mL) was carefully transferred into a centrifuge tube containing 2.5 mL of heptane and 0.31 mL of 0.1 M hydrochloric acid. The mixture was subjected to vigorous shaking for 10 minutes, followed by centrifugation under the same conditions to facilitate phase separation. The upper organic phase was discarded, and the remaining aqueous phase was collected for subsequent assays to measure levels of serotonin (5-HT), norepinephrine (NA), and dopamine (DA). All procedures were conducted on ice to maintain the integrity of the neurotransmitters. [28, 29]

**Statistical Analysis:** Data analysis was performed using GraphPad Prism version 9.5, and the results were presented as mean  $\pm$  SEM. The significance of the results was verified by computing P values using two-way analysis of variance (ANOVA). In addition, error bars were displayed to show the results from our animal experiments' standard error mean. [30]

## RESULTS AND DISCUSSION

**Physical Examination of the Extract:** The physical examination of plant extracts is a preliminary but essential step in phytochemical and pharmacological studies. It provides basic information about the extract's appearance, which can offer clues about its chemical composition, stability, and purity. This examination involves evaluating several key parameters, including color, odor, texture, solubility, and consistency. The dried extracts of the drugs were evaluated for physical parameters such as consistency, color, odor and taste. The results are shown in Table 1.

**Table 1: Characterization of extract**

Name of Extract	Color	Odor	Taste	Consistency	Extractive value (%w/w)
Ethanol extract	Dark Brown	Characteristic	Bitter	Semi- Solid	9.65%

**Thin Layer Chromatography (TLC):** the TLC analysis of the ethanol extract using the ethyl acetate: chloroform: water (5:3:1) solvent system has provided preliminary information about the chemical composition of the plant extract. Further studies, including isolation and characterization of individual compounds, are necessary to fully understand the pharmacological properties and therapeutic potential of the plant.

**Table 2: TLC Studies of ethanolic extract of *V. trilobata***

Solvent system	Detecting agent	No. of spots	Colour of spots	Rf values
Ethyl Acetate: Chloroform: Water (5: 3: 1)	Under UV at 366nm	5	Dark Yellow	0.92
			Yellowish brown	0.85
			yellowish green	0.78
			Light yellow	0.57
			Light yellow	0.46
	Under Visible light	4	Dark Yellow	0.92
			Yellowish brown	0.78
			yellowish green	0.56
Light yellow			0.46	

## Pharmacological Activity

**Acute Toxicity Study/I** An acute oral toxicity study was conducted to evaluate the safety profile of EEVT in Wistar albino rats, following OECD guideline 423.

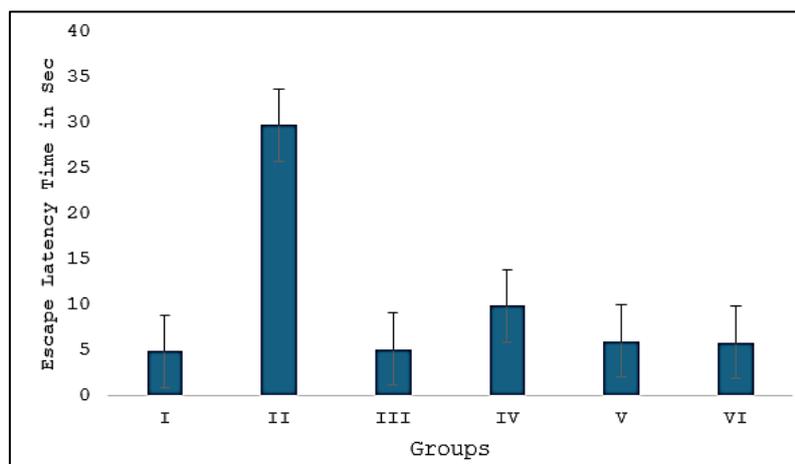
- **Dosing:** Rats were administered EEVT orally at doses of 5mg/kg, 50 mg/kg, 300 mg/kg, and 2000 mg/kg body weight.
- **Observation Period:** Animals were observed for 14 days for any signs of toxicity, behavioral changes, and mortality.

**Table 3: Observations**

Dose (mg/kg)	Mortality	Behavioral Changes	Other Observations
Control	0/3	None	Normal feeding and activity
5	0/3	Slight drowsiness (temporary)	Normal after 2 hours
50	0/3	Reduced locomotion (1–2 h)	Recovered by Day 1
300	0/3	Transient piloerection, drowsy	No mortality, full recovery
2000	0/3	Transient piloerection, drowsy	No mortality, full recovery

No deaths were recorded at any tested dose. Minor behavioral changes such as reduced activity and drowsiness were observed at higher doses, particularly at 2000 mg/kg, but these symptoms resolved spontaneously within 24 hours. EEVT was found to be safe up to a dose of 2000 mg/kg in Wistar albino rats, with no mortality or significant clinical toxicity observed. The LD<sub>50</sub> of EEVT is considered to be greater than 2000 mg/kg, confirming its suitability for further pharmacological testing.

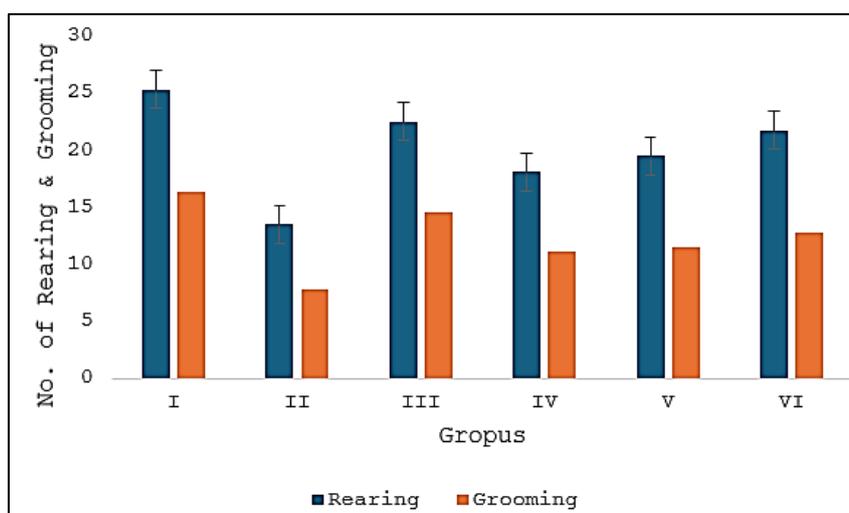
**Screening of anti-amnesic activity by Morris water maze:** The data presented in figure 1 illustrate the impact of various doses of EEVT on learning and spatial memory in rats. Scopolamine administration significantly increased the escape latency time to  $29.876 \pm 1.654$  seconds, indicating impaired memory function. Treatment with low ( $9.983 \pm 0.258$ ), medium ( $6.095 \pm 0.288$ ), and high ( $5.895 \pm 0.431$ ) doses of EEVT progressively decreased the escape latency time, suggesting dose-dependent improvement in memory performance. Notably, the high dose of EEVT exhibited an escape latency time ( $5.895 \pm 0.431$ ) comparable to that of the donepezil-treated group ( $5.211 \pm 0.312$ ), a standard acetylcholinesterase inhibitor known to enhance cognitive function. These findings align with existing literature demonstrating that scopolamine-induced memory deficits can be ameliorated by acetylcholinesterase inhibitors like donepezil. For instance, a study by Snyder et al. (2005) reported that a single dose of donepezil significantly reversed scopolamine-induced cognitive deficits in healthy elderly subjects. Similarly, the present results suggest that EEVT may possess cholinergic properties capable of mitigating scopolamine-induced memory impairments.



**Figure 1: Effect of various doses of EEVT (100 mg/kg, 200 mg/kg, 400 mg/kg) on learning and special memory on Morris water maze.** Each value represents the mean ± SEM (n=6). \*p<0.1, \*\* p<0.01 and \*\*\* p<0.001 in comparison with scopolamine treated group

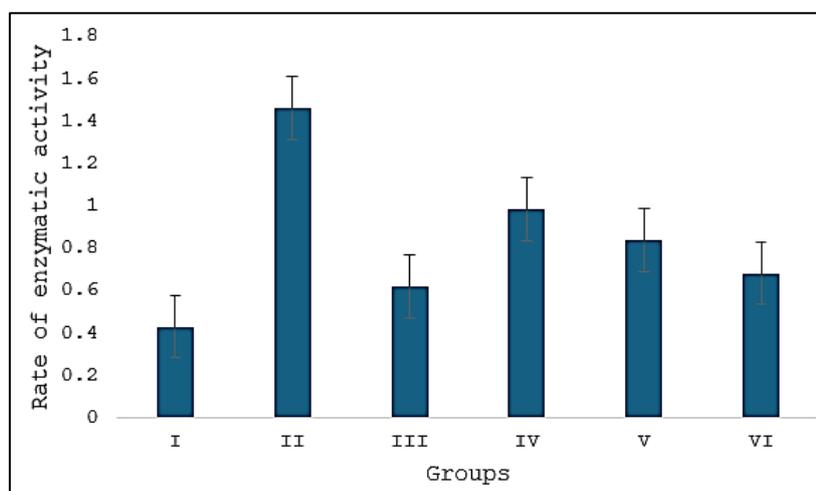
In conclusion, the data indicate that EEVT exhibits potential cognitive-enhancing effects, with its high dose demonstrating efficacy comparable to donepezil in reversing scopolamine-induced memory deficits. Further studies are warranted to elucidate the underlying mechanisms and to explore the therapeutic potential of EEVT in treating cognitive disorders.

**Screening of anti-amnesic activity by open Field:** The data presented in Table 5.14 illustrate the effects of various doses of EEVT on exploratory behavior. Scopolamine induction significantly reduced the number of rearing and grooming behaviors to  $13.534 \pm 1.106$  and  $7.754 \pm 0.387$ , respectively. Treatment with different doses of EEVT resulted in a significant increase in both rearing and grooming: low dose ( $18.123 \pm 0.672$  and  $11.121 \pm 0.312$ ), medium dose ( $19.533 \pm 0.564$  and  $11.563 \pm 0.643$ ), and high dose ( $21.744 \pm 0.432$  and  $12.785 \pm 0.421$ ), respectively. Notably, the highest dose of EEVT ( $21.744 \pm 0.432$  and  $12.785 \pm 0.421$ ) produced values comparable to those observed in the Donepezil-treated group ( $22.563 \pm 0.764$  and  $14.564 \pm 0.422$ ), indicating a similar improvement in exploratory behavior. In conclusion, the data indicate that EEVT exhibits potential cognitive-enhancing effects, with its high dose demonstrating efficacy comparable to donepezil in reversing scopolamine-induced memory deficits. Further studies are warranted to elucidate the underlying mechanisms and to explore the therapeutic potential of EEVT in treating cognitive disorders.



**Figure 2: Effect of various doses of EEVT (100 mg/kg, 200 mg/kg, 400 mg/kg) on exploratory behavior on open field apparatus.** Each value represents the mean ± SEM (n=6). \* p<0.1, \*\* p<0.01 and \*\*\* p<0.001 in comparison with scopolamine treated group.

**Effect of Acetylcholinesterase level on rat brain:** The data illustrating the impact of various doses of EEVT on acetylcholinesterase (AChE) levels in the brain are presented in Fig. 3. The results indicate that scopolamine administration significantly elevates AChE levels in the brain to  $1.464 \pm 0.0032$ . Conversely, treatment with different doses of EEVT markedly reduces the AChE concentration (low dose:  $0.987 \pm 0.032$ , medium dose:  $0.842 \pm 0.056$ , and high dose:  $0.685 \pm 0.018$ ), respectively. Furthermore, the highest dose of EEVT ( $0.685 \pm 0.018$ ) demonstrates an AChE level comparable to that of the Donepezil-treated group ( $0.623 \pm 0.0932$ ), suggesting a similar efficacy in lowering brain AChE.

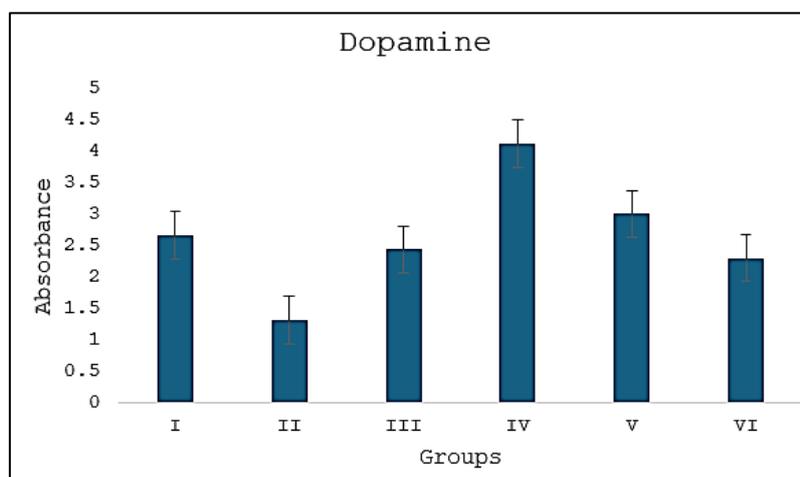


**Figure 3: Effect of various doses of EEVT (100 mg/kg, 200 mg/kg, 400 mg/kg) acetyl cholinesterase levels.** Each value represents the mean ± SEM (n=6). \* p<0.1, \*\* p<0.01 and \*\*\* p<0.001 in comparison with scopolamine treated group.

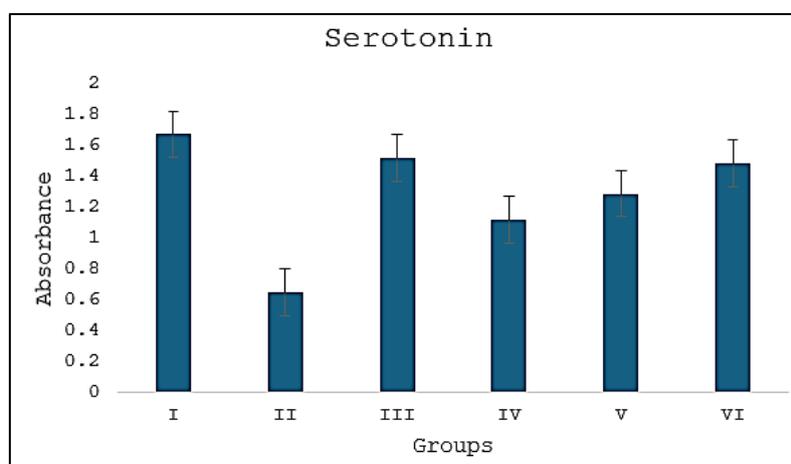
### Estimation of neurotransmitters

**I) Effect of EEVT on dopamine on amnesic rat brain:** The data illustrating the effect of various doses of EEVT on dopamine levels in the brain are presented in Fig. 4. The findings indicate that scopolamine administration significantly reduces dopamine concentration in the brain to  $1.321 \pm 0.064$ . Treatment with different doses of EEVT markedly elevates dopamine levels (low dose:  $4.121 \pm 0.053$ , medium dose:  $3.009 \pm 0.036$ , and high dose:  $2.31 \pm 0.054$ ), respectively. Notably, the highest dose of EEVT ( $2.31 \pm 0.054$ ) exhibits a dopamine level slightly higher than that observed in the Donepezil-treated group ( $2.443 \pm 0.321$ ).

**II). Effect of EEVT on level of serotonin on amnesic rat brain:** The data illustrating the effect of various doses of EEVT on serotonin levels in the brain are presented in Fig. 5. The results demonstrate that scopolamine administration significantly reduces serotonin concentration in the brain ( $0.652 \pm 0.013$ ). Treatment with different doses of EEVT significantly increases ( $P < 0.1$ ) serotonin levels ( $1.121 \pm 0.32$ ,  $1.289 \pm 0.0621$ , and  $1.487 \pm 0.159$ ) for the low, medium, and high doses, respectively. Furthermore, the highest dose of EEVT exhibits a comparable significant effect to that observed in the Donepezil-treated group ( $1.521 \pm 0.15$ ).

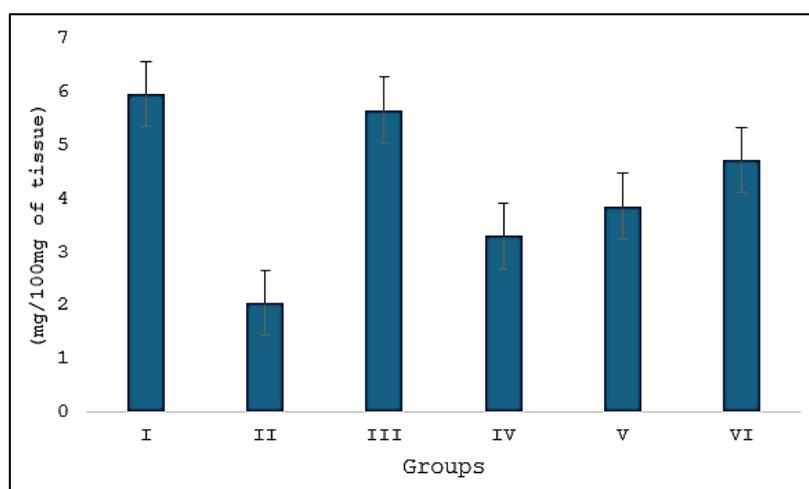


**Figure 4: Effect of various doses of EEVT (100 mg/kg, 200 mg/kg, 400 mg/kg) on dopamine levels.** Each value represents the mean  $\pm$  SEM (n=6). \*  $p < 0.1$ , \*\*  $p < 0.01$  and \*\*\*  $p < 0.001$  in comparison with scopolamine treated group.



**Figure 5: Effect of various doses of EEVT (100 mg/kg, 200 mg/kg, 400 mg/kg) on serotonin levels.** Each value represents the mean  $\pm$  SEM (n=6). \*  $p < 0.1$ , \*\*  $p < 0.01$  and \*\*\*  $p < 0.001$  in comparison with scopolamine treated group.

**Estimation of total Proteins:** The data depicting the effect of various doses of EEVT on total protein levels are shown in Fig. 6. The results indicate that scopolamine administration significantly reduces total protein levels to  $2.052 \pm 0.004$ . Treatment with different doses of EEVT significantly elevates total protein levels (low dose:  $3.312 \pm 0.0059$ , medium dose:  $3.876 \pm 0.095$ , and high dose:  $4.743 \pm 0.052$ ), respectively. Moreover, the highest dose of EEVT ( $4.743 \pm 0.052$ ) demonstrates a total protein level approaching that of the Donepezil-treated group ( $5.680 \pm 0.061$ ).



**Figure 6: Effect of various doses of EEVT (100 mg/kg, 200 mg/kg, 400 mg/kg) on total protein levels.** Each value represents the mean  $\pm$  SEM (n=6). \*  $p < 0.1$ , \*\*  $p < 0.01$  and \*\*\*  $p < 0.001$  in comparison with scopolamine treated group.

## CONCLUSION

The present study was conducted to evaluate the nootropic activity of the ethanolic extract of *Vigna trilobata* in experimental animals. Nootropic agents are substances that enhance memory, learning, and cognitive functions, and this study aimed to scientifically validate the traditional use of *Vigna trilobata* in improving brain function. Healthy adult albino rats (or mice) were used as the animal model, and the study involved various behavioral models commonly employed for assessing memory and learning, including the elevated plus maze, and Y-maze. The plant extract showed a dose-dependent improvement in learning and memory parameters, such as reduced transfer latency in the elevated plus maze and increased time spent in the correct arm of the Y-maze. These results indicate enhanced spatial memory and learning ability in treated animals. From the present study, it can be considered that the ethanolic extract high dose (400 mg/kg) exhibited significant anti-amnesic activity in scopolamine induced rat model. All the Parameters of formulation treated group have shown better results when compared with scopolamine induced -group.

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