

Original Article

## Pharmacological assessment of *Amaranthus viridis* Linn against scopolamine-induced amnesia in experimental rat model

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### ABSTRACT

**Objectives:** Alzheimer's disease (AD) is one of the many neurological conditions that impair memory and cognitive function and get worse with age. *Amaranthus viridis* Linn is a medicinal plant used as intervention for a wide varieties of ailment. A natural constituent omega 3 fatty acid present in *A. viridis* Linn possesses strong anti-inflammatory and antioxidant properties. Most of the drugs showed neuroprotective activity by inhibiting inflammation and oxidative stress in brain cells. Thus, we intended to evaluate pharmacological assessment of *A. viridis* Linn against scopolamine-induced amnesia in experimental rat model.

**Materials and Methods:** The current study investigates whether *A. viridis* Linn can prevent scopolamine (SCP)-induced amnesia in female Wistar rats. The Morris water maze (MWM) and the Novel object recognition tests were used to assess memory-related behavioural factors. Every group except the control group received three oral doses of *A. viridis* extract (AVE) (200, 400 and 800 mg/kg) and daily intraperitoneal injections of scopolamine and donepezil at a dose of 1 mg/kg each for a total of 17 days.

**Results:** When compared to the inducer group, all three doses showed shorter escape latency designating enhanced scopolamine-induced impairment. The long-term memory's ability to recognise novel objects was also enriched by AVE, as shown by an improvement in the recognition index against chronic scopolamine-induced memory impairments. Acetylcholinesterase enzyme in particular brain areas (cortex, hippocampus) was dose-dependently inhibited by AVE.

**Conclusion:** According to these research results, the AVE showed an improvement in memory function and may therefore represent a promising targeted therapy for neurodegenerative diseases.

**Keywords:** Alzheimer's disease, *Amaranthus viridis* Linn, Learning and memory, Neurodegenerative illness

### INTRODUCTION

The most prevalent form of dementia is Alzheimer's disease (AD). This slowly progressing neurodegenerative disorder is identified by the occurrence of neuritic plaques and neurofibrillary tangles, predominantly in the medial temporal lobe and neocortical structures of the brain, which are the region's most commonly impacted.<sup>[1]</sup> The first patient of Alois Alzheimer had amyloid plaques and a considerable loss of neurons in the brain. Alzheimer diagnosed the patient with a serious cerebral cortex condition. This condition was first referred to as AD by Emil Kraepelin in the ninth edition of his psychiatric handbook.<sup>[2]</sup> Progressive cognitive decline can be caused by

intoxications, infections, abnormalities in the pulmonary and circulatory systems that reduce oxygen supply to the brain, nutritional deficiencies, vitamin B12 shortages, tumours and various other conditions. Globally, there are about 50 million people who have AD, and by 2050, that number is projected to increase by a factor of four every 5 years, to 152 million.<sup>[3]</sup> The *Amaranthus viridis* Linn belongs to the *Amaranthaceae* family and is widespread in the tropics. *A. viridis*, often known as slender amaranth, is a plant that is indigenous to Brazil, the Caribbean (West Indies) and other tropical and subtropical regions of the world. The majority of the time, it is eaten as a leafy vegetable in South India. The leaves contain vitamins that are high in fibre amongst their nutritional components. Along with vitamins A, B, B2 and C, calcium, phosphorus, iron and amino acids valine, arginine, histidine, lysine, cystine, methionine, phenylalanine, leucine and isoleucine are also essential nutrients.<sup>[4]</sup> Diuretics, analgesics, antipyretics, vermifuge, anti-ulcer, hypoglycaemia, hypolipidaemic, laxatives, asthma and venereal illnesses are some of the traditional applications. *A. viridis* Linn, both raw and branched, was found to have antioxidant activity and phenolic content. In addition, *A. viridis* Linn demonstrates a wide range of beneficial activities, including anti-inflammatory, antinociceptive, antipyretic, antihelmintic, antifungal, hepatoprotective, antihyperglycaemic, hypolipidaemic, antidiabetic, antiviral, antihyperlipidaemic and cardioprotective effects.<sup>[5]</sup> By blocking acetylcholinesterase (AChE), and free radicals and altering signalling pathways, flavonoids have been shown to elicit cognitive and neuroprotective effects. *A. viridis* plant is enriched with flavonoids. However, there is no systematic report to prove the anti-amnesic properties of ethanolic leaf extract of *A. viridis*. Therefore, the current study has been planned to discover the possible role of *A. viridis* against scopolamine-induced cognitive impairment in female Wistar rats.

## MATERIALS AND METHODS

### Chemicals and reagents

Scopolamine (SCP), donepezil, acetylthiocholine iodide, monobasic phosphate buffer, dibasic phosphate buffer (PH 7.4), normal saline and formaldehyde (10% formalin) were purchased from Yarrow Chem Products, Mumbai, India. The remaining reagents were of analytical grade.

### Instrumentation

Novel object recognition model (Orchid Scientific, India), Morri's water maze (San Diego Instruments, United States), UV spectrophotometer (SHIMADZU UV-1700, Japan), Centrifugation apparatus (Eppendorf, centrifuge 5430 R, India), Water bath (Shital Scientific Industries, India),

Rotary evaporator (Rotavapor R-3, Buchi), Heating mantle (T S P, S.S 104, India) and Sonicator (AnaMatrix Instrument Technologies Pvt Ltd, LMUC-3, India).

### Plant material

*A. viridis* Linn was collected from B. G. Nagara in the Mandya district. The plant parts were washed, shade-dried and then reduced to coarse powder for storage in an airtight container at room temperature for future use. The plant was subjected to authentication by Dr. Pradeep from the Department of Dravyaguna at S D M College of Ayurveda and Hospital, B.M. Road, Thanniruhalla, Hassan-573201, Karnataka (Voucher Specimen number of the plant: SDMCAH-DG/2022/55).

### Preparation of extract

*A. viridis* leaves powder has been extracted by cold maceration technique using 80% ethanol. The extracted solvent was filtered using Whatman filter paper. The solvent was evaporated by a rotary evaporator to obtain dry crude extract. The acquired plant extract has been stored in a dark amber coloured flask and then preserved for further use.<sup>[6]</sup>

### Qualitative phytochemical analysis of *A. viridis* Linn

A preliminary phytochemical evaluation of ethanolic extract of *A. viridis* Linn was performed by the standard procedures for tannins, phlorotannins, saponin, flavonoid, alkaloids, steroids, terpenoids, glycosides, phenolic compounds, proteins and also anthraquinones.<sup>[7]</sup>

### Animal housing

Wistar rats (Female), aged 6–8 weeks and weighing around 180–200 g, were obtained from Vaarunya Biolabs Private Limited, Bangalore, and housed in the animal facility at Faculty of Pharmacy, Sri Adichunchanagiri College of Pharmacy, B.G. Nagara, Mandya, Karnataka. The rats were kept in polypropylene cages under a 12:12 h light/dark cycle, with a temperature of  $23 \pm 2^\circ\text{C}$ . They were provided with a regular feed and water. To minimise stress, the rats were given a week to acclimatise before the experiments began. All experimental procedures were approved by the Institutional Animal Ethics Committee (IAEC) of Sri Adichunchanagiri College of Pharmacy, Karnataka, India (IAEC Approval number: SACCP-IAEC/2022-02/70).

### Experimental design

Female Wistar rats were randomly divided into six groups with six animals each. The groups were designated as follows.

- Group I: Normal (Saline)
- Group II: Inducer group (Scopolamine 1 mg/kg)
- Group III: Standard group (Donepezil 1 mg/kg)

- Group IV: *A. viridis* 200 mg/kg Inducing Agent
- Group V: *A. viridis* 400 mg/kg Inducing Agent
- Group VI: *A. viridis* 800 mg/kg Inducing Agent.

### Morris water maze (MWM) test

Before starting the experiment, the rats were trained for 1 week. The rats were administered with *A. viridis* extract (AVE) from day 1 to day 17 at doses of 200, 400 and 800 mg/kg bodyweight. Scopolamine was given from day 9 to day 17, 30 min after AVE administration. MWM test was done on days 7 and 14. A circular pool of 120 cm (diameter) and 50 cm (height) made up the MWM. The water pool was divided into four equal sectors, which are numbered from 1 to 4, and a platform with 7.5 cm (diameter) was positioned in the third sector, just 2 cm below the water's surface, where a temperature of  $24 \pm 1^\circ\text{C}$  was maintained. Each rat was submerged in the water during the positioning navigation stage from one of four different sectors each day. When the rats discovered the platform within 90 s and remained there for 3 s, the time was recorded as escape latency. We conducted a test of space exploration, at this point, the platform was taken away, and each rat was dropped into the MWM from the first sector and given 90 s to roam around at their leisure.<sup>[8]</sup>

### Novel object recognition test (NORT)

The experimental setup for the object recognition task consisted of a black acrylic open field box measuring (40 × 40 × 40 cm). The test was carried out in low red-light illumination between the hours of 9:00 am and 6:00 pm. Before starting the experiment, the rats were trained for 1 week. The rats were administered with *A. viridis* from day 1 to day 17 at doses of 200, 400 and 800 mg/kg. Scopolamine was given from day 9 to day 17, 30 min after AVE administration. On the 17<sup>th</sup> day of a NORT, researchers used two similarly shaped, clear cultured flasks filled with water and Lego building set of the same height as discriminating objects. Each rat was made to acclimate to an open field box without any objects for 10 min the day before the experiment. During the first experiment on day 17, each rat was put in the open field box for 5 min to explore two identical objects (the clear cultured flasks filled with water). After this session, one of the flasks was replaced with a new object (the Lego set), which differed in texture, colour and size. Each rat was then allowed to investigate the objects for 2 min, with exploration defined as the rat touching the object with its forepaws or nose. The time spent exploring each object was recorded. To reduce scent trails, the open field box was cleaned with 70% ethanol. The recognition index, calculated using the formula  $(\text{TB}/[\text{TA} + \text{TB}] \times 10)$ , where TA is the time spent investigating the familiar object (flask) and TB is the time

spent investigating the novel object (Lego set), was used to assess the rats' ability to recognise and differentiate between familiar and novel objects based on their exploration time.<sup>[9]</sup>

### Measurement of AChE in rat's brain

Animals were killed by cervical dislocation, and the brains were carefully removed. A phosphate buffer solution with a pH of 7.4 was used to homogenise isolated brains. The clear supernatant from the centrifugation of the brain homogenate at 3000 rpm for 15 min at  $4^\circ\text{C}$  was utilised to calculate the brain AChE activities.<sup>[10]</sup>

### Histopathology of brain tissues

The brain tissues were placed in a freshly made Bouin's solution and dehydrated with progressively higher alcohol concentrations. Fixed tissues were sectioned at a thickness of 5 m, fixed in paraffin and stained with haematoxylin and eosin (H&E).<sup>[11]</sup>

### Statistical analysis

Data were expressed as mean  $\pm$  standard error of the mean. Differences between the groups were statistically determined using analysis of variance followed by Tukey's test, with  $P < 0.001$  considered statistically significant.

## RESULTS

A preliminary phytochemical evaluation of ethanolic extract of *A. viridis* Linn confirmed the presence of flavonoids, tannins, phenols, alkaloids, saponins, phenolic compounds, proteins and anthraquinones but does not contain phlorotannins, terpenoids, cardiac glycosides and reducing sugars.

### MWM test

When compared to the controls, scopolamine treatment led to noticeably longer escape latencies during the acquisition sessions (days 7 and 14); however, this impact was lessened by the administration of the AVE. In the control group, but not in the scopolamine-treated rats, significant reductions in escape latencies were seen on day 14 compared to day 7 of the acquisition sessions. The results of MWM in rats after administration of AVE are given in Table 1a-c.

On day 17, scopolamine-exposed rats significantly decreased their swimming time in the target quadrant compared to the controls. However, rats that received the test formulation alongside scopolamine showed a dose-dependent increase in the amount of time spent in the target quadrant compared to the scopolamine-treated disease controls.

## NORT

In comparison to the scopolamine-treated group, all three groups administered an ethanolic extract of *A. viridis* Linn displayed a dose-dependent increase in the recognition index. The results of Novel object recognition in rats after administration of AVE are given in Table 2a and b and depicted in Figure 1a and b.

### Estimation of acetylcholinesterase enzyme levels in the brain

When compared to the disease control group (Scopolamine), all three test doses of the ethanolic leaf extract of *A. viridis* Linn

**Table 1a:** Effect of AVE on ETL of rats by using Morris water maze on the 7<sup>th</sup> day.

Groups	Treatment	ETL mean±SEM (n=6)
I	Control	50.37±0.5909
II	Inducer (Scopolamine)	70.21±0.7155
III	Standard (Donepezil)	41.58±1.265***
IV	AVE 200 mg/kg+(SCP 1 mg/kg)	60.46±1.154*
V	AVE 400 mg/kg+(SCP 1 mg/kg)	50.88±2.771**
VI	AVE 800 mg/kg+(SCP 1 mg/kg)	42.55±0.6847***

The behavioural analysis was compared to the Inducer group. Data are expressed as Mean±SEM, n=6, and statistical analysis by one-way ANOVA followed by Tukey's test. \*\*\*P<0.001 when compared with Inducer control, the result is highly statistically significant, indicating a difference between the Donepezil-treated group and the Inducer. \*\*P < 0.01. The result is statistically significant, showing improvement compared to the Inducer group. \*P < 0.05 indicates mild statistical significance compared to the Inducer group. AVE: *Amaranthus viridis* extract, ETL: Escape time latency, ANOVA: Analysis of variance, SEM: Standard error of the mean, SCP: Scopolamine

**Table 1b:** Effect of AVE on ETL of rats using Morris water maze on the 14<sup>th</sup> day.

Groups	Treatment	ETL mean±SEM (n=6)
I	Control	45.32±3.045
II	Inducer (Scopolamine)	60.76±2.322
III	Standard (Donepezil)	36.31±0.5069***
IV	AVE 200 mg/kg+(SCP 1 mg/kg)	50.85±1.745*
V	AVE 400 mg/kg+(SCP 1 mg/kg)	42.93±1.326**
VI	AVE 800 mg/kg+(SCP 1 mg/kg)	39.53±0.7741***

The behavioural analysis was compared to the Inducer group. Data are expressed as Mean±SEM, n=6, and statistical analysis by one-way ANOVA followed by Tukey's test. \*\*\*P<0.001 when compared with Inducer control, the result is highly statistically significant, indicating a difference between the Donepezil-treated group and the Inducer. \*\*P < 0.01. The result is statistically significant, showing improvement compared to the Inducer group. \*P < 0.05 indicates mild statistical significance compared to the Inducer group. AVE: *Amaranthus viridis* extract, ETL: Escape time latency, ANOVA: Analysis of variance, SEM: Standard error of the mean, SCP: Scopolamine

demonstrated a dose-dependent reduction in AChE level. The results are mentioned in Table 3 and represented in Figure 2.

### Histopathology of brain tissues

- Normal control - A compactly arranged layer of pyramidal cells with a prominent nucleus was observed in the hippocampus
- Standard (Donepezil) - Neuronal cells are well organised with layers of pyramidal cells and the absence of apoptotic cells was observed in the hippocampus

**Table 1c:** Effect of AVE on TSTQ of rats using Morris water maze on the 17<sup>th</sup> day.

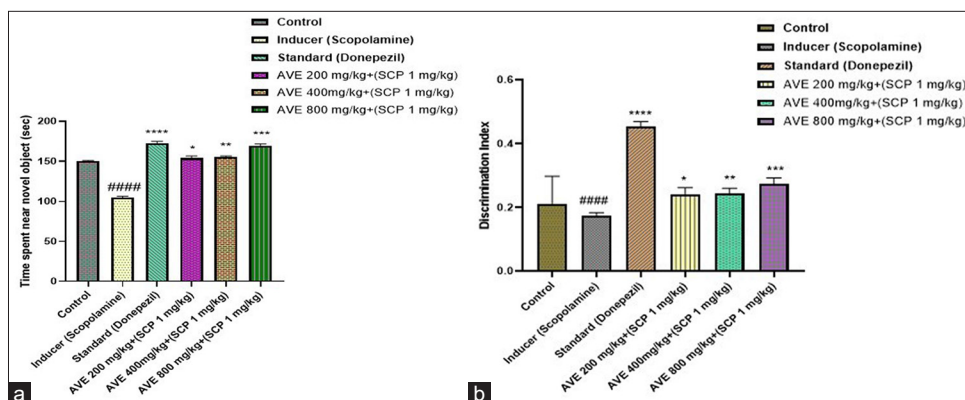
Groups	Treatment	On 17 <sup>th</sup> day TSTQ (sec) mean±SEM (n=6)
I	Control	40.34±0.7706
II	Inducer (Scopolamine)	25.21±1.324
III	Standard (Donepezil)	50.52±20.059***
IV	AVE 200 mg/kg+(SCP 1 mg/kg)	36.85±0.1800*
V	AVE 400 mg/kg+(SCP 1 mg/kg)	39.28±0.9493**
VI	AVE 800 mg/kg+(SCP 1 mg/kg)	45.45±0.9279***

The behavioural analysis was compared to the Inducer group. Data are expressed as Mean±SEM, n=6, and statistical analysis by one-way ANOVA followed by Tukey's test. \*\*\*P<0.001 when compared with Inducer control. the result is highly statistically significant, showing improvement compared to the Inducer group. \*\*P < 0.01 The result is statistically significant, showing improvement compared to the Inducer group. \*P < 0.05 indicates mild statistical significance compared to the Inducer group. AVE: *Amaranthus viridis* extract, ANOVA: Analysis of variance, TSTQ: Time spent in target quadrant, SEM: Standard error of the mean, SCP: Scopolamine

**Table 2a:** Effect of AVE on time spent near the novel object of rats using novel object recognition test.

Groups	Treatment	Time spent near novel object Mean±SEM (n=6)
I	Control	150.2±0.4034
II	Inducer (Scopolamine)	104.7±0.6424
III	Standard (Donepezil)	172.8±0.8568****
IV	AVE 200 mg/kg+(SCP 1 mg/kg)	154.2±0.9107*
V	AVE 400 mg/kg+(SCP 1 mg/kg)	155.3±0.5634**
VI	AVE 800 mg/kg+(SCP 1 mg/kg)	169.0±1.024***

The behavioural analysis was compared to the Inducer group. Data are expressed as Mean±SEM, n=6, and statistical analysis by one-way ANOVA followed by Tukey's test. \*\*\*\* P < 0.0001 result is highly statistically significant, indicating a very strong difference between the Donepezil-treated group and the Inducer \*\*\*P<0.001 when compared with Inducer control. P < 0.01 The result is statistically significant, showing improvement compared to the Inducer group. \*P < 0.05 indicates mild statistical significance compared to the Inducer group. AVE: *Amaranthus viridis* extract, ANOVA: Analysis of variance, SEM: Standard error of the mean, SCP: Scopolamine



**Figure 1:** (a) Effect of *Amaranthus viridis* extract on time spent near the novel object of rats, (b) Effect of *A. viridis* extract on discrimination index of rats using Novel object recognition test. where in (a) and (b) the data are represented as mean  $\pm$  SEM ( $n = 6$ ). Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. "####" denotes comparison with normal control. \*\*\* $P < 0.001$  when compared with the Inducer control, indicating a highly significant difference compared to the Donepezil-treated group and the Inducer. \*\* $P < 0.01$  shows statistically significant improvement compared to the Inducer group. \* $P < 0.05$  indicates mild statistical significance compared to the Inducer group. AVE: *Amaranthus viridis* extract, SCP: Scopolamine

**Table 2b:** Effect of AVE on discrimination index object of rats using novel object recognition test.

Groups	Treatment	Discrimination index mean $\pm$ SEM ( $n=6$ )
I	Control	0.2125 $\pm$ 0.03250
II	Inducer (Scopolamine)	0.1733 $\pm$ 0.004179
III	Standard (Donepezil)	0.4533 $\pm$ 0.006424****
IV	AVE 200 mg/kg+(SCP 1 mg/kg)	0.2417 $\pm$ 0.007993*
V	AVE 400 mg/kg+(SCP 1 mg/kg)	0.2450 $\pm$ 0.006075**
VI	AVE 800 mg/kg+(SCP 1 mg/kg)	0.2733 $\pm$ 0.007454***

The behavioural analysis was compared to the Inducer group. Data are expressed as Mean $\pm$ SEM,  $n=6$ , and statistical analysis by one-way ANOVA followed by Tukey's test. \*\*\*\* $P < 0.0001$  result is highly statistically significant, indicating a very strong difference between the Donepezil-treated group and the Inducer \*\*\* $P < 0.001$  when compared with Inducer control. \*\* $P < 0.01$  The result is statistically significant, showing improvement compared to the Inducer group. \* $P < 0.05$  indicates mild statistical significance compared to the Inducer group. AVE: *Amaranthus viridis* extract, ANOVA: Analysis of variance, SEM: Standard error of the mean, SCP: Scopolamine

- *A. viridis* 200 mg/kg b.w - Cells are well organised, less shrunken and appropriate intracellular spaces are visible
- *A. viridis* 400 mg/kg b.w - Cells are well organised, less shrunken and sufficient intracellular spaces are visible
- *A. viridis* 800 mg/kg b.w - sufficient intracellular spaces, well-organised cells with fewer shrunken cells and no alterations in cell shape are found
- Inducer (Scopolamine) - The number of intracellular

**Table 3:** Effect of AVE on AChE activity on rat brain.

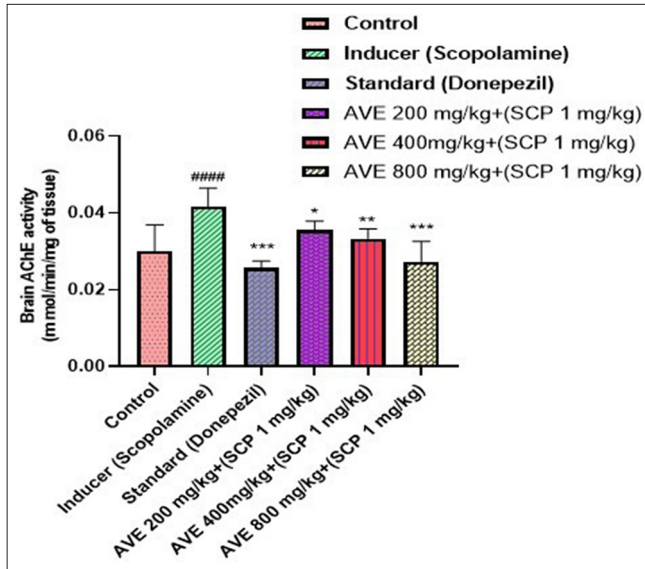
Groups	Treatment	AChE $\mu$ M/min/1/g tissue
I	Control	0.03000 $\pm$ 0.002610
II	Inducer (Scopolamine)	0.04167 $\pm$ 0.001808
III	Standard (Donepezil)	0.02567 $\pm$ 0.0006785***
IV	AVE 200 mg/kg+(SCP 1 mg/kg)	0.03550 $\pm$ 0.0008931*
V	AVE 400 mg/kg+(SCP 1 mg/kg)	0.03333 $\pm$ 0.0009428**
VI	AVE 800 mg/kg+(SCP 1 mg/kg)	0.02733 $\pm$ 0.001996***

The behavioural analysis was compared to the Inducer group. Data are expressed as Mean $\pm$ SEM,  $n=6$ , and statistical analysis by one-way ANOVA followed by Tukey's test. \*\*\* $P < 0.001$  when compared with Inducer control, the result is highly statistically significant, indicating a difference between the Donepezil-treated group and the Inducer. \*\* $P < 0.01$ . The result is statistically significant, showing improvement compared to the Inducer group. \* $P < 0.05$  indicates mild statistical significance compared to the Inducer group., AVE: *Amaranthus viridis* extract, ANOVA: Analysis of variance, SEM: Standard error of the mean, SCP: Scopolamine

spaces is more, the arrangement of the cells is not linear, the pyramidal cells are disorganised and the size of the cells is less [Figure 3].

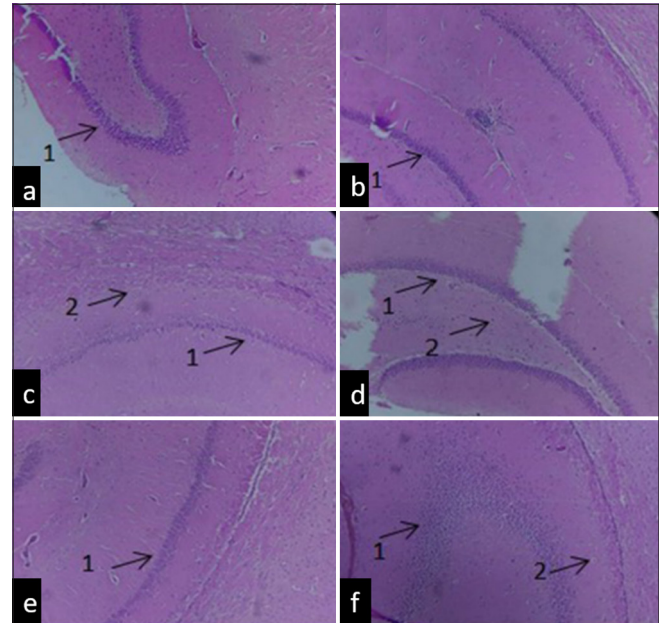
## DISCUSSION

Memory is the process by which experiences are stored in the brain so that responses to the environment can be adapted. The ability to define one's identity and place in addition to carrying out regular work duties is quite important.



**Figure 2:** Estimation of AChE enzyme levels in the brain before histopathology. Effect of *Amaranthus viridis* extract on acetylcholinesterase on rat brain. The analysis was compared to the control group and the data are represented as mean  $\pm$  SEM ( $n = 6$ ). Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. "####" denotes comparison with normal control. \*\*\* $P < 0.001$  when compared with the Inducer control, indicating a highly significant difference compared to the Donepezil-treated group and the Inducer. \*\* $P < 0.01$  shows statistically significant improvement compared to the Inducer group. \* $P < 0.05$  indicates mild statistical significance compared to the Inducer group. AVE: *Amaranthus viridis* extract, SCP: Scopolamine.

The most significant neurotransmitter involved in the control of cognitive processes is thought to be the central cholinergic system. The primary enzyme involved in the hydrolysis of acetylcholine (ACh), which ends cholinergic transmission, is AChE. Neurodegenerative illnesses like AD have been documented to cause a decrease in cholinergic conduction, which is linked to cognitive impairment.<sup>[12]</sup> About 50 million people worldwide suffer from AD, which is characterised by cognitive deficits, memory problems and other forms of cognitive dissonance. Death usually occurs 3–9 years following the diagnosis.<sup>[13]</sup> The pathophysiology of AD is complex and multifaceted, making it a dangerous neurodegenerative disorder. The clinical development of AD is characterised by a clear and distinct deterioration in cognitive function, and different motor dysfunctions occur across the disease spectrum.<sup>[14-16]</sup> Scopolamine, an anti-muscarinic agent, competes with ACh at muscarinic receptors by binding to postsynaptic receptor sites with high affinity and enhancing AChE activity in the cortex and hippocampus, thereby inhibiting AChs effects on these receptors. Due to cholinergic hypofunction, scopolamine stops cerebral blood flow. Along with causing reactive oxygen



**Figure 3:** Histopathology of rat brain where (a) the photomicrograph shows the molecular layer (1) with preserved neuronal architecture, (b) the granular cell layer (1) is clearly visible, showing intact histoarchitecture, (c) the polymorphic layer (2) and granular layer (1) are shown with organized cellular layers, (d) the granular layer (1) and polymorphic layer (2) show pyknotic granular cells (black arrows), indicating neurodegeneration, (e) the granular layer (1) appears intact, lacking signs of degeneration, and (f) both the granular layer (1) and polymorphic layer (2) are visible, with black arrows indicating peripherally located nuclei in pyramidal cells. Stained with Hematoxylin & Eosin at 40 $\times$ .

species, scopolamine also causes free radical damage, an increase in the brain MDA levels of the scopolamine-treated group and a decline in antioxidant status.<sup>[17]</sup> In response to increased AChE activity, scopolamine was observed to lower ACh levels. This enzyme is in charge of destroying ACh, a neurotransmitter necessary for cognitive function.<sup>[18]</sup> *A. viridis* belongs to the *Amaranthaceae* family. According to preliminary phytochemical screening, AVE contains tannins, saponins, flavonoids, alkaloids, steroids, phenolic compounds, proteins and anthraquinones, but not reducing sugars, cardiac glycosides and terpenoids. Rodent's spatial learning and memory have frequently been tested using the MWM experiment. It demonstrated a reduction in escape latency time and increased time spent in the target quadrant using ethanolic extract of *A. viridis* Linn leaves in a dose-dependent way. NORT showed that when compared to the scopolamine-treated group, Donepezil and AVE group rats significantly reduced the amount of time spent examining novel objects. As the cognitive impairment is caused by the loss of ACh, which occurs from the hydrolytic action of AChE, an ethanolic extract of leaves from *A. viridis* Linn exhibited a dose-dependent reduction in AChE level in

addition to behavioural testing. An increase in vacuolated cytoplasm and a decrease in the number of pyramidal cells were found in the hippocampus and cortex areas of rats whose brains had been given scopolamine by H&E staining. The modification in brain structure also demonstrates that scopolamine-induced memory impairment has occurred, and the involvement of these brain regions in cognitive processes provides more evidence. In response to AVE pre-treatment, the number of pyramidal cells increased while cytoplasm vacuolation was reduced. By lowering brain AChE activity and oxidative stress, AVE corrected the memory impairment caused by scopolamine with maintaining brain structure and preventing neurodegeneration, according to our findings.

## CONCLUSION

This study showed that all three doses of AVE show good nootropic activity compared to the inducer group by keeping this as a benchmark we can focus on identifying and isolating the active phytoconstituent, and the researcher can further focus on nanoformulations to increase the efficacy and bioavailability of AVE. The oxidative stress and AChE activity in the brain caused by scopolamine are likewise restored to normal by AVE. As a result, it appears that the AVE may be a good option for use as a memory booster or as a treatment for neurodegenerative conditions like AD. More research is required to completely understand how and which phytoconstituents of AVE interact with biochemical processes in the brain.

**Ethical approval:** The research/study was approved by the Institutional Animal Ethical Committee at Sri Adichunchanagiri College of Pharmacy, approval number SACCP-IAEC/2022-02/70, dated 20th January 2023.

**Declaration of patient consent:** Patient's consent is not required as there are no patients in this study.

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**Conflicts of interest:** There are no conflicts of interest.

**Use of artificial intelligence (AI)-assisted technology for manuscript preparation:** The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

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