Antioxidative and Neuroprotective Potential of *Salvia plebeia* R. Br. in Scopolamine Induced Amnesia in Rat

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Abstract

This study aim to investigate the antioxidative and neuroprotective potential of *Salvia plebeia* R. Br. in a scopolamine-induced amnesia in rats. Memory impairment is closely associated with oxidative stress and cholinergic dysfunction, as seen in Alzheimer's disease (AD). The ethanolic extract of *S. plebeia* (SPEE), rich in flavonoids and phenolic compounds, was evaluated for its ability to ameliorate scopolamine-induced cognitive deficits. The plant aerial parts were extracted by Soxhlet extraction and subjected to qualitative and quantitative phytochemical screening. Acute oral toxicity studies (OECD-423) confirmed the extract's safety up to 2000 mg/kg. The wistar rats were divided into five groups: normal control, disease control, standard (donepezil, 5 mg/kg), and two test groups (SPEE 200 mg/kg and 400 mg/kg). Behavioral performance was assessed using the Novel Object Recognition, Y-maze, and Passive Avoidance tests. Biochemical estimations of hippocampal acetylcholinesterase (AChE), malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) were performed, alongside histopathological analysis. Scopolamine-treated rats showed significant impairment in memory, elevated AChE and MDA levels, and decreased antioxidant enzyme activity. Treatment with SPEE significantly reversed these changes, improving behavioral scores, normalizing AChE activity, reducing lipid peroxidation, and enhancing endogenous antioxidant levels in a dose-dependent manner. Histological examination further confirmed neuroprotection of hippocampal neurons. The findings suggest that *S. plebeia* exerts potent antioxidative and neuroprotective effects, likely mediated through cholinergic modulation and oxidative stress attenuation, supporting its therapeutic potential in cognitive disorders such as AD.

Keywords: Acetylcholinesterase, Amnesia, Antioxidant, *Salvia plebeia* R. Br., Scopolamine, Neuroprotection

Introduction

Memory is a fundamental cognitive function essential for learning, adaptation, and survival. Amnesia, defined as the impairment or loss of memory, can occur as a consequence of aging, neurodegenerative diseases, brain injury, or drug-induced cognitive dysfunction. Among neurodegenerative disorders, Alzheimer's disease (AD) represents the most prevalent cause of dementia worldwide, characterized by progressive memory loss, cognitive decline, and behavioral disturbances [1]. According to the World Health Organization, nearly 55 million people suffer from dementia globally, and this number is projected to reach 139 million by 2050 [2]. The pathogenesis of amnesia, particularly in AD-related conditions, is multifactorial and involves, degeneration of basal forebrain cholinergic neurons leads to decreased acetylcholine (ACh) levels, impairing learning and memory processes [3]. Excessive reactive oxygen species (ROS) production causes neuronal lipid peroxidation, protein oxidation, and mitochondrial dysfunction, contributing to neuronal apoptosis [4]. The hyper activation of microglia and astrocytes releases pro-inflammatory cytokines (TNF-α, IL-1β, IL-6), worsening neuronal damage and neuroinflammation. Aggregation of amyloid-β peptides and hyper phosphorylation of tau proteins impair synaptic function and neuronal communication. Among these, oxidative stress and cholinergic deficits are considered major contributors to memory impairment, making them attractive therapeutic targets [5-7].

Presently available drugs for managing cognitive decline include cholinesterase inhibitors (donepezil, rivastigmine, galantamine) and NMDA receptor antagonists (memantine). These drugs offer only symptomatic relief and do not halt or reverse disease progression. Moreover, their long-term use is associated with adverse effects such as gastrointestinal disturbances, hepatotoxicity, and cardiovascular complications [8, 9]. Hence, there is a growing demand for safer, cost-effective, and multi-targeted therapeutic options derived from natural sources.

Herbal medicines, rich in flavonoids, phenolic acids, terpenoids, and alkaloids, have demonstrated significant neuroprotective and memory-enhancing properties [10]. Their

mechanisms of action include antioxidant activity, modulation of cholinergic transmission, and inhibition of acetyl cholinesterase (AChE), anti-inflammatory effects, and protection against neuronal apoptosis. Several plant extracts have shown efficacy in reversing scopolamine-induced amnesia, supporting the exploration of ethno medicinal plants for developing novel cognitive enhancers [10, 11].

Salvia plebeia R. Br., commonly known as "East Indian sage," belongs to the family Lamiaceae and is widely distributed across Asia, including India, China, Korea, and Japan. In traditional medicine, *S. plebeia* has been used to treat inflammation, liver ailments, respiratory disorders, fever, and skin diseases. In Ayurveda and traditional Chinese medicine, it is valued for its rejuvenating and protective properties [12, 13]. Phytochemical studies reveal that *S. plebeia* contains flavonoids (luteolin, apigenin, hispidulin), phenolic acids (rosmarinic acid, caffeic acid), terpenoids, and essential oils. These compounds exhibit strong antioxidant, anti-inflammatory, hepatoprotective, antimicrobial, and anticancer activities. Notably, flavonoids like luteolin and apigenin have been reported to enhance memory, inhibit acetylcholinesterase, and protect neurons from oxidative stress, making them particularly relevant in the context of neurodegenerative disorders [14, 15].

Given the central role of oxidative stress and cholinergic dysfunction in amnesia, agents that can scavenge free radicals, enhance antioxidant defense, and improve cholinergic neurotransmission hold great therapeutic promise. *S. plebeia*, being rich in bioactive flavonoids and phenolic acids, is hypothesized to exert neuroprotective effects through its antioxidative and acetylcholinesterase-inhibiting properties. However, systematic evaluation of its potential against scopolamine-induced amnesia is limited. This study aims to fill this gap by exploring its antioxidative and neuroprotective efficacy in an established rodent model.

Materials and Methods

Collection and authentication of *S. plebeia*

The aerial parts of *Salvia plebeia* were collected from Eastern Ghats range of Hoganekkal reserve forest, Dharmapuri district, Tamil Nadu. The aerial parts included leaves, stems and flower. It was collected during the month of April 2025. Then the collected plant material was

botanically identified and authenticated by The Joint Director, Botanical Survey of India, Coimbatore, Tamil Nadu, India.

Preparation and Extraction of *S. plebeia*

The collected fresh plant materials were thoroughly washed in running tap water in order remove sand and dust. Then the plant material were dried and coarsely powdered by a mechanical grinding and sieved (mesh no. 40). The powdered materials were initially defatted with petroleum ether (40-60^{0}c) and then extracted with 70% v/v ethanol, by continuous hot percolation techniques using soxhelt apparatus until the siphon tube clear. After completion of the extraction, it was filtered and dried at 60°C in rotary evaporator to produce a semisolid mass. The dried extract was stored at 4°C in an air tight container until further use.

Phytochemical analysis

Qualitative analysis

The *S. plebeia* aerial parts ethanolic extract (SPEE) was subjected to preliminary phytochemical screening for the identification of phytoconstituents present in the SPEE by the standard laboratory methods [16].

Quantitative analysis

Estimation of total alkaloid content

To determine the total alkaloid content in the *S. plebeia* ethanolic extract (SPEE) was dissolved with 1ml of 2N Hcl and filtered. The filtered solution was transferred into the separating funnel, and then 5 ml of phosphate buffer and 5 ml bromocresol green solution (BCG) were added. Then this mixer was diluted with chloroform. The absorbance of the test and standard solutions were determined at 470 nm. Atropine 20-100 µg/ml was used as standard. The total alkaloid content was expressed as mg of Atropine (AE)/g of extract [17].

Estimation of total flavonoid content

Total flavonoid content in the SPEE was estimated by $AlCl_3$ method. Quercetin was used as standard. The quercetin 1 mg/ml methanolic solution was prepared and different aliquots 20-100µg/ml from this solution was prepared with methanol. 3 ml of SPEE solution (1mg/ml solution in methanol) or standard solution was added into the test tube containing 1 ml of 2%

$AlCl_3$ methanolic solution and allowed to stand for 1 hour at room temperature. The absorbance of the solution was measured at 420 nm. The total flavonoid content was expressed as mg of Quercetin (QE)/g of the extract [18].

Estimation of total phenolic content

The total phenolic content in the SPEE was determined by the Folin-Ciocalteu method with slight modification. The 0.1 ml of extract (0.1 mg/ml in distilled water) was treated with 0.5 ml of Folin-Ciocalteau reagent and 1.5 ml of 7% sodium carbonate. The combined solution was shaken well and made up to 10 ml with distilled water. Then it was incubated in dark at room temperature for 2 hrs. Then the absorbance of the test and standard was taken at 725 nm in spectrometer against a reagent blank. Gallic acid (20-100 µg/ml) was used as standard. The total phenolic content was expressed as mg of gallic acid (GAE)/gm of extract [19].

Acute oral toxicity studies

Acute oral toxicity of *S. plebeia* ethanolic extract (SPEE) was evaluated by the acute toxic class method as per OECD (Organization for Economic Co-operation and Development) test guideline 423 [20]. Three female rats were treated with a single oral dose of SPEE 2000 mg/kg was administered. After administration, each animal was individually observed for the first 30 minutes, followed by special attention for the first 4 hours and periodically for 24 hours, thereafter daily for 14 days. The evaluation parameters include changes in skin and fur, mucous membrane, eye, respiration, circulatory, central, peripheral nervous system, and somatomotor activity and behavioral changes. The occurrence of salivation, diarrhea, lethargy, tremors, convulsions, sleep, and coma should be monitored closely.

***In vivo* scopolamine induced amnesia model**

Experimental animals

The colony inbred either sex of albino wistar rats (200-250 gm) were included in this study. The animals were kept under standard environmental conditions of 12/12 light/dark rhythm, maintained under controlled room temperature (23 ± 2^0C) and a relative humidity of 60±10%, in polypropylene cages. The rats were given three days to acclimate to the behavior testing apparatus before being evaluated. The experimental protocol was carried out according to the guidelines of the Committee for Control and Supervision of Experiments on Animals (CCSEA),

India and approved by the Institutional Animal Ethical Committee (IAEC) of Sri Abirami College of Pharmacy, Coimbatore; (IAEC Reference No.: SACOP/Re/M. Pharm/08/2025 Dated: 21.06.2025).

Experimental Design

The thirty animals were divided into five groups of six each. Group I served as normal control had no treatment and were intraperitoneally injected with normal saline (0.9 % NaCl) on days 8–14. Group II served as disease control received normal saline, Group III to V served as treatment group donepezil Hcl at a dose of 5 mg/kg, SPEE extract low dose 200 mg/kg and high dose 400 mg/kg respectively. The drugs were administered between 9.00 and 10.00 a. m. for 14 consecutive days. Scopolamine is used to induce memory deficits. Scopolamine, at a dose of 3 mg/kg body weight (*i. p.*) administered to the Group II-V animals from day 8 to day 14 to induce cognitive impairment. Oral doses of vehicle, donepezil, and *S. plebeia* extracts were given 30 min before the scopolamine injection. Behavioral tests were carried out 1 hr after the treatment on days 7 and 14.

Behavioral assessment and Cognition tests

The behavioral tests, which comprised the novel object recognition test (NORT), Y-maze test (YMT), and open field test (OFT), were conducted on days 7 and 14.

Novel object recognition test (NORT)

To evaluate cognitive function, Novel object recognition test NORT was employed. The test was carried out in a transparent plexiglass box with a consistent light condition (40 lux), measuring 40 cm in length, 40 cm in width, and 40 cm in height. The NORT consisted of three phases: habituation, training, and testing. During the habituation phase, which took place on the first day, the animals were permitted to explore the empty box for 5 min before being returned to their initial cage. During the training phase, the animals were placed in the box for 5 min to explore two familiar objects. On the testing day, which occurred 24 hr after the training, the animals were exposed to one object with which they were familiar (object A) and another object with which they were unfamiliar (object B). The rats directed their noses towards the objects and sniffed them from a distance of less than 2 cm. Each rat investigated the objects, and afterward,

the box and objects were cleaned with a 70 % ethanol solution [21]. The discrimination ratio (DR) was determined using the following formula:

$$DR = \frac{Time\ Spent\ exploring\ object\ B - Time\ spent\ exploring\ object\ A}{Time\ Spent\ exploring\ object\ B + Time\ spent\ exploring\ object\ A}$$

Y-maze test

Y-maze test was used to evaluate short-term working memory by measuring spontaneous alternation behavior. Spontaneous alternation behavior (SAB) was used as a measure of exploratory behavior and the rats' ability to explore a novel area. Typically, rats tend to explore a different arm of the maze than the one they previously visited. After being placed in the center of the maze, the rats were allowed to freely explore the three arms of the maze for 8 min. The number of arm entries and the number of triads will be recorded to determine the percentage of spontaneous alternation behavior. Arm entries were counted when all four paws of the rat were within the arm [22]. The following formula was used to calculate the percentage of spontaneous alternation behavior [SAB (%)]:

$$\text{SAB (\%)} = \frac{\text{Number of alterations}}{\text{Total arm enteries} - 2} \text{x } 100$$

Step-through Passive Avoidance Test

The step-through passive avoidance test was a fear-motivated test that evaluates the long-term avoidance memory ability of animals. The experimental apparatus consisted of a dark chamber separated by an automatic guillotine door and a steel rod on the bottom that gave an electric shock to rats. Experimental rats underwent two separate trials: a training trial for the acquisition of fear and a retention trial to estimate whether the memory of the fear remains. In the training trial, a rat was placed in a bright chamber. After 20s of adaptation time, the entrance to the dark chamber opened with a little noise. When there at moved into the dark room, the middle partition closed mildly, and then a 0.5mA of electric foot shock was delivered to the rat for 5s through the steel rod on the bottom. After the training trials, rats returned to the cage and received a retention trial to measure the memory abilities after 24 hr. In the retention trial, the rat moved to the bright chamber, and after 20 s, the passage to the dark room opened with a sound. The time of step-through latency of the rat were measured for 300s [23].

Biochemical Estimation

Collection of Rat Hippocampus

At the end of the experiment on day 15 of the study, the rats were intra peritoneally (*i.p.*) injected with thiopental sodium at a dose of 80 mg/kg *b.w.* After that the hippocampus, the frontal cortex and the amygdale complex were removed in accordance with the rat brain atlas and were stored at -80^{o}C. The hippocampus was homogenized with lysis buffer. The homogenized tissues in the lysis buffer were sonicated and centrifuged at 18,403xg for 20 min at 4^{o}C using a cooling centrifuge. The supernatant of tissue lysate was collected and stored at -80^{o}C prior to analysis. The supernatant was further used for the estimation of the following AChE, MDA, SOD, GSH, GPx and catalase,

Measurement of AChE Activity

The determination of AChE activity of the hippocampus was measured according to the modified method of Ellman's with slight modifications [24]. Acetylthiocholine iodide was used as a substrate for the measurement of AChE activity. Five microliters of appropriately diluted hippocampus lysate and 65 µL of 50 mM sodium phosphate buffer (pH 7.4) were incubated for 15 min at 37^{0}C. After incubation, 70 µL of Ellman's reaction mixture [0.5 mM acetylthiocholine and 1 mM 5,5-dithio-bis(2-nitrobenzoic acid) in a 50 mM sodium phosphate buffer (pH 7.4)] was added and incubated for 10 min at 37^{0}C. The mixture was then measured at 415 nm using the microplate reader. AChE activity was expressed nM/min/mg tissue.

Measuring malondialdehyde (MDA) level

Brain tissue homogenate (25 µL) was mixed with 1.5mL of acetic acid 20%, 1.5mL of 0.8mM thiobarbituric acid (TBA), and 200 µL of SDS 1.8% solution. The sample was then placed in boiling water for 60 min. The sample was cooled and 1mL of distilled water and 5mL of n-butanol-pyridine solution added to it and the resulting mixture was shaken. The mixture was then centrifuged for 10 min at 4000 rpm and the optical absorbance of the supernatant was recorded at 523nm [25].

Measurement of antioxidant enzyme activities

SOD assay

The SOD activity was determined in the supernatant using the nitro blue tetrazolium by method of Kakkar *et al.,* [26]. This method employs xanhine and xanthine oxidase to generate

superoxide radicals which react with 2-(4-iodo-phenyl)-3-(4-nitrophenole)-5-phenyl-tetrazolium chloride (INT) to form a red formazan dye. The SOD activity is then measured by the degree of inhibition of this reaction. One unit of SOD is that which causes a 50% inhibition, based on the 50% inhibition of the formation of NADH- phenazine methosulfate-nitroblue tetrazolium formazan at 520 nm. One unit of the enzymes is taken as the amount of enzyme for 50% inhibition of NBT reduction/min/mg protein.

Reduced glutathione (GSH) assay

Reduced glutathione (GSH) content was determined via the method of Ellman [27]. GSH determination is based on the development of yellow color when 5, 5' dithio 2-nitro benzoic acid (DTNB) is added to compounds containing sulfhydryl groups. The values are expressed as nmoles g-1wet tissue.

Glutathione peroxidase (GPx) assay

Glutathione peroxidase (GPx) activity was assayed via the method of Rotruck [28] with a modification: a known amount of enzyme preparation was incubated with H_2O_2 in the presence of GSH for a specified time period. The amount of H_2O_2 utilized was determined via the method of Ellman [27]. The values are expressed as μmoles of GSH utilized/min/mg Hb or protein.

Catalase assay

The activity of catalase was determined in 3 mL of reaction media, which contained 2 mL of homogenizing medium (phosphate buffer; pH 7.0) in a test tube followed by 1 mL of H_2O_2 solution [29]. The blank was composed of one mL buffer pH 7.0 and 2 mL tissue homogenate (pH 7.0). The extinction was measured at a wavelength of 240 nm using ultraviolet-visible spectro-photometer. The catalase activity was expressed as U mg^{-1} protein.

Estimation of Total protein concentration

The supernatant obtained from the right hippocampal homogenate prepared with lysis buffer solution was used to prepare a whole-cell protein lysate. The total protein concentration of the lysate was measured using a commercially available protein assay kit (E-BC-K168-S) and the colorimetric Bradford method (Elabsciences, USA), with bovine serum albumin (BSA) as a standard control.

Rat Brain tissue Histopathological analysis

The rats were sacrificed (after being anesthetized) by decapitation at the end of study. For each rat, the skull was opened and the brain was cut along the sagittal plain; three of the resulting half-

brains were selected at random from each group of rats and fixed in formalin (10% solution in saline) for histopathology, where they were sectioned 5µm thickness section. The section were stained with hematoxylin and eosin, stain and examined by an electric light microscope.

Statistical Analysis

For statistical analysis GraphPad software (Prism 3.8; GraphPad Software, San Diego, CA, USA) was used. All data were expressed mean ± standard error of the mean SEM. The groups of data were compared with analysis repeated measures ANOVA followed by Tukey's multiple comparison tests. The values of $P< 0.05$ were considered as a statistical significant difference.

Results

Extraction Value and Qualitative Phytochemical Analysis

The *Salvia plebeia* ethanolic extract (SPEE) cumulative extraction value was calculated as 20.5% w/w yield percentage. The result of primary phytochemical screening of SPEE showed the presence of alkaloids, carbohydrates, flavonoids, steroids, glycosides, proteins, tannins, phenols, terpens & steroiols.

Quantitative Phytochemical Estimation

The major phytoconstituents alkaloids, flavonoids, and phenolic compounds total content in the *S. plebeia* ethanolic extract is mentioned in Table 1.

Table 1: Quantitative Phytochemical Estimation of *S. plebeia* ethanolic extract

S. No.	Phytoconstituents	Quantity (mg/g)
1.	Total Alkaloid content	22.08 ± 3.22
2.	Total Flavonoid content	53.21 ± 4.20
3.	Total Phenolic content	36.32 ± 1.34

N=3, values are expressed as mean ± SEM.

Acute oral toxicity study

The acute oral toxicity results of *S. plebeia* ethanolic extract were showed the limited dose of 2000 mg/kg single dosing revealed that normal weight gain in the treated animals. There were also no toxic symptoms, mortality, observational, behavioral and somatomotor changes observed after single dosing of SPEE. These results indicate that the SPEE was found to safer for acute use

at the tested dose level of 2000 mg/kg. Hence the lethal dose of 50% (LD50) of *S. plebeia* ethanolic extract is greater than 2000 mg/kg.

Effects of *S. plebeia* ethanolic extract on recognition memory

The novel object recognition test (NORT) was used to measure their capacity to memorize the studied objects, as determined by the increasing discrimination ratio in the behavioral test. On day 7 of drugs treatment, there were no significant differences in the rats' recognition memories among the groups. However, on day 14 post scopolamine administration, the rats that received scopolamine injection alone (group-II) demonstrated memory impairment, as shown by a significant ($P<0.001$) poorer discrimination ratio, compared to the control rats. The scopolamine-injected rats that were treated with donepezil and *S. plebeia* extract at doses of 200 and 400 mg/kg showed significant ($P<0.001$) recognition memory compared to disease control groups (Table 2).

Table 2: Effects of *S. plebeia* ethanolic extract on recognition memory in rats, using the novel object recognition test

Treatment	Discrimination ratio	
	Day 7	Day 14
Group-I (Vehicle Control)	0.25 ± 0.04	0.35 ± 0.01
Group-II (Disease Control- Scopolamine 3 mg/kg *i.p.*)	0.30 ± 0.01	-0.15 ± 0.02[c]
Group-III (Standard – Donepezil 5 mg/kg *p.o.*)	0.23 ± 0.02	0.28 ± 0.04[f]
Group-IV (SPEE-200 mg/kg *p.o.*)	0.26 ± 0.02	0.29 ± 0.01[f]
Group-V (SPEE-400 mg/kg *p.o.*)	0.28 ± 0.03	0.25 ± 0.04[af]

Values are expressed as mean ± SEM, N=6, Statistical significance represented as [a]$P<0.05$; [b]$P<0.01$; [c]$P<0.001$ Vs Group I. [d] $P<0.05$; [e]$P<0.01$; [f]$P<0.001$ Vs Group II. Data were analyzed by one-way ANOVA followed by Tukey's multiple comparison tests

Effect of *S. plebeia* ethanolic extract on spatial working memory

To measure the rats' short-term memory, the Y-maze test was employed. Spontaneous alternation behavior, which measures spatial working memory and is based on rats' natural exploratory behavior to investigate new environments, was used as an indicator. On day 7, rats treated with donepezil or SPEE at doses of 200 and 400 mg/kg does not exhibit any changes in the spontaneous alternation behavior compared to the vehicle group. On day 14, rats injected with scopolamine and given vehicle displayed significant ($P<0.001$) memory impairment compared to the control group, as evidenced by a lower percentage of spontaneous alternation behavior. Treatment with donepezil or SPEE at the doses of 200 and 400 mg/kg in scopolamine-injected rats resulted in higher spontaneous alternation behavior than those of the scopolamine alone administrated disease control group (Table 3).

Table 3: Effects of *S. plebeia* ethanolic extract on percentage of spontaneous alternation behavior in Y-maze test in rats

Treatment	Spontaneous alternation behavior [SAB (%)]	
	Day 7	Day 14
Group-I (Vehicle Control)	66.34 ± 3.65	72.45 ± 2.01
Group-II (Disease Control- Scopolamine 3 mg/kg *i.p.*)	65.38 ± 7.10	45.50 ± 3.52[c]
Group-III (Standard – Donepezil 5 mg/kg *p.o.*)	69.45 ± 5.33	67.35 ± 6.04[f]
Group-IV (SPEE-200 mg/kg *p.o.*)	72.34 ± 6.28	66.90 ± 3.21[f]
Group-V (SPEE-400 mg/kg *p.o.*)	74.80 ± 5.12	69.35 ± 3.45[f]

Values are expressed as mean ± SEM, N=6, Statistical significance represented as [a]$P<0.05$; [b]$P<0.01$; [c]$P<0.001$ Vs Group I. [d] $P<0.05$; [e]$P<0.01$; [f]$P<0.001$ Vs Group II. Data were analyzed by one-way ANOVA followed by Tukey's multiple comparison tests

Effect of *S. plebeia* ethanolic extract on long term avoidance memory

Long-term avoidance memory was measured by passive avoidance test. Long term memory was measures by condition avoidance response in re-exposure in the memorized condition in rat. On day 7, rats treated with donepezil or SPEE at doses of 200 and 400 mg/kg does not exhibit any changes in the latency time compared to the vehicle group. On day 14, in rats injected with scopolamine and given vehicle displayed significant ($P<0.001$) decreased latency time compared to the control group, as evidenced by a impaired memory. Treatment with donepezil or SPEE at the doses of 200 and 400 mg/kg in scopolamine-injected rats resulted in higher latency time than those of the scopolamine alone administrated disease control group (Table 4).

Table 4: Effects of *S. plebeia* ethanolic extract on long term avoidance memory in passive avoidance test in rats

Treatment	Latent time (in Sec)	
	Day 7	Day 14
Group-I (Vehicle Control)	50.00 ± 4.50	140.55 ± 8.22
Group-II (Disease Control- Scopolamine 3 mg/kg *i.p.*)	65.30 ± 2.35	56.70 ± 6.45^{c}
Group-III (Standard – Donepezil 5 mg/kg *p.o.*)	60.52 ± 4.23	138.50 ± 7.07^{f}
Group-IV (SPEE-200 mg/kg *p.o.*)	55.30 ± 4.20	124.43 ± 4.35^{f}
Group-V (SPEE-400 mg/kg *p.o.*)	52.40 ± 5.00	129.50 ± 6.25^{f}

Values are expressed as mean ± SEM, N=6, Statistical significance represented as $^{a}P<0.05$; $^{b}P<0.01$; $^{c}P<0.001$ Vs Group I. $^{d}P<0.05$; $^{e}P<0.01$; $^{f}P<0.001$ Vs Group II. Data were analyzed by one-way ANOVA followed by Tukey's multiple comparison tests

Effects of *S. plebeia* ethanolic extract on acetylcholinesterase (AchE) and malondialdehyde (MDA) levels in rat's hippocampus

The AChE activity was found to be significantly (P<0.001) higher in scopolamine-injected rats compared to the control group. However, treatment with donepezil or SPEE at doses of 200 and 400 mg/kg significantly reduced AChE activity in scopolamine-injected rats compared to the scopolamine alone administrated disease control group.

The rats administered with scopolamine injections showed significantly (P<0.001) higher level of MDA compared to the control rats. Conversely, rats that received donepezil or SPEE at doses of 200 and 400 mg/kg in combination with scopolamine injections exhibited significant (P<0.001) decreased MDA levels in the hippocampus when compared to the scopolamine alone administrated disease control group (Table 5).

Table 5: Effects of *S. plebeia* ethanolic extract on acetylcholinesterase (AchE) and malondialdehyde (MDA) levels in rat's hippocampus

Treatment	AchE (U/mg protein)	MDA (µmol/mg protein)
Group-I (Vehicle Control)	34.02 ± 2.34	2.80 ± 0.05
Group-II (Disease Control- Scopolamine 3 mg/kg *i.p.*)	65.09 ± 4.56^{c}	5.32 ± 0.35^{c}
Group-III (Standard – Donepezil 5 mg/kg *p.o.*)	38.12 ± 6.74^{f}	3.50 ± 0.23^{e}
Group-IV (SPEE-200 mg/kg *p.o.*)	41.45 ± 8.97^{e}	3.68 ± 0.20^{e}
Group-V (SPEE-400 mg/kg *p.o.*)	36.23 ± 3.65^{f}	3.06 ± 0.06^{f}

Values are expressed as mean ± SEM, N=6, Statistical significance represented as ^{a}P<0.05; ^{b}P<0.01; ^{c}P<0.001 Vs Group I. ^{d} P<0.05; ^{e}P<0.01; ^{f}P<0.001 Vs Group II. Data were analyzed by one-way ANOVA followed by Tukey's post hoc multiple comparison tests

Effects of *S. plebeia* ethanolic extract on the activity of antioxidant enzymes in rat's hippocampus

The results depicted in Table 6 showed that rats treated with scopolamine displayed significant ($P < 0.001$) reduced activities of SOD, GSH, GSH-Px and Catalase when compared to the control group. However, rats treated with donepezil or *S. plebeia* extract at doses of 200 and 400 mg/kg showed increased activities of SOD, GSH, GSH-Px, and Catalase when compared to the scopolamine-injected rats. In comparison between the treatments the SPEE showed better dose dependent increased effect on antioxidant levels.

Table 6: Effects of *S. plebeia* ethanolic extract on the activity of antioxidant enzymes in rat's hippocampus

Treatment	SOD (U/mg protein)	GSH (nmol/g tissue)	GPx (U/mg protein)	Catalase (U/mg protein)
Group-I (Vehicle Control)	84.72 ± 4.42	8.70 ± 0.50	27.43 ± 1.30	180.35 ± 7.43
Group-II (Disease Control-Scopolamine 3 mg/kg *i.p.*)	49.43 ± 6.46^{c}	3.42 ± 0.35^{c}	11.20 ± 3.42^{c}	114.56 ± 6.34^{c}
Group-III (Standard: Donepezil 5 mg/kg *p.o.*)	68.42 ± 1.40^{f}	7.56 ± 0.23^{f}	21.77 ± 2.45^{e}	162.45 ± 4.56^{f}
Group-IV (SPEE-200 mg/kg *p.o.*)	74.50 ± 2.43^{f}	6.85 ± 0.30^{f}	23.42 ± 3.56^{f}	159.00 ± 5.60^{f}
Group-V (SPEE-400 mg/kg *p.o.*)	79.37 ± 3.50^{f}	8.67 ± 0.10^{f}	26.89 ± 2.23^{f}	173.24 ± 7.32^{f}

Values are expressed as mean ± SEM, N=6, Statistical significance represented as [a]P<0.05; [b]P<0.01; [c]P<0.001 Vs Group I. [d] P<0.05; [e]P<0.01; [f]P<0.001 Vs Group II. Data were analyzed by one-way ANOVA followed by Tukey's multiple comparison tests.

Effects of *S. plebeia* ethanolic extract on total protein content in rat's hippocampus

Brain lysates were prepared from the brain tissue in ice cold homogenizing buffer (Phosphate Buffer, 50 mM, pH 7.4) and the total protein content was estimated by Lowry *et al* method. The result showed significant reduction in the protein content in rat hippocampus in the subsequent higher and lower doses of SPEE treated rats groups as compared to scopolamine alone treated disease control group and normal control group rats (Table 7).

Table 7: Effects of *S. plebeia* ethanolic extract on total protein content in rat's hippocampus

Treatment	Protein content (mg/gm) in hippocampus tissue
Group-I (Vehicle Control)	4.22 ± 0.42
Group-II (Disease Control- Scopolamine 3 mg/kg *i.p.*)	5.07 ± 0.56
Group-III (Standard – Donepezil 5 mg/kg *p.o.*)	3.92 ± 0.07[d]
Group-IV (SPEE-200 mg/kg *p.o.*)	2.56 ± 0.12[be]
Group-V (SPEE-400 mg/kg *p.o.*)	2.05 ± 0.32[cf]

Values are expressed as mean ± SEM, N=6, Statistical significance represented as [a]P<0.05; [b]P<0.01; [c]P<0.001 Vs Group I. [d] P<0.05; [e]P<0.01; [f]P<0.001 Vs Group II. Data were analyzed by one-way ANOVA followed by Tukey's multiple comparison tests

Histopathological Changes

Histopathological studies of the brain tissues hippocampal region from the rats supported the above results. No changes in the histopathological structures were observed in control, donepezil-treated, or SPEE high dose 400 mg/kg treated groups. The normal structure of the different neuronal sections was recorded. Some nuclear pyknosis and degeneration were observed only in the striatum neurons of the donepezil-treated group (Figure 4). On the contrary, scopolamine administration intraperitoneally to the rats for a week resulted in alterations in the normal features of the brain tissues. The striatum demonstrated small eosinophilic plaques in addition to neuronal nuclear pyknosis and degeneration were observed (Figure 1).

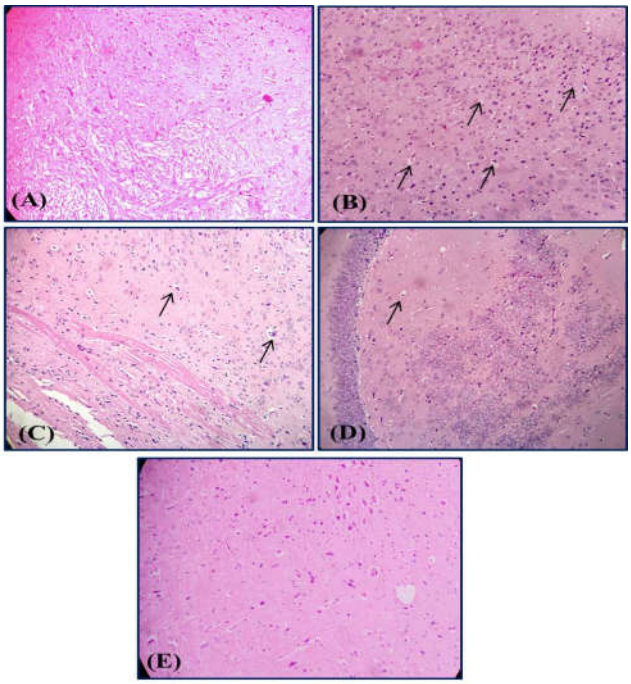

Figure 1: Histopathological images (stained by hematoxylin and eosin) showing hippocampal reign in the brain of rats. (A) Control, (B) Scopolamine, (C) Donepezil, (D) SPEE-200 mg/kg, (E) SPEE-400 mg/kg. Black arrows indicates the neuronal degeneration

Discussion

Cognitive impairment and amnesia represent major clinical challenges, particularly in the context of neurodegenerative disorders such as Alzheimer's disease (AD). The multifactorial pathogenesis of amnesia involves cholinergic dysfunction, oxidative stress, mitochondrial impairment, and neuroinflammation, all of which ultimately lead to progressive neuronal loss

and memory deficits. Scopolamine-induced amnesia in rodents has been widely adopted as an experimental model because it closely mimics the cholinergic and oxidative disturbances observed in AD. This model provides a reliable platform for evaluating the efficacy of potential therapeutic agents, including natural products with multitarget pharmacological activities [30, 31].

In the present study, the *Salvia plebeia* R. Br., a traditional medicinal herb rich in flavonoids and phenolic acids, was evaluated for its neuroprotective potential. Phytoconstituents such as luteolin, apigenin, and rosmarinic acid are well documented for their antioxidant, anti-inflammatory, and acetylcholinesterase (AChE)-inhibiting properties, making *S. plebeia* a rational candidate for managing memory deficits [32–34].

The primary phytochemical analysis of *S. plebeia* ethanoic extract (SPEE) showed the presence of alkaloids, carbohydrates, flavonoids, steroids, glycosides, proteins, tannins, phenols, terpens & steroiols. The quantitative estimation of key phytoconstituents in *S. plebeia* ethanoic extract showed the rich content of total flavonoids 53.21 mg/g, followed by total phenolic content 36.32 mg/g and total alkaloid content of 22.08 mg/g.

The safety of the *S. plebeia* ethanoic extract was evaluated by acute oral toxicity study as per OECD guideline 423. The SPEE did not show any signs of toxic symptoms or mortality at the limit dose of 2000 mg/kg *p. o.*, which showed that the SPEE is safe to use up to 2000 mg/kg. According to the Globally Harmonized System (GHS) toxicity classification the tested *S. plebeia* ethanoic extract was classified as category 5 or unclassified (2000 mg/kg < LD50 <5000 mg/kg).

Scopolamine-induced amnesia is widely used to model memory deficits because of its ability to selectively impair muscarinic cholinergic neurotransmission, leading to learning and memory impairments [35, 36]. In the current study, scopolamine administration significantly impaired recognition memory (novel object recognition test), working memory (Y-maze), and long-term memory (passive avoidance test). These findings are in agreement with earlier studies where scopolamine produced deficits resembling early AD-like pathology [37, 38].

The treatment of *S. plebeia* ethanoic extract at the doses of 200 and 400 mg/kg significantly improved memory performance in all the behavioral paradigms. Similar memory-enhancing effects have been documented with other flavonoid-rich extracts such as *Bacopa monnieri* and *Ginkgo biloba*, which improved scopolamine-induced deficits via antioxidant and cholinergic mechanisms [39, 40]. The improvement in recognition and working memory in SPEE-treated

groups aligns with the nootropic potential of luteolin and apigenin, reported to enhance cognitive performance in rodents [41, 42].

The cholinergic hypothesis of AD proposes that degeneration of basal forebrain cholinergic neurons and reduced acetylcholine (ACh) levels are central to memory decline [43]. In line with this, scopolamine-treated rats in this study exhibited elevated AChE activity, reflecting impaired cholinergic function.

Administration of SPEE significantly reduced AChE activity in a dose-dependent manner, comparable to donepezil, a clinically used AChE inhibitor. The AChE inhibitory effect is attributed to flavonoids and phenolic acids present in *S. plebeia*, particularly luteolin and apigenin, which have shown AChE inhibitory potential [33, 44]. Kim et al. (2021) further demonstrated that nepetoidin B, a diterpenoid from *S. plebeia*, modulated cholinergic signaling while simultaneously enhancing antioxidant defense [45]. These findings support the cholinesterase-inhibiting activity of SPEE as a dual mechanism of action.

Oxidative stress contributes significantly to both scopolamine-induced neuronal injury and AD progression [46, 47]. In this study, scopolamine increased hippocampal malondialdehyde (MDA) levels and reduced antioxidant enzyme activities (SOD, CAT, GPx, GSH). These biochemical alterations confirm oxidative stress as a mechanism of cognitive decline. Treatment with SPEE restored antioxidant enzyme activities and reduced MDA concentrations, indicating strong antioxidative potential. The present findings corroborate these earlier reports, suggesting that the antioxidative action of SPEE is a major contributor to its neuroprotective effects [48, 49].

Neuroinflammation is a key factor in AD and drug-induced amnesia, mediated by glial activation and pro-inflammatory cytokine release [50]. Although cytokine markers were not assessed in this study, the observed behavioral and histological protection suggest indirect modulation of neuroinflammatory cascades. Zou et al. (2018) demonstrated that sesquiterpenoids from *S. plebeia* suppressed NF-κB and MAPK pathways, thereby reducing pro-inflammatory mediator production [51]. Similarly, Kim et al. (2021) reported that nepetoidin B inhibited NF-κB activation while up-regulating Nrf2/HO-1 antioxidant signaling [45]. These mechanisms provide a plausible explanation for the observed neuroprotective efficacy of SPEE in the present study.

Histological assessment revealed severe neuronal degeneration and pyknosis in the hippocampus of scopolamine-treated rats. These observations are consistent with reports of scopolamine-induced neuronal apoptosis and hippocampal dysfunction [52]. Conversely, SPEE-treated rats

showed preserved neuronal morphology, comparable to donepezil treatment. Similar neuroprotection has been reported with other polyphenolic-rich extracts such as *Rosmarinus officinalis* and rosmarinic acid, which maintained hippocampal structure by suppressing oxidative stress and apoptotic pathways [53, 54]. The histopathological evidence in the present study supports the biochemical and behavioral outcomes.

Hence this study concluded that the results of this study establish *Salvia plebeia* R. Br. ethanolic extract as a safe and effective natural agent with significant cognitive-enhancing and neuroprotective properties. By simultaneously targeting oxidative stress and cholinergic dysfunction-two major pathological mechanisms of amnesia and Alzheimer's disease, SPEE demonstrates therapeutic promise as a multi targeted intervention. Further studies using chronic neurodegenerative models and molecular pathway validation are warranted to advance its potential for clinical application.

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