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Anti-oxidative, anti-AChE and neuroprotective effect of hydro-alcoholic extract of *Sansevieria cylindrica* leaves and *Plumeria obtusa* seed pods in Wistar rats

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Abstract The rising incidence of neurodegenerative diseases represents a significant global health concern. The prognosis for such diseases is often unfavorable, and patients require supportive treatment to manage their condition effectively. The present study investigated the anti-oxidative, anti-acetylcholinesterase (anti-AChE) effect of hydro-alcoholic extract of *Sansevieria cylindrica* leaves and *Plumeria obtusa* seed pods in Wistar rats. Plants were authenticated followed by pharmacognostic evaluation. The antioxidant and anti-AChE activities were evaluated by scopolamine-induced oxidative stress and acetylcholine level alterations in Wistar rats. On the 0th, 7th, and 14th days during study period behavioral parameters were evaluated, including open field test, new object recognition test, and Morris water maze task. Different antioxidant enzymes were also evaluated in brain homogenate post 7 days treatment. AChE levels in rats' brains were assessed post 14 days of treatment. Finally, histopathology of brains samples was performed. Correspondingly, *in-vitro* and *in-silico* studies were done to support the study findings.

Results *Sansevieria cylindrica* and *Plumeria obtusa* exhibited strong antioxidant and anti-AChE activities due to secondary metabolites content. Rearing and, line crossing by rats showed substantial alteration ($p < 0.05$) by combination of both plants at high dose. The escape latency found to be reduced significantly ($p < 0.05$) by *Sansevieria cylindrica* individually and in combination with *Plumeria obtusa* at all doses. The lipid peroxidation level in brain was decreased significantly than standard at 1:1 combination of plants extracts. The levels of GSH, CAT and SOD were attenuated significantly by different concentration of extracts ($p < 0.05$ and $p < 0.001$, respectively). The reduction in AChE levels was around 34.62% and 31.10% in male and female rats, respectively in combination of both plants at high dose compared to disease control group. It was further evident in brain histopathology. All *in-vivo* results were supported by *in-vitro* free radicals scavenging effect and *in-silico* study.

Conclusion The hydro-alcoholic extracts from *Plumeria obtusa* seed pods and *Sansevieria cylindrica* leaves showed a strong natural antioxidant, and acetylcholinesterase inhibitions which can aid in the treatment of neurodegenerative diseases including Alzheimer.

Keywords *Sansevieria cylindrica*, *S. cylindrica*, *Plumeria obtusa*, *P. obtusa*, Antioxidant, Anti-AChE

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Introduction

Many neurodegenerative diseases are marked by a gradual decline in learning and memory, cognitive function, as well as difficulties with reading, writing, and motor skills. Additionally, these conditions often lead to significant personality changes. The reduction in reactive oxygen species (ROS) like H_2O_2 , O_2 , and OH^- are one of the causes of such ailments. These conditions are also linked to diabetes, cancer, and cardiovascular diseases [1, 2]. Various substances that mitigate oxidative stress, including catalase, glutathione peroxidase, and superoxide dismutase, are currently being investigated for their potential to counteract free radicals. [3]. Another significant neuroprotective approach involves modifying specific neurotransmitters using acetylcholine esterase (AChE) inhibitors [4]. These approaches are further useful as potential therapeutic or preventive option for neurodegenerative diseases like Alzheimer's (AD). Moreover, inhibition of AChE is also considered to be beneficial in glaucoma, Parkinson's disease, Myasthenia gravis, and other disorders [5].

For thousands of years, various natural herbs remain an important source to treat central nervous system disorders and enhance memory and cognitive function [6]. This tendency is further supported by the natural antioxidants such as phenolics, flavonoids, vitamin C, carotenoids, tannins, and proanthocyanins found in these plants, which reduces the free radical formation [7]. A literature survey, also found usage of plants parts in inhibition of cholinesterase enzymes [8]. African origin *Sansevieria cylindrica* (*S. cylindrica*) plant (Asparagaceae) is commonly cultivated as indoor ornamental plants in India, Egypt and other countries [9, 10]. Traditionally, this plant's parts are used as anti-tumor, antibacterial, antidiabetic, anti-inflammatory, and free radical scavenging agent [11]. In line with the current study, existing literature reports the isolation of steroidal saponin from *S. cylindrica*, which has significant neuroprotective effects and helps in mitigating central nervous system disorders [11]. Biflavocochins, a substance from *Dracaena cochinchinensis* (Lour.) S.C. Chen, demonstrated neuroprotection against cellular damage to neuroendocrine PC12 cells generated by serum deprivation in an *in-vivo* experiment conducted in brown rats. This result supported the neuroprotective effect of *S. cylindrica* as, *Dracaena* and *Sansevieria* plants have the structural similarities of flavonoids resulting in inclusion of *Sansevieria* species in the genus *Dracaena* based on chemotaxonomy [12]. Another plant *Plumeria obtusa* (*P. obtusa*) (Apocynaceae), is found in the Greater antilles, Florida, northern central America, and southern Mexico [13]. Biological studies reported various health benefits of *Plumeria* [14, 15]. As like other plants, the methanolic

extract of *P. obtusa* was evaluated for actions related to AD's treatment which reported improvement in behavioral alterations associated with AD in rats and reverse hyperglycemia produced by alloxan [16].

Given the substantial traditional claims and medicinal potential of both plants, the current study investigates the neuroprotective effects and antioxidant activities of *Sansevieria cylindrica* leaves and *Plumeria obtusa* seed pods in animal models. In addition, *in-vitro* and *in-silico* techniques were utilized to find chemical constituents in both plants that may contribute to the inhibition of free radicals and AChE. Phenols and flavonoids are recognized to benefit human health and have antioxidant and anti-Alzheimer effects, and hence, the phenolic and total flavonoids content of these plants was also evaluated during various pharmacognostic assessment.

Material

Collection, authentication and pharmacognostic evaluation of plant material

S. cylindrica and *P. obtusa* were collected as complete, fresh plants at Akole, District-Ahmednagar, Maharashtra, India. For authentication, these plants' herbariums were prepared and sent to the Western Regional Center located in Pune, Maharashtra. Following this, various pharmacognostic assessments were carried out, including morphology, microscopy, quantitative and qualitative analysis of phytoconstituents, and preliminary phytochemical tests.

Chemicals and reagents

The investigation utilizes analytical grade chemicals and reagents, such as 20% acetic acid solution, 1 mM acetylthiocholine iodide, 10 mM DTNB, DPPH solution (125 μM dissolved in methanol), Griess reagent (consisting of 0.1% naphthyl ethylene diamine hydrochloride and 1% sulfanilamide in 2% H_3PO_4), 10 mM HCL, 10 mM H_2O_2 , 0.1 M NaOH, pyrogallol solution (2.6 mM in 10 mM HCL), 8.1% SDS, 10 mM Sodium nitroprusside, 0.8% aqueous solution of TBA, and 0.15 M Tris-hydrochloride, etc. S.D. Fine Chemicals, based in Mumbai, India, provided the majority of the chemicals and reagents. The other chemicals were made in laboratory for the investigation.

Methodology

The study was carried into three steps as shown in Fig. 1. The detailed descriptions of all steps are given below.

In-vitro anti-oxidant activity

S. cylindrica leaf and *P. obtusa* seed pod hydroalcoholic extracts were employed for a variety of *in-vitro* and *in-vivo* activity. Shewale et al. [17], discussed the process

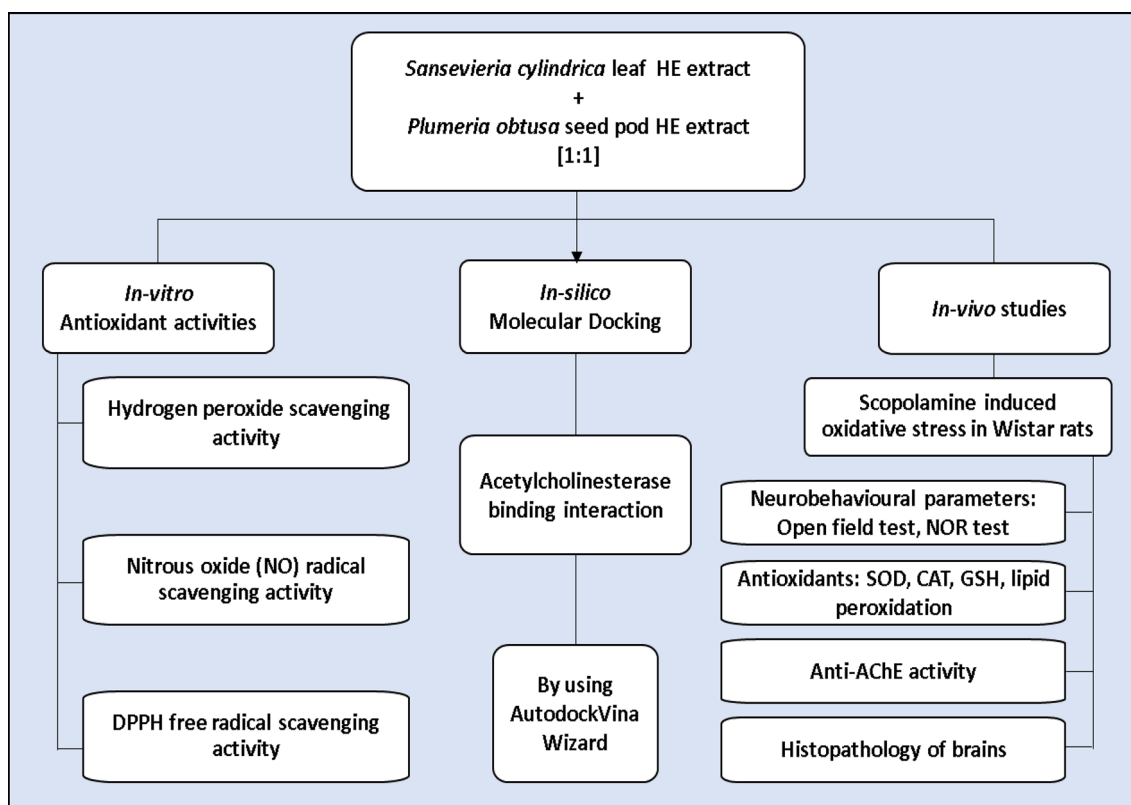


Fig. 1 Flow chart of various activities undertaken during *in-vitro*, *in-silico* and *in-vivo* assessments

for making the hydroalcoholic extract of both plants. The antioxidant effect of the hydro-alcoholic extracts from both plants were assessed using a variety of assay techniques as described by Hebbani et al. [18] and Bhatti et al. [19] with minor modifications. Below is a description of the protocols for these assays.

Hydrogen peroxide scavenging activity

In the experiment, 3.4 ml of plant extracts at concentrations ranging from 0.2 to 1.0 mg/ml were mixed with 0.6 ml of hydrogen peroxide and allowed to incubate at room temperature for 10 min. A butylated hydroxyanisole was used as a reference. The absorbance of the mixture after undergoing oxidation was measured at 230 nm using a spectrophotometer against a blank solution (consisting of phosphate buffer alone). Subsequently, the percentage of inhibition was calculated using the following formula:

$$\text{Percent inhibition} = (\text{OD}_{\text{control}} - \text{OD}_{\text{extract}} / \text{OD}_{\text{control}}) \times 100.$$

Nitrous oxide (NO) radical scavenging activity

The evaluation was carried out using a colorimetric technique. During this process, 800 μL of different extracts (0.2 to 1.0 mg/ml) diluted in phosphate-buffered saline

(25 mM, pH 7.4) were combined with 200 μL of sodium nitroprusside (SNP). After that, each mixture was exposed to visible light for 2.5 h at 37 $^{\circ}\text{C}$. It was then kept for incubation at room temperature for 20 min in the dark. The samples were then mixed together with 300 μL of Griess reagent (1% sulfanilamide and 0.1% naphthyl ethylene diamine hydrochloride in 2% H_3PO_4) and incubated again for 40 min. At 546 nm, color intensity of each sample was measured in relation to a blank solution made up of 0.6 ml Griess reagent and 2 ml H_2O . Ascorbic acid was used as a reference standard for comparison. Combination of 300 μL of Griess reagent, 800 μL of H_2O , and 200 μL of SNP was used as the control sample. Subsequently, the percentage of inhibition was calculated using the following formula:

$$\text{Percent inhibition} = (\text{OD}_{\text{control}} - \text{OD}_{\text{extract}} / \text{OD}_{\text{control}}) \times 100.$$

DPPH free radical scavenging activity

DPPH free radical scavenging activity assay was performed with minor modifications for evaluation of antioxidant potential of plants extracts. 2 ml of each extract (0.2 to 1.0 mg/ml) was combined with 1 ml of DPPH solution (125 μM dissolved in methanol). After vigorous shaking of the mixture, it was kept at 37 $^{\circ}\text{C}$ in the dark for

Table 1 List of Phytoconstituents/ ligands with PubChem ID and manual code

Phytoconstituents							
<i>Plumeria obtusa</i>				<i>Sansevieria cylindrica</i>			
S. no.	Code	Ligand	Pub Chem ID	S. no.	Code	Ligand	Pub Chem ID
1	PC1	Steroid U	133659	1	SC1	Dihydrochalcone	64802
2	PC1a	beta-Amyrin	73145	2	SC2	Pongol methyl ether	636768
3	PC1b	Triterpenoid	451674	3	SC3	Homopterocarpin	101795
4	PC2	Lupeol	259846	4	SC4	1-Phenylpropane-1,3-diol	572059
5	PC2a	Plumieride	72319	5	SC5	p-Coumaroyl tyramine	5372945
6	PC2b	alpha-Amyrin	73170	6	SC6	7,4'-dihydroxy-homoisoflavanone	3759709
7	PC3	Ursolic acid	64945	7	SC7	N-trans-Feruloyloctopamine	24096391
8	PC3a	Triacetin	5541	8	SC8	3,4-Dihydroxyallylbenzene	53446942
				9	SC9	3-Hydroxychavicol 1-glucoside	78384828
				10	SC10	27-p-Coumaroyloxy ursolic acid	5459189

30 min. The absorbance of each mixture was then measured at 517 nm against a blank solution containing 1 ml of DPPH and 2 ml of methanol. Ascorbic acid prepared within the same concentration ranges was utilized as reference standard. The percentage inhibition was then determined using specific formula.

In-silico acetyl cholinesterase inhibition activity

In this study, acetyl cholinesterase (PDB code 1B41) was searched in the Protein Data Bank (PDB) using the RCSB website (<https://www.rcsb.org/>). Eight compounds from the *P. obtusa* plant and ten compounds from the *S. cylindrica* plant, as reported in the literature [20, 21], were selected for molecular docking with acetyl cholinesterase enzyme. The chemical structures of these phytochemical compounds were sketched by Chem Draw Ultra 12.0 software, USA. All structures were optimized and minimized at 7 to 4 PH by Chimera tool. The standard drug, Physostigmine, was obtained from the PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>). The molecular docking study was performed by using AutodockVina Wizard, the PyRx virtual screening software version 0.8. A grid was set in the region with dimension size $x=58.083$; size $y=39.224$; size $z=78.50$ Å which insures that the grid covers entire region offered for interacting ligands. This was carried out in order to ascertain interactions between the protein and ligands as well as the most likely binding configurations. Afterwards, Biovia Discovery Studio Visualizer was used to visualize the resulting interactions. This method helps in predicting non-covalent interactions that lead to the creation of an intermolecular complex between a drug or chemical molecule and the receptor or active site area of a protein. The names of the phytoconstituents, their corresponding

manual codes along with PubChem IDs are listed in Table 1.

In-vivo assessment of neuroprotective activity in Scopolamine induced oxidative stress in Wistar rats

Experimental animals

In-vivo experiments were carried out using healthy mature Wistar rats of both sex (1:1 proportion), aged between 8 and 12 weeks (weight 180–350 g). The rats were procured from Crystal Biological Solutions in Pune, India (Registration No. 2030/PO/RcBiBt/S/18/CPCSEA). The female rats were nulliparous at the time of testing. Before the experiments, the rats were permitted to acclimatize to the laboratory conditions for 7 days. Three rats were housed in each polypropylene cages maintained under standard conditions, including a temperature of 21 °C, relative humidity ranging from 60 to 70%, and a 12-h light/12-h dark cycle. The rats were provided with fresh paddy husk bedding, and throughout the study, they had unrestricted access to a regular rat pellet diet (supplied by M/s Nutrivet Life Sciences, Pune, India) as well as drinking water ad libitum. All procedures were conducted in accordance with the guidelines established by the CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals), India.

Grouping of animals and dosing regime

The investigations involved six rats per group, consisting of three males and three females, and these rats were randomly assigned to 12 groups as indicated in Table 2. Each rat was further careful allotted to ensure average body weight variation of 25% in each group. The carboxy methyl cellulose (CMC) at concentrations of 1% (v/v) and each plant extract was mixed together to form a suspension. The study doses were chosen based on acute and

Table 2 Study design for *in-vivo* assessment of anti-oxidant and anti-AChE activity

Group	Group name	Dose (mg/kg)	No. of rats	ROA
G1	Normal control (e.g. saline)	1.0	6	PO
G2	Scopolamine	3	6	IP + PO
G3	Scopolamine + Plant 1	3 mg + 100 mg	6	IP + PO
G4	Scopolamine + Plant 1	3 mg + 200 mg	6	IP + PO
G5	Scopolamine + Plant 1	3 mg + 400 mg	6	IP + PO
G6	Scopolamine + Plant 2	3 mg + 100 mg	6	IP + PO
G7	Scopolamine + Plant 2	3 mg + 200 mg	6	IP + PO
G8	Scopolamine + Plant 2	3 mg + 400 mg	6	IP + PO
G9	Scopolamine + Plant 1 + Plant 2	3 mg + 50 mg/plant	6	IP + PO
G10	Scopolamine + Plant 1 + Plant 2	3 mg + 100 mg/plant	6	IP + PO
G11	Scopolamine + Plant 1 + Plant 2	3 mg + 200 mg/plant	6	IP + PO
G12	Scopolamine + Rivastigmine	3 mg + 1.5 mg	6	IP + PO

Plant 1: *Sansevieria cylindrica*; Plant 2: *Plumeria obtusa*; ROA: Route of administration;
PO: Per oral, IP: Intra-peritoneal

sub-acute oral toxicity studies of both the plants [17, 22]. Oral suspension of plant extracts containing different doses of 100 mg/kg, 200 mg/kg, and 400 mg/kg body weight were administered to all rats in group 3 to 11. In a similar way, group 2 also known as the negative control group received scopolamine, while group 1 treated as the vehicle control. As the positive control group, group 12 received rivastigmine as a standard drug. Neurobehavioural changes, *in-vivo* antioxidant activity, anti-AChE activity and histopathological changes in amygdala region were evaluated in the study.

Evaluation of neurobehavioral changes

To assess whether both plant extracts could ameliorate cognitive impairment in a rat model induced by scopolamine, several behavioral tests, including the open field test, novel object recognition (NOR) test, and Morris water maze task, were conducted on the 0th, 7th, and 14th days of the experiment.

Procedure for open-field test In this study, we performed an open field test with some modifications to a previously described method [23]. In brief, rats were placed individually in the left rear quadrant of a 72 × 72 × 36 cm open field enclosure. This enclosure featured a metal square box with a formica surface divided into 16 equal squares, each measuring 18 cm × 18 cm. The rats were kept one at a time into the center of the box and allowed to explore for 90 s. To evaluate their locomotor and exploratory activities, the stopwatches and hand-operated counters were used. The number of line crossings (indicative of locomotor activity) and the number of rearing events (instances when the rat stood on its hind legs, indicating exploratory behavior) were recorded. To ensure a consistent testing

environment and to remove any urine and feces, the apparatus was cleaned with a 10% ethanol and a dry cloth after each test.

Procedure for NOR test The Novel Object Recognition (NOR) test is extensively used to estimate the object recognition capacities of rodents. In this study, the NOR test was performed with minor modifications [23]. An open field arena was made of polyvinyl chloride plastic, plywood, and transparent acrylic, measuring 30 × 30 × 30 cm. The test included two phases familiarization and test phase. During the familiarization phase, rats were allowed to freely explore the arena for 5 min, interacting with two identical objects. In the test phase, one of these objects was replaced with a new one that differed in shape and color, and the rats were given another 5 min to explore. To ensure uniformity, the arena was cleaned with a 10% ethanol and dried after each rat session. Exploration was defined as the rat smelling or touching the objects with its nose and/ or forepaws within 2 cm. A video recording camera fixed above the arena recorded the rats' exploratory behavior. Total time required for object recognition was recorded. The rats object recognition capability was measured as the rate of time spent exploring the two identical objects during the familiarization phase or the new object during the test phase, compared to the total exploration time.

Procedure for Morris water maze task To assess memory ability in rats, we conducted the Morris water maze test, with modifications to a previously described method [24, 25]. The maze consisted of a circular pool with a diameter of 150 cm and a height of 0.47 m (VJ Instruments India Pvt. Ltd., Karanja, India). To create the testing environ-

ment, the pool was filled with water to a depth of 30 cm, maintained at a temperature of $28 \pm 1^\circ\text{C}$, and made opaque by adding milk or milk powder. A 10 cm diameter escape platform with a grooved surface to improve grip was placed 1 cm below the water surface. The goal for the rats was to swim and find this hidden platform. The time taken for each rat to locate the hidden platform and stop swimming (referred to as escape latency) was recorded. Each trial began with placing the rat at the pool's edge, facing the wall in one of the four quadrants. The rats underwent three days of initial training, with each day consisting of 90-s training trials.

Assessment of in-vivo anti-oxidant activity

Antioxidant activity was evaluated with a minor modification to the existing process [26]. The rats were divided randomly into different groups including control, 3 mg/kg scopolamine, and 3 mg/kg scopolamine plus 100 mg/kg, 200 mg/kg, or 400 mg/kg doses of both plants' extracts. Oral administration of the hydro-alcoholic extracts of both plants was done concurrently with an intraperitoneal (*i.p.*) injection of scopolamine. For seven days in a row, each medication was given once daily. 24 h. following the last course of treatment, the rats were sacrificed and each brain was removed. It was then washed with ice-cold normal saline and 0.15 M tris-hydrochloride. The brain was sliced into small sections, the brain homogenates were centrifuged for 10 min at $15,000 \times g$.

Lipid peroxidation assay (thiobarbituric acid reactive substances) The assessment of lipid peroxidation was conducted following the procedures outlined by Khatri et al. in 2016 and Shalavadi et al. in 2012 [27, 28]. The reaction mixture consisted of 0.2 ml of brain tissue homogenate, 8.1% SDS, 1.5 ml of a 20% acetic acid solution adjusted to pH 3.5 with NaOH, and 1.5 ml of a 0.8% aqueous solution of TBA. The final volume of the mixture was adjusted to 4.0 ml using distilled water, and it was then heated at 95°C for 60 min in a water bath. After cooling, 1 ml of distilled water and 5 ml of a mixture of n-butanol and pyridine (in a ratio of 15:1, v/v) were added to the reaction mixture, and the solution was vigorously shaken. Following centrifugation at 4000 rpm for 10 min, the absorbance of the organic layer was measured at 532 nm using a UV-spectrophotometer. The level of lipid peroxidation was reported as nanomoles of MDA (malondialdehyde) per milligram of proteins.

Superoxide dismutase (SOD) assay The ability of the SOD to neutralize superoxide radicals produced by the auto-oxidation of pyrogallol in an alkaline environment was used to measure SOD activity. The SOD assay was performed with a few modifications to earlier reported

procedure [29]. 140 μl of potassium phosphate buffer (0.1 M, pH 7.4), 5 μl of tissue homogenate, and 5 μl of pyrogallol solution (2.6 mM in 10 mM HCl) forms the reaction used in this test. The absorbance of each reaction mixture was measured at 325 nm after every 5 min. The quantity of enzyme needed to achieve a 50% inhibition of pyrogallol autoxidation within a 3 ml assay mixture was used to define the activity of SOD.

Catalase (CAT) assay The catalase activity was assessed following a method given in reference with minor adjustments [30]. In summary, the assay mixture was composed of 145 μl of 10 mM H_2O_2 in a 50 mM potassium phosphate buffer (pH 7), and 50 μl of tissue homogenate. A microplate reader was utilized to monitor the rate of absorbance reduction at 240 nm for duration of 3 min. Catalase activity was quantified as CAT activity per milligram of protein.

Reduced glutathione (GSH) assay Dithiobisnitrobenzoate was used as a substrate for the measurement. As soon as the color turned yellow, the absorbance at 412 nm was quickly measured. The GSH level was expressed as micro-moles of GSH per gram of protein [30].

AChE inhibition assay

Assessment of in-vivo anti-AChE activity The standard experimental protocol was adhered to, for determining anti-AChE activity, as outlined in references [23–25, 31]. The procedure for assessing *in-vivo* anti-AChE activity was similar to the antioxidant experimental procedure reported previously. However, the daily dosing was extended till 14 days. The animals were sacrificed on the last day of the experiment. After that, brain was removed, it was treated with 0.15 M tris-hydrochloride followed by cold normal saline. The midbrain area was immediately segregated. Equal volumes (1 ml) of ice-cold 0.1 M phosphate buffer saline (pH 7.4) were used to homogenize the brain tissues. Homogenization was repeated if any intact tissue was still visible. After centrifuging the brain homogenates for 10 min at 15,000 rpm using the centrifugation (R-248 M of CPR-24 plus Instrument, Remi, Mumbai, India), the supernatants were used for the AChE assay.

Procedure for AChE inhibition assay AChE inhibition assay was conducted using previously reported method [31]. The supernatants collected as per above given procedure was used for assay. The assay mixture included 13.1 μl supernatant, 145 μl of sodium phosphate buffer (pH 8), 5 μl of 1 mM acetylthiocholine iodide, and 5 μl of 10 mM DTNB. Using a microplate reader, the change in absorbance was detected at 412 nm. The results were

Table 3 Qualitative phytochemical analysis of plants

S. no.	Parameters	Method	Positive (+) / Negative (-)	
			<i>Sansevieria cylindrica</i>	<i>Plumeria obtusa</i>
1	Carbohydrates	Molish test	+	+
		Fehling solution test	—	—
		Benedict's	+	+
2	Amino acids	Ninhydrin test	—	—
3	Proteins	Biuret	—	—
4	Flavonoids	Shinoda test	+	+
		Zn. Hydrochloride test	—	—
		Lead acetate test	+	—
5	Alkaloids	Dragendroff's test	+	+
		Mayer's test	+	+
		Hager test	—	+
		Wagner's test	+	+
6	Glycosides	Borntrager's test	—	—
		Keller Killani test	+	+
7	Volatile oil	Stain test	—	—
8	Fixed oils and fats	Spot test	—	—
9	Steroids	LibermannBuchard test	+	+
		Salkowski test	—	—
10	Saponins	Foaming test	+	—
11	Tannins and phenols	FeCl ₃ test	—	+
		Potassium dichromate test	+	+

reported in terms of micromoles of acetylthiocholine iodide hydrolyzed per milligram of protein per minute. The AChE activity was determined using the given formula:

$$\text{Moles/L/min} = \text{Absorbance(per min rate)} / 1.36 \times 10^4$$

Histopathological assessment of brain The brains preserved from all experimental groups underwent standard processing and were embedded in paraffin. Sections with a thickness of 3–5 µm were prepared and stained using hematoxylin–eosin stain. A certified toxicopathologist conducted histopathological examinations of vital organs.

Statistical analysis

The mean and standard deviation were reported against observed results. A one-way analysis of variance (ANOVA) was used to estimate differences between the experimental groups, and post hoc multiple comparisons tests were performed. p-value is less than 0.05 considered as statistically significant.

Results

Authentication of plant and its pharmacognostic evaluation

The Botanical Survey of India (BSI), with certificate number BSI/WRC100-1/TECH/2019/62, dated December 19, 2019, confirmed the authenticity of both plants. According to BSI, the plant species that were submitted are *S. cylindrica* Bojer ex Hook. and *P. obtusa* L., members of the Apocynaceae and Asparagaceae families, respectively. Alkaloids, glycosides, steroids, flavonoids, and tannins were detected during the primary screening of phytochemicals, as shown in Table 3. Furthermore, the plants and their extracts were assessed for a number of pharmacognostic tests. The findings of these assessments have been reported in our earlier publications [32, 33].

In-vitro anti-oxidant assays

We conducted an assessment of the H₂O₂ radical scavenging activity for *S. cylindrica*, *P. obtusa*, and their combination, comparing them to a reference standard. At a concentration of 1 mg/ml, the combination displayed a notable free radical scavenging effect for H₂O₂, measuring 80.56%. However, BHA, the reference standard exhibited a slightly higher free radical scavenging effect at 86.90%. Importantly, the comparison between the combination

and BHA did not show a significant difference ($p > 0.05$). In NO scavenging activity, the combination of both plants demonstrated progressive reductions in NO levels at concentrations of 0.2, 0.4, 0.6, 0.8, and 1.0 mg/ml. The most substantial decrease in NO was observed at 1.0 mg/ml, which was nearly on par with the performance of the ascorbic acid. However, this comparison also showed no significant difference ($p > 0.05$). Furthermore, in terms of scavenging the DPPH radical, the order of effectiveness decreased as follows: *P. obtusa* > Ascorbic acid > Combination (1:1) of plant extracts > *S. cylindrica*. The detailed findings of the *in-vitro* antioxidant effects of both plants can be accessed in our previously reported studies [34].

In-silico binding interaction with Acetyl cholinesterase

Using acetyl cholinesterase with a particular binding cavity, the phytochemical components from *P. obtusa* and *S. cylindrica* were submitted to molecular docking study. The ligand conformations were ranked based on their predicted binding energies using a scoring function. Among the 18 molecules examined, certain molecules exhibited the most favorable docking poses and binding energies. Specifically, the SC10 compound demonstrated promising interactions, prompting further investigation into its molecular interactions.

Notably, we have previously published docking studies involving phytochemical constituents and drug standards, utilizing the 1B41 protein structure from the Protein Data Bank, along with various other enzymes [35]. Our analysis of the 2D pose of 1B41 with physostigmine and SC10 revealed a network of molecular interactions (Fig. 2), including hydrogen bonds, Van der Waals forces, alkyl interactions, π -alkyl interactions, and π -sigma bonds with the active-site residues of 1B41. The results of the binding energy for protein–ligand interactions have been tabulated in Table 4.

Assessment of neurobehavioral parameters

Rearing and line crossing

In the open field test, we tracked the number of line crossings and rearing behavior over 90 s on day 0, day 7, and day 14 of the experimental period. As shown in Fig. 3, nearly all experimental groups of rats displayed significant changes in the number of line crossings or rearing actions after treatment with plant extracts or the standard, compared to the scopolamine-treated group. Regarding rearing on day 14, all plants extracts groups demonstrated a significant difference ($p < 0.05$) compared to the disease control group, except for the 100 mg/kg of *P. obtusa* group. The 1:1 combination of *S. cylindrica* and *P. obtusa* also showed a reduction in rearing pattern. But, at 100 mg/kg, this difference was statistically significant ($p < 0.05$) compared to the

control group (G12). At 200 and 400 mg/kg doses, the combination displayed a reduction in rearing behavior as like rivastigmine, although this difference wasn't statistically significant ($p > 0.05$). Also, observations were reported for line crossing behaviors. The combined ameliorative effect of *S. cylindrica* and *P. obtusa* at 100 mg/kg and 200 mg/kg doses showed a lower impact compared to the standard drug. Additionally, at a 400 mg/kg combination of both plants, the line crossing showed reduction as like standard, although this difference wasn't statistically significant ($p > 0.05$).

Novel object recognition test

On day 0 of the NOR test, rats were introduced to the experimental arena without any specific stimuli. Following this initial familiarization, the familiarization phase commenced, during which the time spent exploring two identical objects was recorded. As shown in Fig. 4, during this phase, all groups exhibited a similar recognition index for the identical objects. The next day, the test phase was conducted. In this phase, the recognition index notably dropped to 45.7% in group 2, indicating a scopolamine-induced decline. However, this decrease in the recognition index was prevented in all groups treated with plant extracts, in a dose-dependent manner. The combination of plant extracts resulted in recognition indices of 58.3%, 61.7%, and 64.3% at doses of 100, 200, and 400 mg/kg, respectively. Although none of the treated groups showed a significant difference from the scopolamine group regarding the recognition index during the test phase, group 12, which received the standard drug rivastigmine, displayed a significant increase ($p < 0.05$) in the recognition index compared to group 2 treated with scopolamine.

Escape latency time test

In order to determine whether the hydro-alcoholic extracts of both plants could decrease the effects of scopolamine on spatial cognition, a Morris water maze (MWM) test was performed. On days 0, 7, and 14, the escape latency time in the MWM was measured. On day 0, the rat's performance across all groups was initially statistically similar. On day 7 and day 14, however, there were notable variations in how much time each group rat spent in the target quadrant during the probe trials. Rats were administered with scopolamine on day 14 showed a significant increase in the amount of escape latency time they spent in the target quadrant. On the other hand, a statistically significant reduction in escape latency time ($p < 0.05$) was seen upon treatment with 200 and 400 mg/kg dose of *S. cylindrica* as shown in Fig. 5.

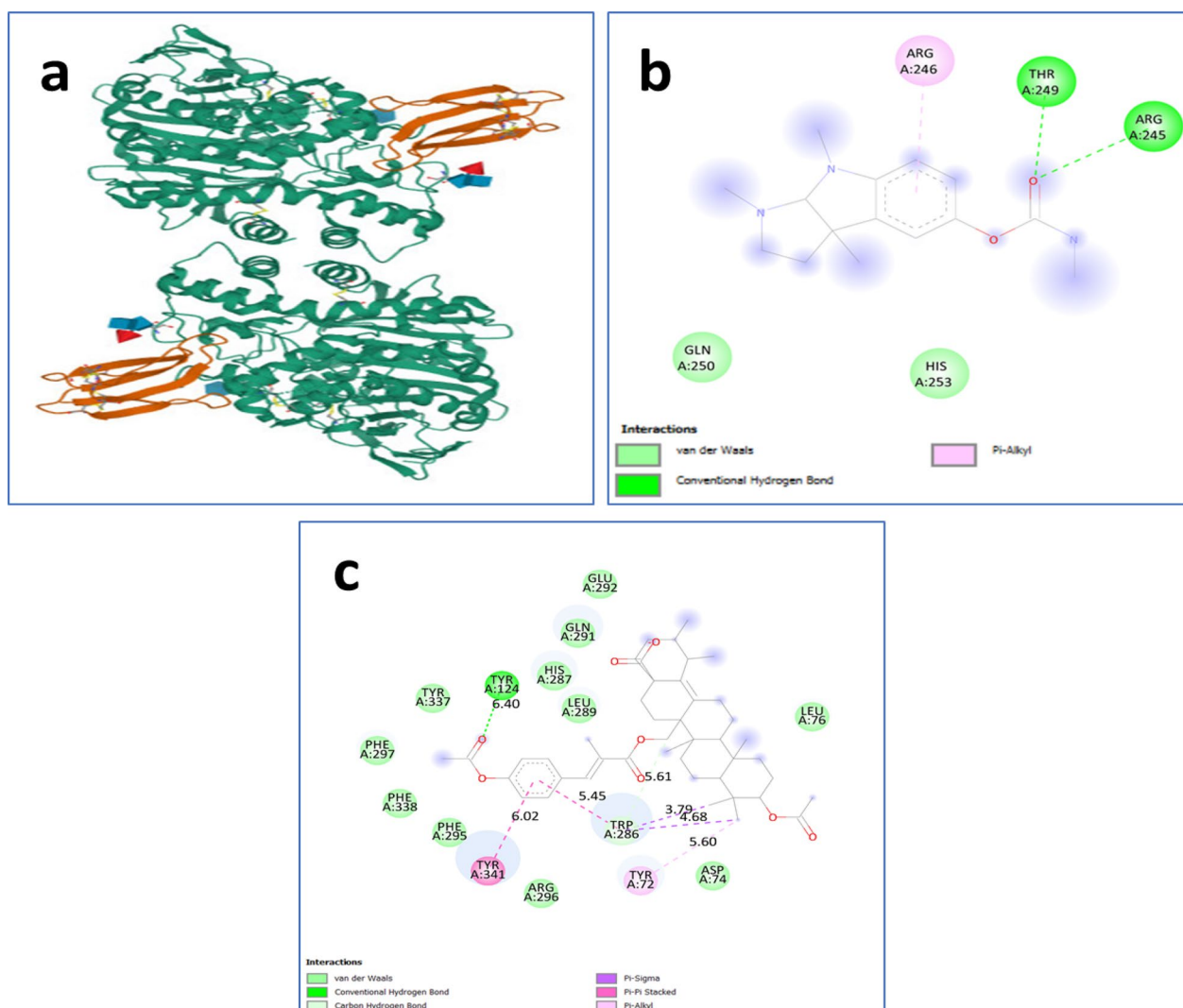


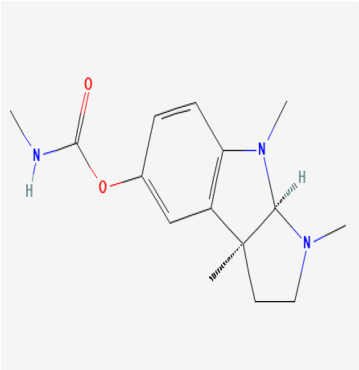
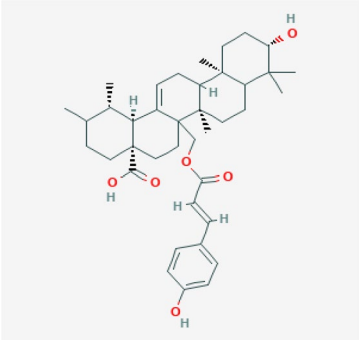
Fig. 2 **a** Cartoon ribbon model of AChE **b** 2D interactions of physostigmine with amino acid residue of AChE **c** 2D interactions of SC10 with amino acid residue of AChE

Evaluation of brain tissue markers of oxidative stress

In this assay, the plant extracts exhibited a dose-dependent inhibition of lipid peroxidation compared to the scopolamine-treated group, as shown in Fig. 6A. Specially, the combination of both plant extracts at a concentration of 100 mg/kg significantly decreased lipid peroxidation ($p < 0.001$). Additionally, in the group treated with the combination of extracts at 200 mg/kg and 400 mg/kg, the levels of lipid peroxidation in brain tissues were markedly reduced, with values of 0.283 ± 0.019 and 0.243 ± 0.052 nmol of MDA per milligram of protein, respectively. This contrasts with the disease control group, which had a higher value of 0.429 ± 0.044 nmol of MDA per milligram of protein. Moreover, the combination of plant extracts showed a significant reduction in lipid peroxidation compared to the rivastigmine,

demonstrating its effectiveness to inhibit ROS. Additionally, there was a significant reduction in SOD activity in the hippocampus of scopolamine-treated rats compared to the normal control group ($p < 0.0001$), as illustrated in Fig. 6B. However, administering various doses of plant extracts reversed this reduction in SOD activity. All groups treated with plant extracts showed a significant increase in SOD levels compared to the scopolamine-treated group ($p < 0.05$, $p < 0.001$, $p < 0.0001$), except for the *P. obtusa* extract at a dose of 100 mg/kg, which exhibited a non-significant increase in SOD levels. Notably, the 1:1 blend of both plant extracts significantly increased SOD levels ($p < 0.001$) compared to the standard drug rivastigmine. The body has developed a sophisticated defense mechanism to minimize the harmful effects of

Table 4 Standard and ligand interactions with amino acid residues of 1B41

S. no.	Ligand	Protein	Binding energy	Interaction	Distance of interaction (Å ^o)
1		1B41	−6.5	H bonding	
				ARG 245	(2.32)
				THR 249	(1.98)
				pi-alkyl	
2		1B41	−8.9	ARG 246	(3.77)
				H bonding	
				TYR 124	(2.36)
				pi-pi stack	
				HIS 57	(4.85 and 4.65)
				pi-sigma interaction	
				TRP 286	(3.79) (4.68)

various oxidants, with a key role played by both non-enzymatic and enzymatic antioxidants. Notable antioxidants include GSH and CAT, which work together to safeguard the organism against oxidative damage [36]. The results obtained in our study echoed those seen with SOD. CAT and GSH levels were observed to decrease significantly in the scopolamine-treated group compared to the normal control group ($p<0.0001$), as depicted in Fig. 6C and D. Most of the treatment groups involving plant extracts exhibited a significant increase in CAT levels, with the combination of plant extracts showing the highest activity, demonstrating a highly significant difference compared to the disease control

group (G2) ($p<0.0001$). Rivastigmine also exhibited an increase in CAT levels, with no significant difference observed between the combination and the standard drug. However, the group receiving a 100 mg/kg dose of *P. obtusa* (group 6) did not show significant changes in brain CAT levels. In this group, the average CAT value was 6.13 ± 0.251 , compared to the value of 4.90 ± 1.058 U of CAT/min/mg of protein in group 2. In terms of GSH levels, *S. cylindrica* extract and the combination of both plants extracts significantly increased GSH levels ($p<0.0001$) compared to the disease control group. The standard drug and the combination of both plant extracts exhibited similar increased in GSH levels without any

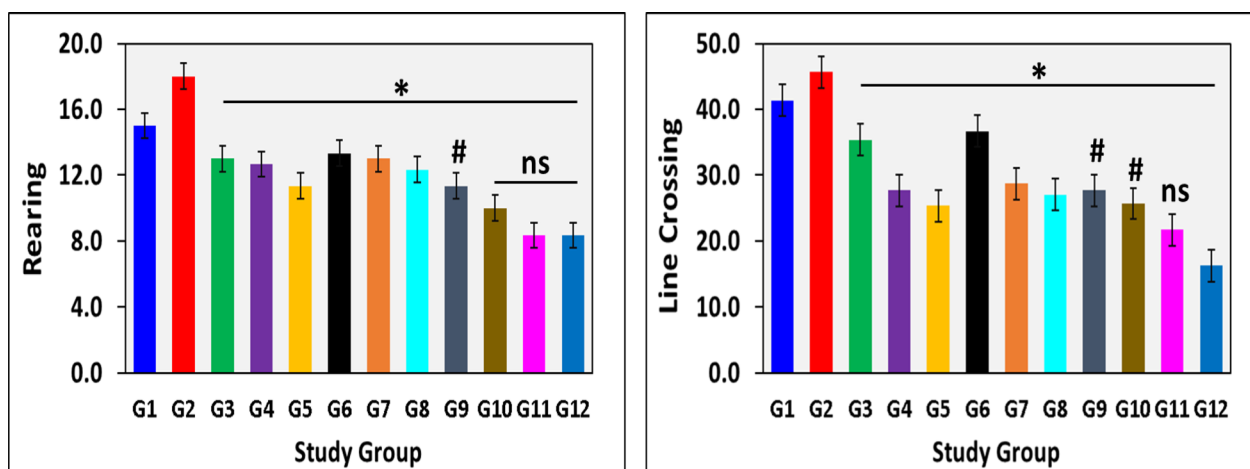


Fig. 3 Locomotor activity of rearing and line crossing on Day 14. SC: *S. cylindrica*. PO: *P. obtusa*. G1: Normal control group. G2: Disease control Scopolamine group (3 mg/kg). G3: Scopolamine + SC (100 mg/kg). G4: Scopolamine + SC (200 mg/kg). G5: Scopolamine + SC (400 mg/kg). G6: Scopolamine + PO (100 mg/kg). G7: Scopolamine + PO (200 mg/kg). G8: Scopolamine + PO (400 mg/kg). G9: Scopolamine + SC + PO (50 mg/kg/plant). G10: Scopolamine + SC + PO (100 mg/kg/plant). G11: Scopolamine + SC + PO (200 mg/kg/plant). G12: Scopolamine + Rivastigmine (3 + 1.5 mg/kg). Results are shown as mean ± SD (n = 3). * $p < 0.05$ versus G2. # $p < 0.05$ versus G12. ns: non-significant vs G12

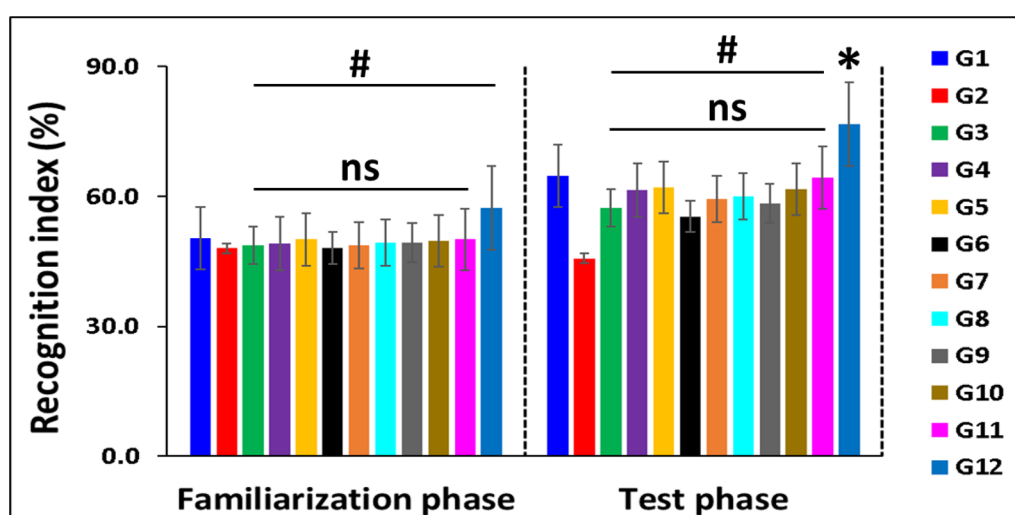


Fig. 4 Prevention of scopolamine-induced recognition dysfunction through NOR. SC: *S. cylindrica*. PO: *P. obtusa*. G1: Normal control group. G2: Disease control Scopolamine group (3 mg/kg). G3: Scopolamine + SC (100 mg/kg). G4: Scopolamine + SC (200 mg/kg). G5: Scopolamine + SC (400 mg/kg). G6: Scopolamine + PO (100 mg/kg). G7: Scopolamine + PO (200 mg/kg). G8: Scopolamine + PO (400 mg/kg). G9: Scopolamine + SC + PO (50 mg/kg/plant). G10: Scopolamine + SC + PO (100 mg/kg/plant). G11: Scopolamine + SC + PO (200 mg/kg/plant). G12: Scopolamine + Rivastigmine (1.5 mg/kg). Results are shown as mean ± SD (n = 3). * $p < 0.05$ versus G2. # $p > 0.05$ (non-significant) versus G2. ns: non-significant versus G12

statistically significant difference. Significant differences in measured GSH levels were also observed at various doses of *P. obtusa* extract, although the activity was lower compared to *S. cylindrica* extract. These results clearly affirm the potential of *S. cylindrica* and *P. obtusa* as promising sources for inhibiting oxidative stress.

AChE inhibitory activity of plants extract

Anti-AChE activity assay

AChE inhibitors are known to counter scopolamine-induced learning and memory impairments [37]. This hypothesis might indicate the usage of various plant extracts against AChE activity. To investigate this, we conducted an AChE activity assay in our current study. Rats were decapitated using a sharp blade to prevent

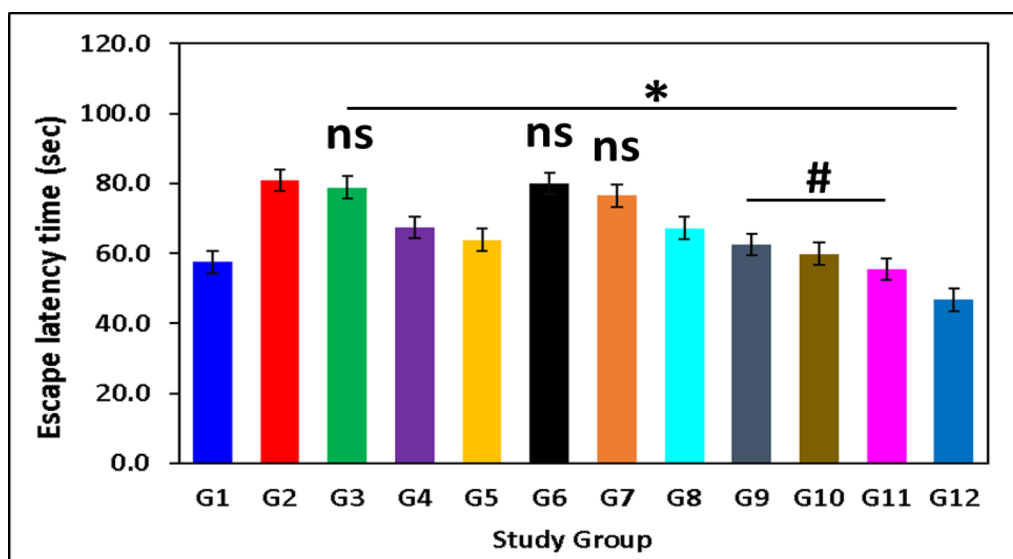


Fig. 5 Prevention of scopolamine-induced recognition dysfunction escape latency by Morris Water Maze test on Day 14. SC: *S. cylindrica*. PO: *P. obtusa*. G1: Normal control group. G2: Disease control Scopolamine group (3 mg/kg). G3: Scopolamine + SC (100 mg/kg). G4: Scopolamine + SC (200 mg/kg). G5: Scopolamine + SC (400 mg/kg). G6: Scopolamine + PO (100 mg/kg). G7: Scopolamine + PO (200 mg/kg). G8: Scopolamine + PO (400 mg/kg). G9: Scopolamine + SC + PO (50 mg/kg/plant). G10: Scopolamine + SC + PO (100 mg/kg/plant). G11: Scopolamine + SC + PO (200 mg/kg/plant). G12: Scopolamine + Rivastigmine (3 + 1.5 mg/kg). Results are shown as mean $\pm$ SD ($n = 3$). * $p < 0.05$ versus G2. # $p < 0.05$ versus G12. ns: non-significant vs G2

contamination of brain tissues and fluids with chemicals such as gases and anesthetics [38]. Our observations showed that different concentrations of both plant extracts resulted in dose-dependent inhibition of AChE activity. Notably, the 1:1 combination of *S. cylindrica* and *P. obtusa*, at a dose of 400 mg/kg, reduced AChE activity to approximately 34.62% and 31.10% in male and female rats, respectively compared to disease control group (Table 5) and it was significant ($p < 0.05$). While most individual plant extract groups also showed reduction in AChE levels, these reductions were found to be non-significant compared to group G2. Furthermore, the combination of both plant extracts decreased AChE levels in both male and female rats, though these reductions were mostly non-significant when compared to the standard control group treated with rivastigmine.

Histopathological observations

Microscopic examination of the brain tissue from the vehicle group revealed a normal cerebral parenchyma with intact neuronal cells in the hippocampus region. In contrast, the negative control group treated with scopolamine exhibited signs of neuronal degeneration and vacuolation in the hippocampus area of the brain. Similar changes were observed in the brains of rats treated with *S. cylindrica* extract and *P. obtusa* extract. However, the severity and incidence rate of these changes were relatively low and exhibited a dose-dependent pattern,

ranging from moderate to minimal, when compared to the negative control group. Remarkably, the combination of both plant extracts provided the highest level of protection to neuronal cells and cerebral parenchyma, suggesting a beneficial effect of the hydro-alcoholic extract (Fig. 7).

Discussion

Various cholinergic drugs have been shown to reduce memory impairment, whereas anticholinergic and anesthetic drugs are associated with adverse effects [39]. Scopolamine, classified as an anticholinergic drug, inhibits acetylcholine action in the nervous system, which can lead to increased oxidative stress in the brain [40]. Oxidative stress triggers the overproduction of reactive oxygen species (ROS) and oxygen-containing radicals, resulting in the peroxidation of molecules like proteins, DNA, and lipids, thereby causing memory impairment. Malondialdehyde (MDA) is a by-product of lipid peroxidation and serves as a biomarker for assessing lipid peroxidation. Superoxide dismutase (SOD) acts as a crucial endogenous antioxidant enzyme, combating ROS by converting superoxide radicals into hydrogen peroxide (H_2O_2) and protecting against radical-induced damage [41, 42]. Additionally, glutathione (GSH) and catalase (CAT) function as antioxidant enzymes by scavenging hydroxyl radicals [7].

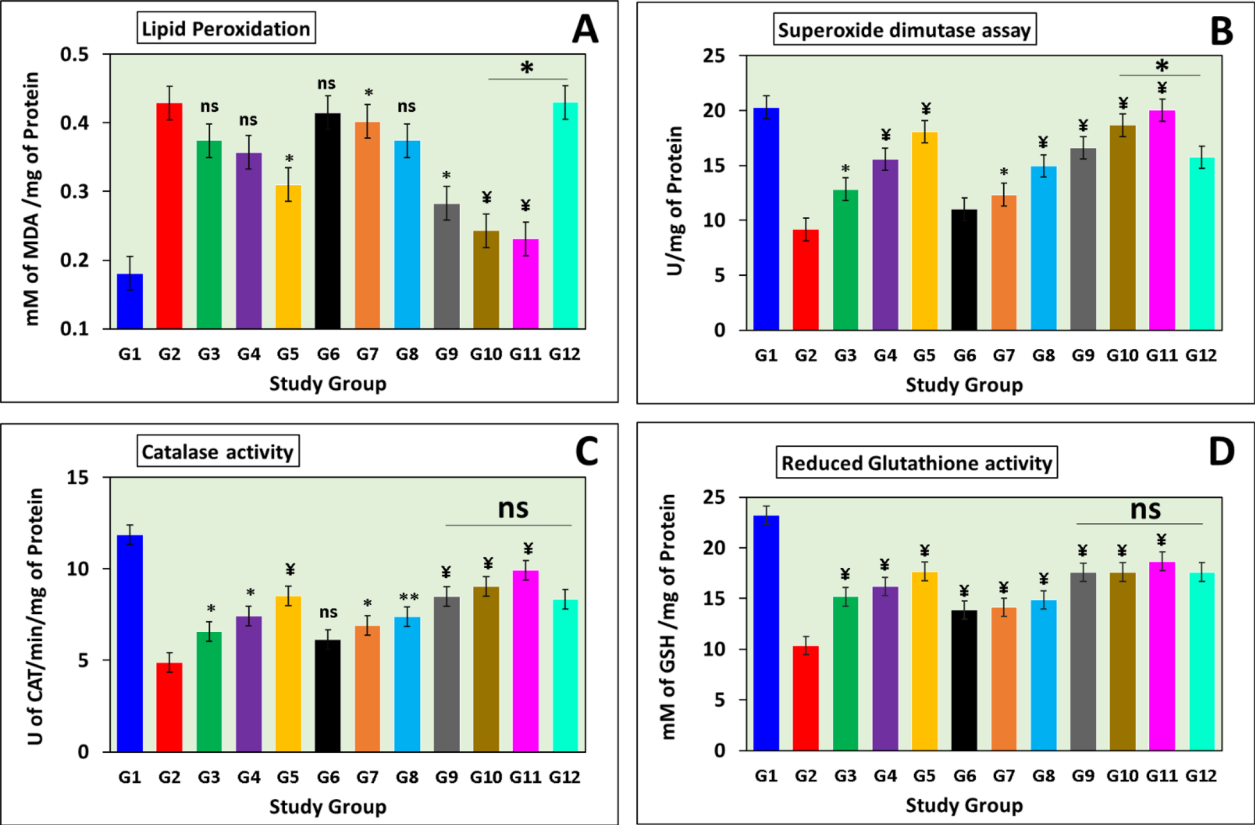


Fig. 6 Assessment of antioxidant parameters from brain tissue of rats from treated groups. SC: *S. cylindrica*. PO: *P. obtusa*. G1: Normal control group. G2: Disease control Scopolamine group (3 mg/kg). G3: Scopolamine + SC (100 mg/kg). G4: Scopolamine + SC (200 mg/kg). G5: Scopolamine + SC (400 mg/kg). G6: Scopolamine + PO (100 mg/kg). G7: Scopolamine + PO (200 mg/kg). G8: Scopolamine + PO (400 mg/kg). G9: Scopolamine + SC + PO (50 mg/kg/plant). G10: Scopolamine + SC + PO (100 mg/kg/plant). G11: Scopolamine + SC + PO (200 mg/kg/plant). G12: Scopolamine + Rivastigmine (3 + 1.5 mg/kg). Results are shown as mean $\pm$ SD (n = 3). * $p < 0.05$ versus disease control group G2 and G12. ** $p < 0.001$ versus G2. $\# p < 0.0001$ versus G2. ns: non-significant versus G2 and G12

Table 5 AChE concentration in rat brain of each group (Mean $\pm$ SD)

Group	Group name	AChE concentration (U/ml/mg of protein)	
		Male	Female
G1	Normal Control (e.g. saline)	10.25 $\pm$ 4.76	20.54 $\pm$ 4.95
G2	Scopolamine	28.42 $\pm$ 3.18	35.27 $\pm$ 3.35
G3	Scopolamine + Plant 1 (100 mg/kg)	26.57 $\pm$ 3.21 (ns)	29.92 $\pm$ 7.28 (ns)
G4	Scopolamine + Plant 1 (200 mg/kg)	21.99 $\pm$ 3.29 (ns)	21.85 $\pm$ 2.21*
G5	Scopolamine + Plant 1 (400 mg/kg)	11.15 $\pm$ 4.02*	17.05 $\pm$ 6.29*
G6	Scopolamine + Plant 2 (100 mg/kg)	35.50 $\pm$ 3.76 (ns)	34.05 $\pm$ 4.84 (ns)
G7	Scopolamine + Plant 2 (200 mg/kg)	30.96 $\pm$ 3.94 (ns)	32.23 $\pm$ 2.77 (ns)
G8	Scopolamine + Plant 2 (400 mg/kg)	14.28 $\pm$ 3.83*	30.15 $\pm$ 5.91 (ns)
G9	Scopolamine + Plant 1 + Plant 2 (50 mg/kg/plant)	26.43 $\pm$ 3.84 (ns) #	25.61 $\pm$ 6.61 (ns) #
G10	Scopolamine + Plant 1 + Plant 2 (100 mg/kg/plant)	21.31 $\pm$ 3.15 (ns)	16.14 $\pm$ 2.90*
G11	Scopolamine + Plant 1 + Plant 2 (200 mg/kg/plant)	9.84 $\pm$ 12.02*	10.97 $\pm$ 5.09*
G12	Scopolamine + Rivastigmine	14.23 $\pm$ 0.34*	13.55 $\pm$ 1.34*

Plant 1: *Sansevieria cylindrica*; Plant 2: *Plumeria obtusa*. Results are shown as mean $\pm$ SD (n = 3). * $p < 0.05$ vs disease control group G2; # $p < 0.05$ vs standard control group G12; ns: non-significant vs disease control group G2

Secondary metabolites from medicinal plants act as antioxidants through various mechanisms, including direct scavenging of free radicals, interaction with transition metals, inhibition of ROS-producing enzymes, and enhancement of endogenous cellular antioxidant systems, such as increasing SOD activity [43]. In the present study, we had induced oxidative stress in rats using scopolamine and evaluated the antioxidant effect of hydro-alcoholic extracts from *P. obtusa* and *S. cylindrica* by measuring levels of MDA, SOD, reduced GSH, and CAT in brain homogenate. The results demonstrated that these extracts restored the antioxidant status, as indicated by elevated levels of SOD, CAT, and GSH, and decrease level of MDA. These findings strongly support the antioxidant properties of these extracts.

The observed antioxidant effects in our study can be attributed to the presence of various phytochemicals in the leaves and seed pods of the experimental plants, as these compounds are known to possess antioxidant activity. Among these phytochemicals, flavonoids have frequently been associated with the antioxidant potential of plant extracts [44, 45]. Additionally, there is a well-established correlation between total phenolic content and antioxidant activity in various plant-based products and ethnopharmacological treatments [46]. Therefore, the antioxidative effect of the hydro-alcoholic extracts of both plants may be attributed to their phenolic and flavonoid components.

Furthermore, our *in-vivo* antioxidant findings are strongly supported by multiple *in-vitro* antioxidant assays conducted using extracts from these plants [35]. Although there is no direct evidence supporting the *in-vivo* antioxidant activity of the two plants studied, existing literature suggests that various plant extracts exhibit strong antioxidant effects in similar animal models. For instance, the ethanolic extract of *M. esculenta* Crantz in Wistar rats demonstrated potent antioxidant effects compared to the standard drug silymarin [7]. Another study reported a reduction in oxidative stress with the aqueous extract from *M. tomentosa* (Benth.) K. Schum. stem bark in a rat model of scopolamine [47]. Alcesefolside, a phytoconstituent isolated from *A. monspessulanus* L., normalized the activity of antioxidant enzymes against carbon tetrachloride-induced brain injury in Wistar rats [48]. Additionally, *Binahong* leaves extract exhibited a

protective effect against chronic kidney injury developed by the unilateral ureteral obstruction method in rats, indicating a potential free radical-scavenging effect [49]. These findings, among others, further support the outcomes of our study.

The *in-vivo* antioxidant activity was also correlated with the inhibition of acetylcholinesterase (AChE) through similar mechanisms. Disruption in the cholinergic system, marked by decreased acetylcholine levels in the synaptic clefts, has a significant impact on cognitive functions, particularly memory recall and learning abilities [50]. Our study aimed to explore the protective effects of both plant extracts against scopolamine-induced behavioral disruptions and conducted a biochemical assay to assess AChE inhibition using brain samples obtained from rats.

The results of our study demonstrated that scopolamine administration led to reduced memory performance in the open-field test, water maze task, and NOR test. Notably, the beneficial effects of *S. cylindrica* and *P. obtusa* on the cognitive performance of scopolamine-treated rats appeared to be comparable to rivastigmine. Additionally, we examined brain tissue sections using histopathological microscopy and found that the extracts had a neuroprotective effect on brain tissue. This is consistent with previous findings demonstrating that many plant extracts or isolated compounds can reverse scopolamine-induced memory impairments. For example, an aqueous extract of *C. edulis* (Forssk.) Valh (Apocynaceae) enhanced memory in mice by reducing AChE activity [51]. *V. herbaceous* extract, rich in indole alkaloids and flavonoids, improved behavioral disturbances and biochemical parameters in Wistar rats [52]. Oral administration of *C. vulgare* increased the discrimination index (DI) by 44.04% compared to the scopolamine group in a rat model, with statistically significant results ($p < 0.05$) [53]. Suffruticosol A, a major compound found in seeds of *P. lactiflora*, increased cell viability at 20 μ M and reversed scopolamine-induced neurodegenerative characteristics in the SH-SY5 cell line. This effect was further confirmed by administering suffruticosol A into the third ventricle beside the brain of C57BL/6 mice for one month, leading to the rescue of cholinergic deficits and BDNF signaling in the hippocampus [54]. Other similar studies also support these findings [55, 56]. Additionally, our current

(See figure on next page.)

Fig. 7 Histopathology effects of different doses of plants extract on rat brain. SC: *S. cylindrica*. PO: *P. obtusa*. G1: Normal control group. G2: Scopolamine group (3 mg/kg). G3: Scopolamine + SC (100 mg/kg). G4: Scopolamine + SC (200 mg/kg). G5: Scopolamine + SC (400 mg/kg). G6: Scopolamine + PO (100 mg/kg). G7: Scopolamine + PO (200 mg/kg). G8: Scopolamine + PO (400 mg/kg). G9: Scopolamine + SC + PO (50 mg/kg/plant). G10: Scopolamine + SC + PO (100 mg/kg/plant). G11: Scopolamine + SC + PO (200 mg/kg/plant). G12: Scopolamine + Rivastigmine (3 + 1.5 mg/kg)

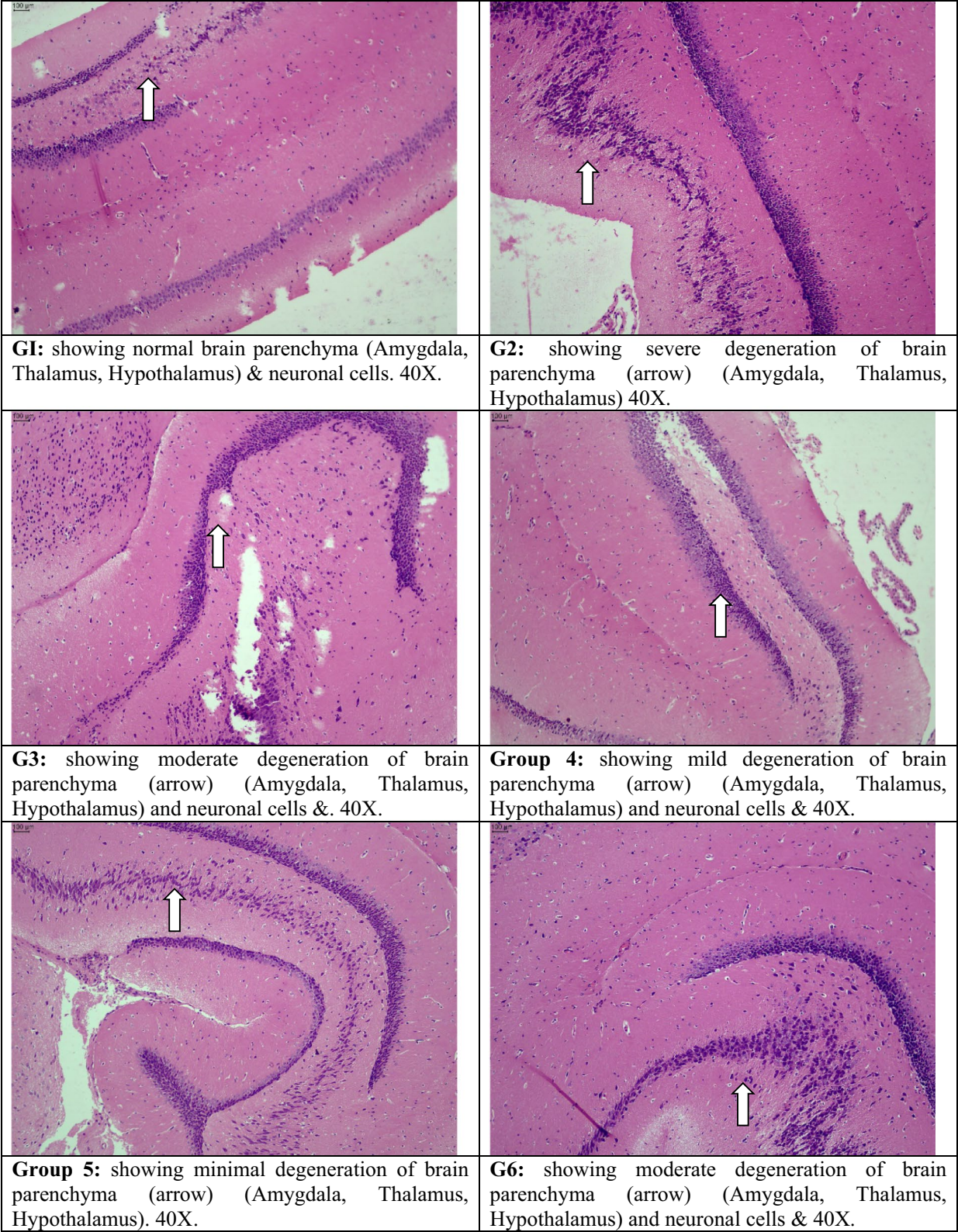


Fig. 7 (See legend on previous page.)

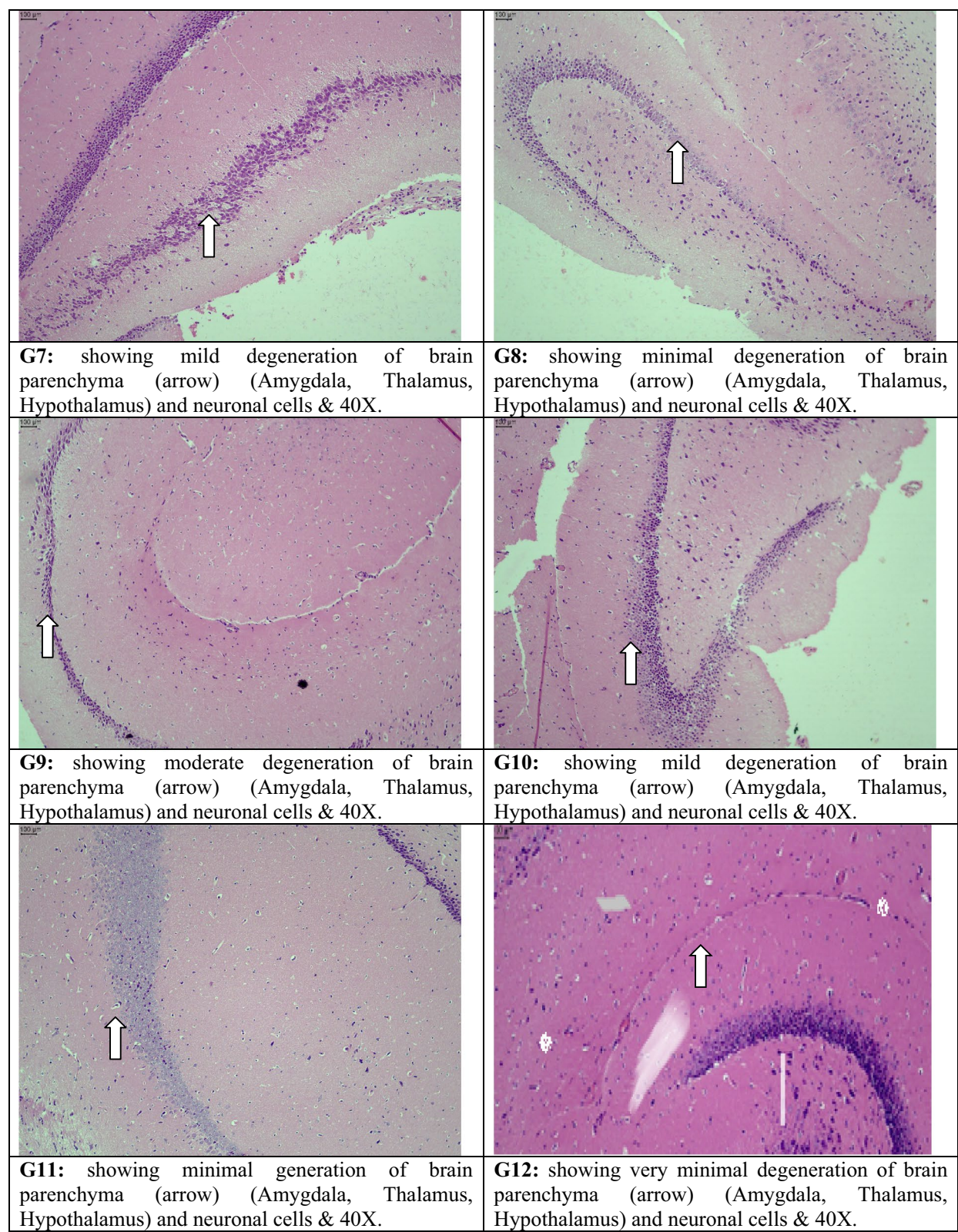


Fig. 7 continued

investigation aligns with *in-silico* studies on AChE inhibition activity of phytoconstituents from both plants, which showed improved binding affinity and inhibitory potential against acetyl cholinesterase [34].

Conclusion

A flourishing field of research is looking for novel compounds to treat diseases, and one such area is the search for new phytochemicals. According to the results of this investigation, extracts from *P. obtusa* and *S. cylindrica* may be able to considerably lower AChE activity and reduced oxidative damage in both *in-vitro* and *in-vivo* environments. The medicinal applications of these plant extracts may provide a pragmatic means of postponing the onset of illnesses in patients and may be a feasible and uncomplicated means of improving memory and learning. Interestingly, neither of the plants extract cholinergic effects have been thoroughly studied before; as a result, it is advised to look into their cholinergic characteristics in clinical contexts.

Abbreviations

AChE	Acetylcholinesterase
AD	Alzheimer's disease
<i>A. monspessulanus</i>	<i>Astragalus monspessulanus</i>
ANOVA	Analysis of variance
BSI	Botanical Survey of India
b.w.	Body weight
<i>C. edulis</i>	<i>Carissa edulis</i>
cm	Centimeter
CPCSEA	Committee for the Purpose of Control and Supervision of Experiments on Animals
<i>C. vulgare</i>	<i>Clinopodium vulgare</i>
DI	Discrimination index
g	Gram
IAEC	Institutional Animal Ethics Committee
ID	Identification
IP	Intra-peritoneal
kg	Kilogram
M	Molar
<i>M. esculenta</i>	<i>Manihot esculenta</i>
mg	Milligram
min	Minutes
μL	Micro liter
ml	Milliliter
mM	Millimole
<i>M. tomentosa</i>	<i>Markhamia tomentosa</i>
MWM	Morris water maze
NOR	Novel object recognition
nm	Nanometer
OD	Optical density
PDB	Protein Data Bank
<i>P. obtusa</i>	<i>Plumeria obtusa</i>
<i>P. lactiflora</i>	<i>Paeonia lactiflora</i>
PO	Per oral
ROA	Route of administration
ROS	Reactive oxygen species
<i>S. cylindrica</i>	<i>Sansevieria cylindrica</i>
<i>V. herbaceous</i>	<i>Vinca herbaceous</i>
v/v	Volume/volume

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Author contributions

Sunil Shewale conceptualized, and designed the study, and prepared the manuscript. Vaishali Undale has done the supervision of experimental work, critical reviewing and editing of manuscript. Mohini Kuchekar, and Bhagyashri Warude performed reviewing and editing of the manuscript. Sunil Shewale and Vrushali Bhalchim conducted the experiment, collected and analyzed the data. Mayur Mali has collated the data and helped in manuscript writing.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Declarations

Ethics approval and consent to participate

The Institutional Animal Ethics Committee (IAEC) of Dr. D. Y. Patil Unitech Society's Dr. D. Y. Patil Institute of Pharmaceutical Sciences and Research, Pimpri, Pune-411018, India (Registration no.: 198/PO/Re/S/2000/CPCSEA) reviewed and approved an experimental design of the animal study with reference no. DYPIPSR/IAEC/20-21/P-23.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no potential competing interests.

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