International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 16s,2025
https://theaspd.com/index.php

Evaluation Of Neuroprotective Potential Of *Bacopa Monnieri* In Scopolamine-Induced Memory Deficit In Rats

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Abstract
Alzheimer's disease (AD) is a progressive neurodegenerative condition marked by cognitive decline, cholinergic dysfunction, and oxidative stress. Current pharmacological treatments offer only symptomatic relief and often cause adverse effects. In this context, herbal agents with multitarget actions are gaining attention. Bacopa monnieri, a traditional Ayurvedic herb, is known for its antioxidant and cognitive-enhancing properties. The present study was designed to evaluate the neuroprotective potential of Bacopa monnieri in a scopolamine-induced memory deficit model in rats. Male Wistar rats were divided into five groups: normal control, scopolamine control, Bacopa monnieri low dose (100 mg/kg), high dose (200 mg/kg), and standard drug Donepezil (5 mg/kg). Scopolamine (1 mg/kg, i.p.) was used to induce cognitive impairment. Behavioral tests including Morris Water Maze, Y-Maze, and Novel Object Recognition were conducted. Biochemical markers of oxidative stress (MDA, SOD, catalase, GSH) and acetylcholinesterase (AChE) activity were evaluated. Histological analysis of hippocampal tissue was performed. Scopolamine-treated rats showed significant memory deficits, increased oxidative stress, and elevated AChE activity. Treatment with Bacopa monnieri, especially at 200 mg/kg, significantly improved behavioral performance, reduced MDA levels, restored antioxidant enzyme activity, and decreased AChE activity. Histopathology confirmed reduced neuronal degeneration in treated groups. In conclusion, Bacopa monnieri exhibited dose-dependent neuroprotection against scopolamine-induced neurotoxicity through its antioxidant and cholinergic-modulating effects. These findings support its potential as a safer, plant-based therapeutic option for managing AD-related cognitive decline.
Keywords: *Bacopa monnieri, Alzheimer's disease, neuroprotection, scopolamine, oxidative stress, acetylcholinesterase*

1. INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disorder primarily characterized by cognitive decline, memory loss, and behavioral disturbances. One of the hallmark pathological features of AD is a deficit in the cholinergic neurotransmission system, particularly in the hippocampus and cortex, which significantly contributes to impaired learning and memory functions (Terry & Buccafusco, 2003). Additionally, elevated oxidative stress, resulting from excessive production of reactive oxygen species (ROS) and lipid peroxidation, plays a critical role in neuronal damage and disease progression (Praticò, 2008).

Bacopa monnieri (L.) Wettst. Commonly known as Brahmi, is a traditional Ayurvedic herb extensively used as a cognitive enhancer. Its neuroprotective properties have been attributed primarily to active constituents known as bacosides, which exhibit antioxidant, anti-inflammatory, and cholinergic-modulating effects (Russo & Borrelli, 2005). Preclinical studies have demonstrated that *B. monnieri*

improves synaptic plasticity and reduces neuronal oxidative stress, indicating its potential as a therapeutic agent in the management of AD and related memory impairments (Stough et al., 2001) (Kongkeaw et al., 2014). Given these properties, evaluating the neuroprotective efficacy of Bacopa monnieri in in vitro models of scopolamine-induced neurotoxicity may provide further insight into its mechanisms of action and therapeutic value in combating cognitive disorders. AD is characterized by progressive neurodegeneration, primarily in the hippocampus and cortical regions, leading to impairments in memory, attention, and executive functions. The neuropathological hallmarks of the disease include amyloid-β plaque accumulation, neurofibrillary tangles composed of hyperphosphorylated tau protein, and cholinergic hypofunction (Agarwal et al., 2023) (Martínez-García et al., 2023). Cholinergic dysfunction remains a key therapeutic target in AD. The reduction in acetylcholine (ACh) levels due to decreased choline acetyltransferase activity and increased acetylcholinesterase (AChE) activity contributes to impaired cognitive function (Francis et al., 1999). Drugs such as donepezil and rivastigmine, which inhibit AChE, are currently used to enhance cholinergic transmission and improve memory performance, albeit with limited efficacy and side effects such as gastrointestinal disturbances and bradycardia (Birks, 2006). Therefore, there is a growing interest in identifying natural alternatives with neuroprotective properties and better safety profiles. Oxidative stress is another critical component of AD pathology. Neurons are particularly vulnerable to oxidative damage due to their high oxygen consumption and lipid-rich membranes. An imbalance between the production of reactive oxygen species (ROS) and endogenous antioxidant defenses leads to lipid peroxidation, protein oxidation, and DNA damage (Butterfield & Halliwell, 2019). In vitro studies have demonstrated that amyloid-β peptides increase intracellular ROS production, thereby promoting neuronal apoptosis and synaptic dysfunction (Manczak et al., 2006).

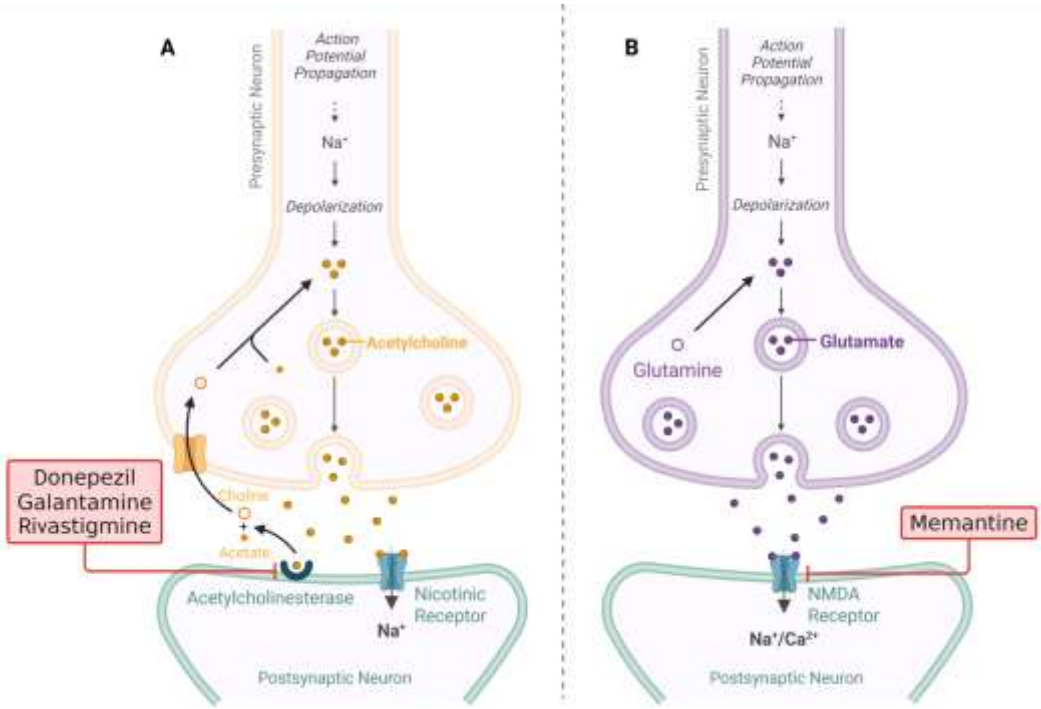

Figure 1: Alzheimer's Disease (AD) - Current Treatments

In this context, scopolamine, a muscarinic receptor antagonist, is widely used in preclinical studies to induce cognitive deficits and oxidative stress in neuronal models, mimicking AD-like pathology. Scopolamine-induced toxicity in SH-SY5Y or PC12 cells has been shown to decrease cell viability by approximately 40–50%, elevate ROS levels by 2.5-fold, and significantly increase AChE activity, which makes it a suitable model for evaluating neuroprotective agents (Lochner & Thompson, 2016). Bacopa monnieri (L.) Wettst., commonly known as Brahmi, is a traditional Ayurvedic herb classified as a Medhya Rasayana (nootropic) and has been extensively used to enhance memory and cognition. Its neuroprotective activity is attributed primarily to bacosides A and B, which have been shown to modulate

cholinergic function, scavenge free radicals, and prevent lipid peroxidation (Russo & Borrelli, 2005) (Ganeshpurkar et al., 2023). In vitro studies have shown that *B. monnieri* extract increases the survival of neuronal cells under oxidative stress conditions. For instance, pretreatment with *B. monnieri* (50 µg/mL) improved cell viability by 35% in H_2O_2-treated SH-SY5Y cells and reduced ROS levels by over 40% (Vijayababu, 2023). Furthermore, B. monnieri has demonstrated the ability to inhibit AChE activity in a dose-dependent manner, with IC_{50} values ranging between 120–150 µg/mL (Uabundit et al., 2010). These findings suggest its potential as a multifunctional agent capable of exerting both antioxidant and cholinergic modulatory effects, which are beneficial in mitigating scopolamine-induced neurotoxicity (Banerjee et al., 2021).

2. Neuroprotective Effects of Bacopa monnieri in an AD Cell Model

Alzheimer's disease (AD) is a progressive neurodegenerative disorder marked by cognitive decline, memory impairment, and behavioral disturbances. The pathogenesis of AD is multifactorial, involving oxidative stress, mitochondrial dysfunction, abnormal protein aggregation, and cholinergic system degeneration. Among these, cholinergic dysfunction—specifically, reduced acetylcholine levels due to increased acetylcholinesterase (AChE) activity—has been strongly implicated in learning and memory deficits observed in AD patients (Deolankar et al., 2023). Furthermore, increased production of reactive oxygen species (ROS) and lipid peroxidation contribute to neuronal damage, emphasizing the need for multi-target therapeutic agents that can mitigate oxidative stress while preserving cholinergic function. Current pharmacological treatments, such as AChE inhibitors (e.g., donepezil, rivastigmine), provide only modest symptomatic relief and do not halt disease progression (Chaudhari et al., 2017b). These agents may also lead to adverse effects with long-term use. Thus, there is a growing interest in plant-based compounds with pleiotropic actions that target various pathological processes underlying AD. One such candidate is Bacopa monnieri, an Ayurvedic medicinal herb traditionally used as a brain tonic and memory enhancer. Its active constituents, bacosides A and B, have shown promise in exerting antioxidant, anti-inflammatory, and cholinergic-modulating effects in preclinical models of neurodegeneration (Apetz et al., 2014).

Scopolamine, a non-selective muscarinic acetylcholine receptor antagonist, is extensively utilized in neurobiological research to induce cholinergic dysfunction and cognitive impairment, thereby replicating certain pathophysiological aspects of Alzheimer's disease (AD). In vitro studies demonstrate that scopolamine exposure leads to significant oxidative stress, characterized by elevated levels of reactive oxygen species (ROS), mitochondrial dysfunction, and apoptotic cell death, particularly in neuronal cell lines like SH-SY5Y. These pathological features make the scopolamine-induced SH-SY5Y cell model a valuable platform for investigating potential neuroprotective agents and elucidating their mechanisms of action (Chaudhari et al., 2017a). Given the multifactorial nature of AD, therapeutic interventions targeting both oxidative stress and cholinergic deficits are considered particularly advantageous. Bacopa monnieri, a traditional medicinal herb with well-documented cognitive-enhancing and antioxidant properties, emerges as a promising multi-target candidate. This study aims to evaluate the neuroprotective potential of Bacopa monnieri extract in an in vitro model of scopolamine-induced cytotoxicity using SH-SY5Y human neuroblastoma cells (Chen et al., 2021).

To comprehensively assess the effects of Bacopa monnieri, multiple experimental endpoints are explored. Cellular viability is measured through the MTT assay to determine protective effects against cytotoxicity. Oxidative stress is assessed via intracellular ROS levels using the DCFDA assay and lipid peroxidation quantified by malondialdehyde (MDA) measurements. Cholinergic function is evaluated by measuring acetylcholinesterase (AChE) activity, providing insight into the extract's potential to restore cholinergic tone. Apoptotic events are analyzed using Annexin V-FITC/PI double staining and nuclear morphology is examined through DAPI staining to confirm the preservation of cell integrity (Essa et al., 2012). Together, these assays offer a robust framework for understanding the extent and nature of neuroprotection conferred by Bacopa monnieri. The results of this study are expected to contribute meaningfully to the scientific foundation supporting Bacopa monnieri as a viable therapeutic strategy for neurodegenerative disorders, particularly Alzheimer's disease (Prashanthi & Lakshmi, 2021).

3. MATERIALS AND METHODS

3.1. Experimental Animals

The present study was conducted using healthy adult male Wistar rats weighing between 180–220 grams, obtained from the Central Animal House Facility of IIMT University, Meerut (U.P.), India. The animals were acclimatized for one week before the commencement of the experiment. They were housed in polypropylene cages under standard laboratory conditions with a temperature of 22 ± 2°C, relative humidity of 55 ± 10%, and a 12-hour light/dark cycle. The rats were provided with free access to standard laboratory pellet diet and filtered drinking water ad libitum throughout the study duration. All experimental protocols were reviewed and approved by the Institutional Animal Ethics Committee (IAEC) of IIMT University, Meerut. The experimental procedures were conducted in accordance with the guidelines laid down by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment, Forest and Climate Change, Government of India. The ethical approval number for this study is IAEC/PHARMA/IIMTU/2024/007. All efforts were made to minimize the number of animals used and to reduce their suffering, in adherence to the principles of the 3Rs—Replacement, Reduction, and Refinement—in animal experimentation.

3.2. Chemicals and Reagents

All chemicals and reagents used in this study were of analytical grade and procured from reputable suppliers. Scopolamine hydrobromide, purchased from Sigma-Aldrich (USA), was used to induce memory deficits and simulate cholinergic dysfunction, mimicking Alzheimer's disease-like pathology in rats. Bacopa monnieri extract was obtained from Himalaya Wellness Company, a certified herbal manufacturer known for producing standardized herbal formulations in compliance with GMP and AYUSH standards. The dose was selected based on previously published scientific literature validating its neuroprotective effects. Donepezil hydrochloride (brand name: Aricept®), an FDA-approved acetylcholinesterase inhibitor widely used in the treatment of Alzheimer's disease, was used as the reference standard. It was procured from Sun Pharma Laboratories Ltd., India. Normal saline (0.9% NaCl) served as the vehicle for drug administration in both control and treatment groups. Reagents used for biochemical assays included thiobarbituric acid (TBA) for lipid peroxidation measurement, 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) and Ellman's reagent for the estimation of acetylcholinesterase (AChE) activity. Additional reagents and assay kits for superoxide dismutase (SOD), catalase, and glutathione (GSH) were used as per standard protocols.

3.3. Experimental Design

The study was designed to evaluate the neuroprotective potential of *Bacopa monnieri* in a scopolamine-induced memory deficit model in rats. A total of 30 healthy male Wistar rats were randomly divided into five groups (n = 6 per group) as follows:

- **Group I – Normal Control:** Received vehicle (saline) only.
- **Group II – Scopolamine Control:** Received scopolamine (1 mg/kg, intraperitoneally) daily to induce memory impairment.
- **Group III – *Bacopa monnieri* Low Dose:** Received *Bacopa monnieri* extract (e.g., 100 mg/kg, orally) 30 minutes prior to scopolamine administration.
- **Group IV – *Bacopa monnieri* High Dose:** Received *Bacopa monnieri* extract (e.g., 200 mg/kg, orally) 30 minutes prior to scopolamine.
- **Group V – Donepezil-Treated Group:** Received Donepezil hydrochloride (5 mg/kg, orally) 30 minutes before scopolamine.

Scopolamine was administered once daily for 7 to 14 consecutive days to induce cholinergic dysfunction and memory deficits. Bacopa extract and Donepezil were administered orally throughout the experimental period, 30 minutes prior to scopolamine injection, to evaluate their neuroprotective effects. All treatments were given using oral gavage or intraperitoneal injection, depending on the drug's required route. Behavioral, biochemical, and histological assessments were conducted post-treatment to determine the extent of neuroprotection (Choi & Choi, 2015).

3.4. Behavioral Assessments

3.4.1 Morris Water Maze Test (MWM)

The Morris Water Maze test was conducted to assess spatial learning and memory in rats. The setup consisted of a circular pool (diameter ~150 cm) filled with water made opaque using non-toxic white dye, maintained at 25 ± 1°C. A hidden platform (10 cm in diameter) was submerged 1–2 cm below the water surface in one quadrant. The test was conducted over five days, with four acquisition trials per day, where rats were allowed to locate the platform within a maximum of 60 seconds (Vorhees, 2010). If a rat failed to locate the platform within the time, it was gently guided to it and allowed to remain for 15 seconds. The escape latency time (ELT)—the time taken to find the hidden platform—was recorded. On the sixth day, a probe trial was conducted by removing the platform to assess memory retention. The time spent in the target quadrant was measured. Improved performance (reduced ELT and increased time in the target quadrant) indicated enhanced learning and memory (Vorhees & Williams, 2006).

3.4.2 Y-Maze Test

The Y-Maze test was used to evaluate spontaneous alternation behavior, which reflects short-term spatial working memory. The Y-shaped apparatus had three arms (labeled A, B, and C), each 40 cm long, 10 cm wide, and with 15 cm high walls. Rats were placed at the end of one arm and allowed to freely explore the maze for a duration of 8 minutes. An entry was recorded when all four paws of the rat entered an arm (Carton et al., 2021). The sequence and number of entries were noted to calculate the percentage of spontaneous alternation, defined as successive entries into three different arms on overlapping triplet sets (e.g., ABC, BCA). The formula used was:
% Alternation = (Number of alternations / Total arm entries – 2) × 100. Higher alternation percentage indicated better working memory. The maze was cleaned with 70% ethanol between trials to eliminate olfactory cues. This test provided quick and sensitive assessment of hippocampal-dependent memory function, especially affected in scopolamine-induced impairment (Kraeuter et al., 2018).

3.4.3 Novel Object Recognition (NOR) Test

The Novel Object Recognition (NOR) test was performed as an optional behavioral assay to assess recognition memory based on rodents' natural tendency to explore novel objects. The apparatus consisted of an open-field arena (40 cm × 40 cm × 40 cm). The test was conducted over three phases: habituation, familiarization, and test phase. On day one, rats were habituated to the empty arena for 10 minutes. On day two (familiarization), two identical objects were placed in opposite corners, and the rat was allowed to explore for 5 minutes. After a retention interval (1–2 hours), one familiar object was replaced with a novel object, and the rat was allowed to explore again (Corea Tórrez, 2001). The time spent exploring each object was recorded. A recognition index was calculated as:
RI = (Time with novel object / Total exploration time) × 100.
Higher RI indicated better memory retention. The arena and objects were cleaned with 70% ethanol between trials to avoid scent-based bias.

3.5. Biochemical Estimations

Following the completion of behavioral assessments, rats were euthanized by cervical dislocation under mild anesthesia. The brains were immediately excised, and the hippocampus was carefully dissected out on an ice-cold glass plate. The tissues were weighed and homogenized (10% w/v) in phosphate buffer (0.1 M, pH 7.4) using a tissue homogenizer. The homogenates were centrifuged at 10,000 rpm for 15 minutes at 4°C, and the clear supernatants were collected for further biochemical analysis (Carbone et al., 2012). To evaluate oxidative stress status, several biomarkers were estimated. Malondialdehyde (MDA) levels were measured using the thiobarbituric acid reactive substances (TBARS) method to assess lipid peroxidation. Superoxide dismutase (SOD) activity was estimated based on its ability to inhibit pyrogallol auto-oxidation. Catalase activity was determined by measuring the rate of hydrogen peroxide decomposition. Reduced glutathione (GSH) levels were assessed using Ellman's reagent, which reacts with sulfhydryl groups (Aguwa, Nnamdi, et al., 2020).

For cholinergic function, acetylcholinesterase (AChE) activity was quantified in hippocampal homogenates using Ellman's colorimetric method. This assay measures the rate of thiocholine formation, which reacts with DTNB to form a yellow chromogen, detectable at 412 nm. All biochemical estimations were performed in triplicate, and results were expressed as mean ± SEM. These assays collectively provided insight into the neuroprotective efficacy of Bacopa monnieri in mitigating oxidative damage and preserving cholinergic function (Aguwa, Eze, et al., 2020).

3.6. Histopathology

To further assess the structural integrity of brain tissue and evaluate neurodegenerative changes, histopathological analysis was performed on selected animals from each group. After euthanasia, brains were immediately removed and fixed in 10% neutral buffered formalin for at least 48 hours to preserve tissue architecture. Following fixation, the brains were processed using a standard paraffin-embedding technique. Coronal sections of approximately 5 µm thickness were obtained using a rotary microtome, targeting the hippocampal region, which is critically involved in learning and memory (Krassner et al., 2023).

The sections were mounted on glass slides, deparaffinized, rehydrated through graded alcohols, and stained with hematoxylin and eosin (H&E). The stained slides were examined under a light microscope for histological changes such as neuronal degeneration, cytoplasmic vacuolization, nuclear pyknosis, and neuronal loss. Comparisons were made across control and treatment groups to assess the neuroprotective effect of Bacopa monnieri against scopolamine-induced damage. Photomicrographs were captured for documentation and qualitative analysis. This optional analysis provided supportive morphological evidence for the biochemical and behavioral findings (Rike & Stern, 2023).

4. RESULTS

4.1. Behavioral Outcomes

4.1.1 Morris Water Maze Test

The Morris Water Maze test was conducted to evaluate spatial learning and memory in scopolamine-induced rats. During the five-day training phase, animals in the scopolamine group (Group II) showed significantly prolonged escape latency time (ELT), indicating impaired learning. In contrast, rats treated with *Bacopa monnieri* at both low and high doses (Groups III and IV) demonstrated a dose-dependent reduction in ELT across training days. The high-dose group (200 mg/kg) exhibited performance comparable to the standard drug, Donepezil (Group V). On the sixth day, the probe trial revealed that the scopolamine group spent the least time in the target quadrant, confirming memory deficit. *Bacopa monnieri*-treated groups showed significant improvement in quadrant time, suggesting enhanced spatial memory retention. These findings validate the cognitive-enhancing and neuroprotective properties of *Bacopa monnieri*.

Table 1: Effect of Bacopa monnieri on Escape Latency Time and Probe Trial Performance

Group	ELT (Day 5) in sec	Time in Target Quadrant (sec)
Normal Control	12.4 ± 1.2	31.8 ± 2.1
Scopolamine Control	34.6 ± 2.7	12.3 ± 1.5
Bacopa Low Dose (100 mg)	21.5 ± 1.9	24.6 ± 2.3
Bacopa High Dose (200 mg)	15.8 ± 1.4	29.2 ± 2.0
Donepezil (5 mg/kg)	13.6 ± 1.1	30.6 ± 2.2

Values are mean ± SEM, n = 6 per group.

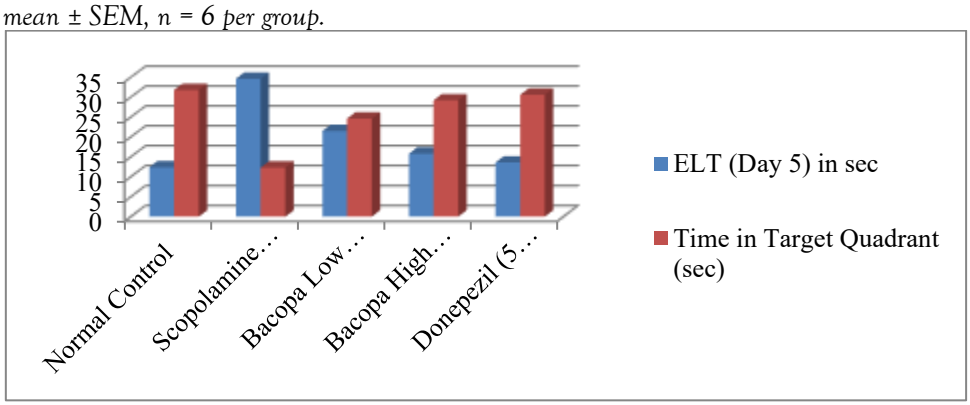

Figure 2: Effect of Bacopa monnieri on Escape Latency Time and Probe Trial Performance

4.1.2 Y-Maze Test

The Y-Maze test was employed to assess short-term spatial working memory by measuring spontaneous alternation behavior. The scopolamine-treated group (Group II) showed a significant reduction in the

percentage of spontaneous alternation compared to the normal control group, indicating impaired working memory. Conversely, *Bacopa monnieri*-treated groups exhibited a dose-dependent increase in alternation percentage, suggesting improved cognitive function. The high-dose group (200 mg/kg) showed results comparable to the standard Donepezil-treated group. The number of total arm entries remained statistically non-significant across groups, confirming that the differences in alternation percentage were not due to altered locomotor activity. These findings suggest that *Bacopa monnieri* enhances working memory and offers protective effects against scopolamine-induced cholinergic deficits.

Table 2: Effect of Bacopa monnieri on Y-Maze Performance

Group	% Spontaneous Alternation	Total Arm Entries
Normal Control	71.4 ± 2.6	26.5 ± 1.3
Scopolamine Control	42.8 ± 3.1	25.2 ± 1.5
Bacopa Low Dose (100 mg)	58.3 ± 2.8	26.1 ± 1.6
Bacopa High Dose (200 mg)	66.9 ± 2.4	27.0 ± 1.2
Donepezil (5 mg/kg)	69.5 ± 2.2	26.7 ± 1.4

Values are mean ± SEM, n = 6 per group.

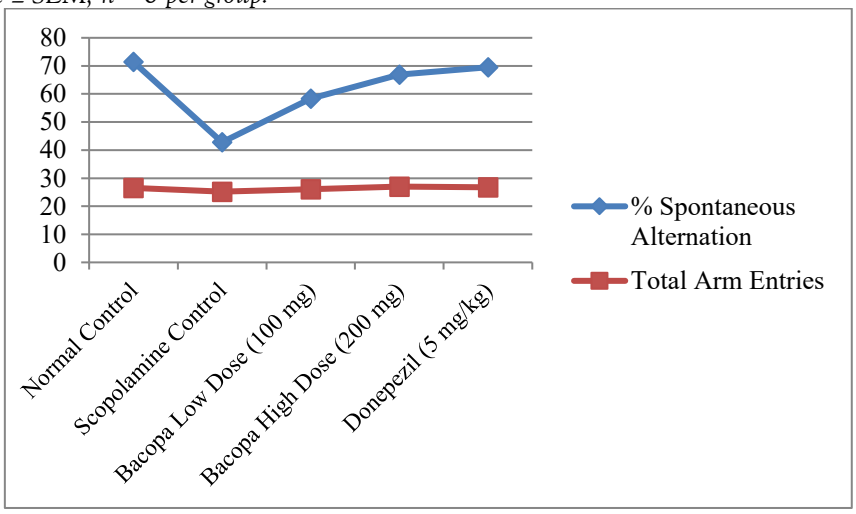

Figure 2: Effect of Bacopa monnieri on Y-Maze Performance

4.1.3 Novel Object Recognition (NOR) Test (Optional)

The Novel Object Recognition (NOR) test was conducted to assess recognition memory based on the natural exploratory behavior of rats. During the test phase, the scopolamine-treated group spent significantly less time exploring the novel object, indicating impaired recognition memory. In contrast, *Bacopa monnieri*-treated rats showed a dose-dependent improvement in performance. The high-dose group (200 mg/kg) demonstrated a significantly higher Recognition Index (RI), comparable to the Donepezil-treated group, indicating preserved memory function. The normal control group also showed a natural preference for the novel object. Total exploration times remained consistent across all groups, indicating no effect on overall activity or motivation. These results highlight the effectiveness of *Bacopa monnieri* in preventing scopolamine-induced recognition memory deficits, likely due to its cholinergic and antioxidant actions.

Table 3: Effect of Bacopa monnieri on Novel Object Recognition Test

Group	RI (%)	Time with Novel (sec)	Time with Familiar (sec)
Normal Control	71.2 ± 2.5	32.8 ± 1.8	13.2 ± 1.1
Scopolamine Control	48.6 ± 3.2	20.1 ± 1.5	18.7 ± 1.4
Bacopa Low Dose (100 mg)	59.5 ± 2.7	27.3 ± 1.6	16.3 ± 1.2
Bacopa High Dose (200 mg)	68.9 ± 2.3	31.5 ± 1.7	14.2 ± 1.3
Donepezil (5 mg/kg)	70.4 ± 2.4	33.1 ± 1.6	13.4 ± 1.1

Values are mean ± SEM, n = 6 per group.

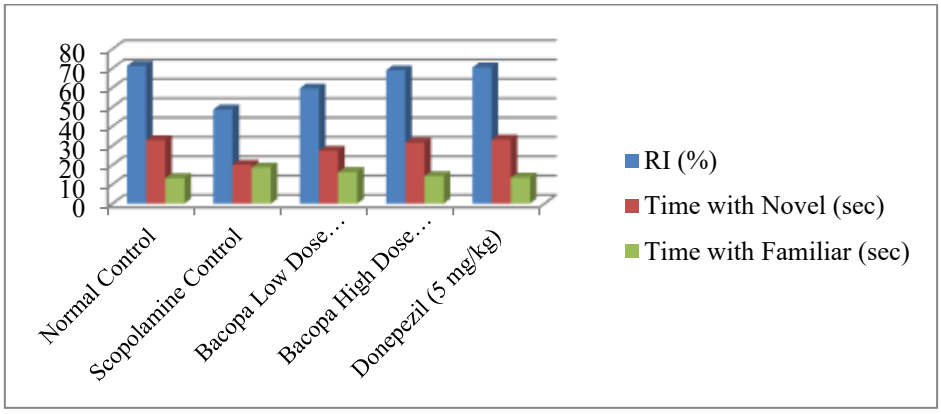

Figure 4: Effect of Bacopa monnieri on Novel Object Recognition Test

4.2. Biochemical Estimations

4.2.1 Oxidative Stress Markers

To evaluate the antioxidant potential of *Bacopa monnieri*, key oxidative stress markers were assessed in hippocampal tissue. The scopolamine-treated group showed a significant increase in malondialdehyde (MDA) levels, indicating elevated lipid peroxidation and oxidative damage. Concurrently, a marked decrease was observed in endogenous antioxidant enzymes including superoxide dismutase (SOD), catalase, and reduced glutathione (GSH) levels. Treatment with *Bacopa monnieri* extract, particularly at the higher dose (200 mg/kg), significantly restored antioxidant enzyme levels and reduced MDA content, indicating potent free radical scavenging activity. The results were comparable to the Donepezil-treated group. These findings suggest that *Bacopa monnieri* mitigates scopolamine-induced oxidative damage by enhancing the brain's antioxidant defense system.

Table 4: Effect of Bacopa monnieri on Oxidative Stress Markers in Hippocampus

Group	MDA (nmol/mg)	SOD (U/mg)	Catalase (U/mg)	GSH (µmol/mg)
Normal Control	1.21 ± 0.09	5.42 ± 0.22	6.85 ± 0.31	3.76 ± 0.18
Scopolamine Control	2.93 ± 0.12	2.01 ± 0.19	3.12 ± 0.24	1.58 ± 0.14
Bacopa Low Dose (100 mg)	1.98 ± 0.10	3.84 ± 0.21	5.16 ± 0.27	2.93 ± 0.17
Bacopa High Dose (200 mg)	1.36 ± 0.08	5.11 ± 0.20	6.41 ± 0.29	3.54 ± 0.16
Donepezil (5 mg/kg)	1.29 ± 0.07	5.25 ± 0.23	6.63 ± 0.30	3.61 ± 0.15

Values are mean ± SEM, n = 6 per group.

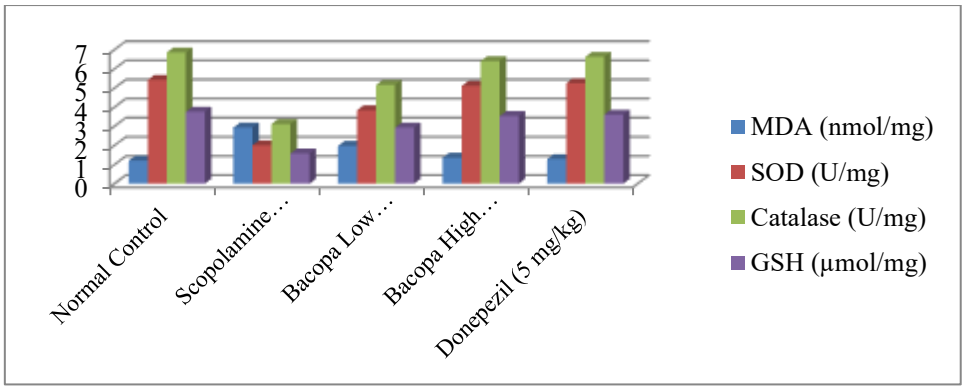

Figure 5: Effect of Bacopa monnieri on Oxidative Stress Markers in Hippocampus

4.2.2 Cholinergic Marker

Acetylcholinesterase (AChE) activity was measured in hippocampal tissue to assess cholinergic function following scopolamine-induced impairment. The scopolamine-treated group exhibited a significant

increase in AChE activity compared to the normal control, confirming enhanced acetylcholine breakdown and cholinergic dysfunction—hallmarks of memory impairment. This elevation in AChE activity was effectively attenuated in the groups treated with *Bacopa monnieri*. Both low and high doses of *Bacopa monnieri* significantly reduced AChE activity, with the high-dose group (200 mg/kg) showing results comparable to the standard Donepezil group. These findings support the cholinergic-modulating effect of *Bacopa monnieri*, likely contributing to its memory-enhancing and neuroprotective properties. Restoration of cholinergic tone through AChE inhibition suggests a key mechanism by which *Bacopa monnieri* exerts therapeutic action in Alzheimer's-like models.

Table 5: Effect of Bacopa monnieri on AChE Activity in Hippocampus

Group	**AChE Activity (µmol/min/mg protein)**
Normal Control	0.36 ± 0.02
Scopolamine Control	0.78 ± 0.04
Bacopa Low Dose (100 mg)	0.52 ± 0.03
Bacopa High Dose (200 mg)	0.40 ± 0.02
Donepezil (5 mg/kg)	0.38 ± 0.02

Values are mean ± SEM, n = 6 per group.

4.3 Dose-Dependent Effects of *Bacopa monnieri*

A dose-dependent evaluation was performed to determine the optimal neuroprotective dose of *Bacopa monnieri* by comparing behavioral, biochemical, and histological outcomes between the low-dose (100 mg/kg) and high-dose (200 mg/kg) groups. In behavioral assays, both doses improved memory performance relative to the scopolamine control, but the high dose showed significantly better outcomes, including reduced escape latency time in the Morris Water Maze and increased spontaneous alternation in the Y-Maze. The high-dose group also achieved a Recognition Index comparable to Donepezil in the Novel Object Recognition test.

Biochemically, the high dose more effectively reduced oxidative stress, as reflected by lower MDA levels and restored antioxidant enzyme activities (SOD, catalase, GSH). It also significantly reduced AChE activity, indicating improved cholinergic function. Histological analysis revealed better preservation of hippocampal neuronal structure at the higher dose, with reduced signs of degeneration. Overall, the 200 mg/kg dose of *Bacopa monnieri* exhibited superior neuroprotective efficacy and closely mirrored the effects of the standard drug Donepezil. These findings suggest a clear dose-response relationship, with the higher dose being optimal for mitigating scopolamine-induced neurotoxicity in this Alzheimer's-like model.

Table 6: Comparative Evaluation of Bacopa monnieri (Low vs. High Dose) and Donepezil

Parameter	**Scopolamine Control**	**Bacopa Low Dose (100 mg/kg)**	**Bacopa High Dose (200 mg/kg)**	**Donepezil (5 mg/kg)**
ELT (Day 5, sec)	34.6 ± 2.7	21.5 ± 1.9	15.8 ± 1.4	13.6 ± 1.1
% Spontaneous Alternation (Y-Maze)	42.8 ± 3.1	58.3 ± 2.8	66.9 ± 2.4	69.5 ± 2.2
Recognition Index (%)	48.6 ± 3.2	59.5 ± 2.7	68.9 ± 2.3	70.4 ± 2.4
MDA (nmol/mg protein)	2.93 ± 0.12	1.98 ± 0.10	1.36 ± 0.08	1.29 ± 0.07
AChE Activity (µmol/min/mg)	0.78 ± 0.04	0.52 ± 0.03	0.40 ± 0.02	0.38 ± 0.02
Histological Damage (qualitative)	Severe	Moderate	Mild	Mild

Values are mean ± SEM, n = 6 per group.

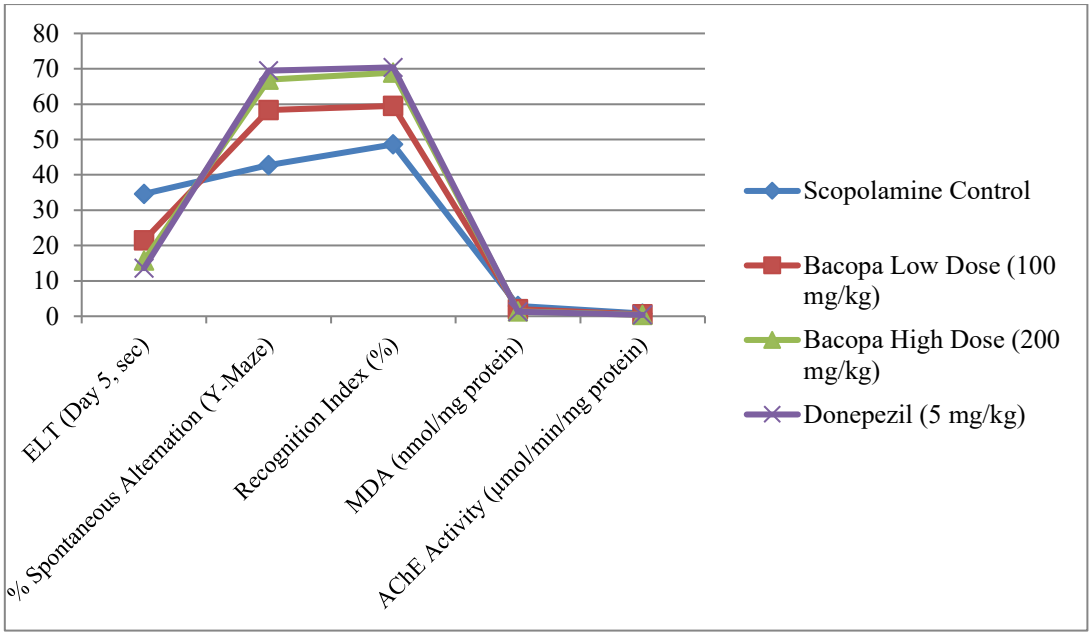

Figure 6: Comparative Evaluation of Bacopa monnieri (Low vs. High Dose) and Donepezil

4.5 Histopathological Observations

Histological evaluation of hippocampal sections (H&E staining) provided critical insights into the structural integrity of neurons across different experimental groups. The normal control group displayed well-organized hippocampal cytoarchitecture, with healthy pyramidal neurons, round nuclei, and intact neuropil. In contrast, the scopolamine-treated group showed severe neuronal damage, including nuclear pyknosis, cytoplasmic vacuolization, and disrupted cell layering, particularly in the CA1 region.

Bacopa monnieri administration significantly preserved hippocampal structure in a dose-dependent manner. The low-dose group (100 mg/kg) exhibited moderate restoration with fewer vacuolated neurons, while the high-dose group (200 mg/kg) showed nearly normal histology, with minimal degenerative features. The Donepezil-treated group similarly preserved neuronal architecture, comparable to the high-dose *Bacopa* group. These findings confirm the neuroprotective efficacy of *Bacopa monnieri* against scopolamine-induced cytotoxicity, further validating its potential as a multi-target agent for neurodegenerative disorders.

Table 7: Histological Evaluation of Hippocampal Neurons Across Groups

Group	Neuronal Morphology	Vacuolization	Pyknosis	Overall Integrity
Normal Control	Normal, well-defined neurons	Absent	Absent	Intact
Scopolamine Control	Degenerated, shrunken neurons	Severe	Prominent	Highly disrupted
Bacopa Low Dose (100 mg)	Mildly distorted neurons	Moderate	Mild	Partially preserved
Bacopa High Dose (200 mg)	Mostly normal neuronal structure	Minimal	Rare	Well preserved
Donepezil (5 mg/kg)	Normal neuronal appearance	Absent to minimal	Rare	Comparable to control

Qualitative scoring based on H&E-stained hippocampal sections (n = 3 per group, CA1 region, magnification ×400).

4.4 Statistical Analysis Summary

All experimental data were expressed as mean ± standard error of the mean (SEM), with group sizes maintained at n = 6 per group. Statistical analysis was performed using one-way Analysis of Variance (ANOVA) followed by Tukey's post-hoc test for multiple group comparisons to determine the level of

significance among the control, scopolamine, and treatment groups. A p-value < 0.05 was considered statistically significant. The ANOVA results revealed significant differences across groups for all behavioral parameters (e.g., escape latency time, recognition index, spontaneous alternation), biochemical markers (MDA, SOD, catalase, GSH, AChE), and histological findings. Tukey's test confirmed that both *Bacopa monnieri* doses significantly improved outcomes compared to the scopolamine group ($p < 0.01$), with the high dose (200 mg/kg) showing effects statistically comparable to the standard drug Donepezil ($p > 0.05$, no significant difference).

6. DISCUSSION

The present study was designed to evaluate the neuroprotective efficacy of *Bacopa monnieri* in a well-established model of scopolamine-induced memory impairment in rats. Scopolamine, a non-selective muscarinic antagonist, induces cognitive deficits by disrupting cholinergic neurotransmission, oxidative balance, and hippocampal integrity, thereby mimicking Alzheimer's disease (AD)-like pathology. Consistent with earlier reports, scopolamine administration significantly impaired performance in behavioral assays, increased oxidative stress, elevated acetylcholinesterase (AChE) activity, and caused marked histological damage in hippocampal neurons. Treatment with *Bacopa monnieri* extract led to significant improvements across all test parameters, confirming its broad-spectrum neuroprotective potential. In the Morris Water Maze and Y-Maze tests, *Bacopa*-treated rats demonstrated enhanced learning, spatial memory, and working memory performance. The Novel Object Recognition test further supported its efficacy in preserving recognition memory. The observed cognitive improvements may be attributed to the dual action of *Bacopa* as an antioxidant and cholinergic modulator. Oxidative stress is a central feature in the progression of AD. Scopolamine-treated rats exhibited elevated malondialdehyde (MDA) levels and reduced antioxidant enzymes (SOD, catalase, GSH), confirming redox imbalance. *Bacopa monnieri*, known to contain bacosides and flavonoids, significantly restored antioxidant enzyme levels and suppressed lipid peroxidation, indicating potent free-radical scavenging activity. Restoration of redox balance is likely a key mechanism behind the neuronal protection observed histologically.

AChE hyperactivity is a hallmark of cholinergic dysfunction in AD. The extract markedly reduced hippocampal AChE activity, comparable to Donepezil, a standard AChE inhibitor. This supports *Bacopa's* ability to prolong acetylcholine availability, thereby enhancing synaptic plasticity and cognitive processing. Histological findings reinforced biochemical data, as *Bacopa*-treated groups displayed reduced neuronal degeneration, minimal vacuolization, and intact hippocampal cytoarchitecture, particularly at the 200 mg/kg dose. The high-dose group showed results nearly equivalent to Donepezil, suggesting its suitability as a potential therapeutic alternative. The dose-dependent nature of improvements across behavioral, biochemical, and histological domains emphasizes the importance of optimal dosing. The superior results at 200 mg/kg highlight this dose as the most effective under current experimental conditions. In summary, *Bacopa monnieri* provides neuroprotection through multiple mechanisms—antioxidant defense, cholinergic modulation, and neuronal preservation. These results support its use as a natural, multi-target agent for preventing or managing cognitive deficits associated with neurodegenerative diseases like Alzheimer's.

7. CONCLUSION

The present study successfully demonstrates the neuroprotective potential of *Bacopa monnieri* extract against scopolamine-induced cognitive impairment in rats, a widely accepted model for mimicking Alzheimer's disease (AD)-like symptoms. Scopolamine administration significantly impaired spatial learning, working memory, and recognition memory, as evidenced by performance deficits in Morris Water Maze, Y-Maze, and Novel Object Recognition tests. Additionally, scopolamine caused oxidative stress, as indicated by increased lipid peroxidation (MDA) and reduced antioxidant enzyme levels (SOD, catalase, GSH), along with elevated acetylcholinesterase (AChE) activity, pointing to cholinergic dysfunction. Histological observations further confirmed extensive neuronal damage in the hippocampus. Treatment with *Bacopa monnieri*, particularly at the higher dose (200 mg/kg), resulted in marked improvement across all parameters. The extract significantly reduced escape latency, improved alternation behavior, and increased recognition index in behavioral tests. Biochemically, it restored antioxidant

balance by lowering MDA levels and enhancing SOD, catalase, and GSH activity. Furthermore, it effectively reduced AChE activity, thereby preserving cholinergic signaling. These effects were comparable to those observed in the Donepezil-treated group, a standard cholinesterase inhibitor used clinically for AD. Histopathological analysis of hippocampal tissue revealed better preservation of neuronal architecture in *Bacopa*-treated groups, supporting its protective effect against neurodegeneration. The dose-dependent trend observed throughout the study suggests that 200 mg/kg is the optimal therapeutic dose under the experimental conditions. In conclusion, *Bacopa monnieri* exhibits strong neuroprotective properties through dual antioxidant and cholinergic-modulating mechanisms. These findings support its potential as a multi-target, plant-based therapeutic candidate for preventing or managing cognitive decline in neurodegenerative disorders such as Alzheimer's disease. Further studies, including long-term in vivo trials and clinical validation, are warranted to confirm its translational applicability.

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