TO STUDY THE NEUROPROTECTIVE EFFECT OF HERBAL EXTRACT OF BACOPA MONNIERI IN SCOPOLAMINE INDUCED MEMORY IMPAIRMENT IN MICE

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Abstract:

Neurodegenerative disorders, particularly Alzheimer's disease, are characterized by progressive cognitive decline associated with cholinergic dysfunction and oxidative stress. Bacopa monnieri (Brahmi), a traditional Ayurvedic medicinal herb rich in bacosides, has been widely recognized for its memory-enhancing and neuroprotective properties.The present study aimed to evaluate the neuroprotective and cognition-enhancing effects of hydroalcoholic extract of Bacopa monnieri against scopolamine-induced memory impairment in mice. A hydroalcoholic extract of Bacopa monnieri was prepared by Soxhlet extraction, yielding 14.4% extract. Acute oral toxicity testing revealed no mortality or significant behavioral abnormalities, confirming its safety at the tested doses. Swiss albino mice were divided into five groups: normal control, scopolamine control, donepezil-treated standard group, and two groups receiving low and high doses of Bacopa monnieri extract. Memory impairment was induced using scopolamine (1 mg/kg, i.p.). Cognitive performance was assessed using Y-maze, Morris water maze, Novel Object Recognition, and Passive Avoidance tests. Biochemical parameters including acetylcholinesterase (AChE) activity, lipid peroxidation (MDA), and antioxidant enzyme levels (SOD, CAT, and GSH) were estimated. Histopathological examination of hippocampal tisSue was also performed.Scopolamine administration produced significant memory deficits, elevated AChE activity, and increased lipid peroxidation. Treatment with Bacopa monnieri extract significantly improved cognitive performance in behavioral tests, evidenced by enhanced spontaneous alternation, reduced escape latency, improved object recognition, and increased passive avoidance latency. The high dose of Bacopa monnieri produced effects comparable to donepezil. Biochemical analysis demonstrated significant reduction in AChE activity (15.81 ± 0.96 µmol/min/mg protein) and MDA levels (2.96 ± 0.16 nmol/mg protein) compared with the scopolamine control group (28.34 ± 1.12 µmol/min/mg protein and 6.75 ± 0.28 nmol/mg protein, respectively). Furthermore, treatment restored endogenous antioxidant defenses and protected hippocampal neurons from degeneration.The hydroalcoholic extract of Bacopa monnieri exhibits significant neuroprotective and memory-enhancing effects against scopolamine-induced cognitive impairment in mice. These effects may be mediated through enhancement of cholinergic neurotransmission, antioxidant activity, inhibition of lipid peroxidation, and neuronal protection. The findings scientifically

validate the traditional use of Bacopa monnieri as a cognitive enhancer and suggest its potential as a therapeutic candidate for the management of Alzheimer's disease and other neurodegenerative disorders.

***Keywords*:**

Bacopa monnieri, Alzheimer's disease, neuroprotection, scopolamine, memory impairment, acetylcholinesterase, oxidative stress, bacosides, cognition enhancement.

I. Introduction

Neurodegenerative disorders represent a major global health challenge due to their increasing prevalence and the lack of effective disease-modifying therapies. Among these disorders, Alzheimer's disease (AD) is the most common cause of dementia and is characterized by progressive cognitive decline, memory impairment, and deterioration of learning abilities. According to recent estimates, millions of individuals worldwide are affected by dementia, with Alzheimer's disease accounting for approximately 6070% of all cases. The pathological hallmarks of AD include extracellular deposition of amyloid-β plaques, intracellular neurofibrillary tangles, oxidative stress, neuroinflammation, and significant loss of cholinergic neurons in the brain. The cholinergic hypothesis remains one of the most widely accepted mechanisms underlying cognitive dysfunction, suggesting that reduced acetylcholine neurotransmission contributes significantly to memory deficits and impaired cognitive performance. Acetylcholine plays a crucial role in learning, memory consolidation, attention, and other higher cognitive functions. Deficiency of cholinergic neurotransmission in the hippocampus and cerebral cortex has been strongly associated with cognitive impairment in both aging and neurodegenerative conditions. Consequently, enhancement of cholinergic activity through inhibition of acetylcholinesterase (AChE), the enzyme responsible for acetylcholine degradation, has become a major therapeutic strategy in the management of Alzheimer's disease. Currently available drugs such as donepezil, rivastigmine, and galantamine provide symptomatic relief but are often associated with adverse effects, limited efficacy, and inability to halt disease progression. Therefore, there is a growing interest in identifying safer and more effective therapeutic agents, particularly those derived from medicinal plants with established traditional use. Experimental models play an essential role in understanding the mechanisms of cognitive impairment and evaluating potential neuroprotective agents. Scopolamine-induced amnesia is one of the most extensively used pharmacological models for studying learning and memory deficits in laboratory animals. Scopolamine is a non-selective muscarinic acetylcholine receptor antagonist that readily crosses the blood-brain barrier and disrupts cholinergic neurotransmission. Administration of scopolamine produces cognitive deficits resembling those observed in Alzheimer's disease, including impaired acquisition, consolidation, and retrieval of memory. In addition to cholinergic dysfunction, scopolamine has been reported to induce oxidative stress, mitochondrial dysfunction, and neuronal damage in brain regions associated with learning and memory, particularly the hippocampus and cerebral cortex. Owing to these characteristics, the scopolamine model is widely accepted for screening anti-amnesic and neuroprotective compounds. Medicinal plants have gained considerable attention as alternative therapeutic options for neurological disorders due to their diverse pharmacological activities, multitarget mechanisms of action, and favorable safety profiles. Among the numerous herbal remedies used in traditional medicine, Bacopa monnieri (L.) Wettst., commonly known as Brahmi, occupies a prominent position in the Ayurvedic system of medicine. Bacopa monnieri is a small creeping herb belonging to the family Plantaginaceae and has been traditionally used as a "Medhya Rasayana," a class of rejuvenating herbs believed to enhance intellect, memory, and cognitive function. For centuries, it has been prescribed for improving concentration, learning ability, anxiety, epilepsy, and various neuropsychiatric disorders. The pharmacological properties of Bacopa monnieri are primarily attributed to its bioactive constituents, including triterpenoid saponins known as bacosides, particularly bacoside A and bacoside B. These compounds have been reported to possess antioxidant, anti-inflammatory, neuroprotective, adaptogenic, and cognitive-enhancing activities. Several preclinical studies have demonstrated that Bacopa monnieri improves memory retention, enhances synaptic communication, promotes neuronal repair, and protects against neurotoxicity. Furthermore, bacosides have been shown to modulate cholinergic transmission, reduce lipid peroxidation,

increase antioxidant enzyme activity, and improve cerebral blood flow, thereby contributing to enhanced cognitive performance. Oxidative stress is increasingly recognized as a critical factor in the pathogenesis of neurodegenerative diseases. Excessive production of reactive oxygen species (ROS) can damage cellular lipids, proteins, and nucleic acids, ultimately leading to neuronal dysfunction and cell death. The brain is particularly vulnerable to oxidative damage due to its high oxygen consumption, abundant lipid content, and relatively limited antioxidant defenses. Previous investigations have demonstrated that Bacopa monnieri exhibits potent free radical scavenging activity and enhances endogenous antioxidant defense systems, including superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH). These antioxidant properties may play a crucial role in its neuroprotective effects against cognitive impairment. Despite extensive traditional use and growing scientific evidence supporting its cognitive benefits, further investigation is required to elucidate the neuroprotective efficacy of Bacopa monnieri in experimentally induced memory impairment. Evaluation of its effects in validated animal models can provide valuable insights into its mechanisms of action and potential therapeutic applications in neurodegenerative disorders. The scopolamine-induced memory impairment model offers an appropriate platform to assess both behavioral and biochemical alterations associated with cognitive dysfunction.Therefore, the present study was undertaken to investigate the neuroprotective effect of Bacopa monnieri herbal extract against scopolamine-induced memory impairment in mice. The study aimed to evaluate its influence on learning and memory using established behavioral paradigms and to assess its effects on cholinergic function and oxidative stress biomarkers in brain tissue. The findings of this study may contribute to the development of plant-based therapeutic approaches for the prevention and management of cognitive disorders and neurodegenerative diseases.

II. Methods

A. Collection and Authentication of Plant Material

Fresh whole plants of Bacopa monnieri will be collected from a local herbal garden or authenticated crude drug supplier during the appropriate harvesting season. The collected plant material will be thoroughly washed with distilled water to remove adhering soil particles, dust, and other extraneous matter. After cleaning, the plant material will be shade dried at room temperature for about 10-15 days to prevent degradation of thermolabile phytoconstituents. The completely dried material will then be coarsely powdered using a mechanical grinder and passed through sieve no. 40 to obtain uniform particle size.

B. Standardization of Plant Material

Standardization of Bacopa monnieri will be carried out according to Indian Pharmacopoeia guidelines and standard pharmacognostic procedures to ensure the identity, purity, quality, and consistency of the plant material. Various physicochemical parameters such as determination of foreign organic matter, moisture content, total ash value, acid-insoluble ash, water-soluble ash, alcohol-soluble extractive value, and water-soluble extractive value will be evaluated using standard procedures.

C. Tests for Phytoconstituents

1) Test for Alkaloids: The extract will be treated separately with Dragendorffs reagent and Mayers reagent. Formation of orange-brown precipitate with Dragendorffs reagent or cream-colored precipitate with Mayers reagent will indicate the presence of alkaloids.

2) Test for Flavonoids: Flavonoids will be identified by performing Shinoda test and alkaline reagent test. Development of pink or reddish coloration in Shinoda test and formation of intense yellow coloration in alkaline reagent test will confirm the presence of flavonoids.

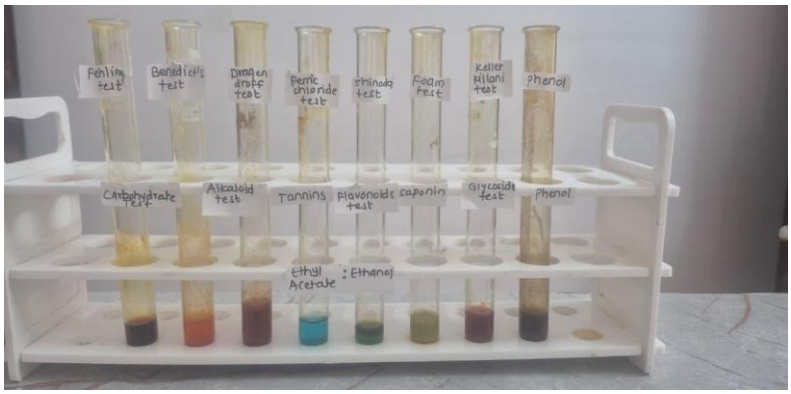


Fig. 1 : Preliminary Phytochemical Screening

3) Test for Saponins: Foam test will be performed by vigorously shaking the extract with distilled water. Formation of stable persistent foam will indicate the presence of saponins.

4) Test for Tannins and Phenolic Compounds: Ferric chloride test and lead acetate test will be carried out for identification of tannins and phenolic compounds. Appearance of blue-black or greenish coloration with ferric chloride and formation of precipitate with lead acetate will indicate positive results.

5) Test for Glycosides: KellerKilliani test will be performed for detection of glycosides. Formation of a brown ring at the junction of two layers will indicate the presence of glycosidic compounds.

D. Soxhlet Extraction Method

Approximately 500 g of coarsely powdered whole plant material of Bacopa monnieri will be subjected to continuous hot extraction using a Soxhlet extraction apparatus. Initially, the powdered drug will be defatted with petroleum ether to remove fatty and non-polar impurities such as waxes, chlorophyll, and lipids. After complete defatting, the marc will be dried and further extracted using hydro alcoholic solvent system containing ethanol and water in the ratio of 70:30. The extraction process will be continued for about 6-8 hours or until complete exhaustion of the plant material is achieved, as indicated by a colorless siphon tube solvent. The obtained extract will then be filtered and concentrated under reduced pressure using a rotary vacuum evaporator to remove the solvent. The concentrated extract will be further dried on a water bath at controlled temperature to obtain a semisolid mass.

E. In vivo Studies

1) Acute Oral Toxicity Study: Acute oral toxicity study of the hydro alcoholic extract of Bacopa monnieri will be carried out according to OECD guideline 423 (Acute Toxic Class Method). Healthy Swiss albino mice will be fasted overnight prior to administration of the extract, while water will be provided ad libitum. The extract will be administered orally at different dose levels, and the animals will be observed carefully for any signs of toxicity. Observations will include changes in behavioral, neurological, and autonomic responses such as alertness, locomotor activity, tremors, convulsions, salivation, respiration, sleep, coma, and mortality. Animals will be continuously monitored during the first 4 hours after administration and periodically observed for 24 hours, followed by daily observation for a total period of 14 days to identify delayed toxic effects or mortality. The results obtained from the toxicity study will be used for selection of safe and effective dose levels for subsequent pharmacological evaluation.

Experimental Design

Animals will be randomly divided into five experimental groups consisting of six animals in each group (n = 6). The treatment schedule will be designed to evaluate the neuroprotective effect of Bacopa monnieri extract against scopolamine-induced memory impairment in mice.

The plant extract and standard drug will be administered orally once daily for 14 consecutive days. Memory impairment will be induced by intraperitoneal administration of scopolamine (1 mg/kg) from day 8 to day 14.

Table 1: Animals Grouping

Group	Treatment
Group I	Normal control (Vehicle only)
Group II	Scopolamine control (1 mg/kg, i.p.)
Group III	Standard treatment (Donepezil 2.5 mg/kg, p.o. + Scopolamine)
Group IV	Bacopa monnieri extract low dose + Scopolamine
Group V	Bacopa monnieri extract high dose + Scopolamine

Result And Discussion

Percentage Yield of Extract

The hydroalcoholic extraction of Bacopa monnieri by Soxhlet apparatus produced a dark green semisolid extract with satisfactory yield **14.4%.**

Acute Oral Toxicity Study

No mortality or significant behavioral abnormalities were observed in animals treated with Bacopa monnieri extract during the 14-day observation period. The extract was found to be safe at the tested dose levels.

Y-Maze Test

Bacopa monnieri extract significantly improved spontaneous alternation behavior in scopolamine-induced memory impaired mice when compared with disease control group

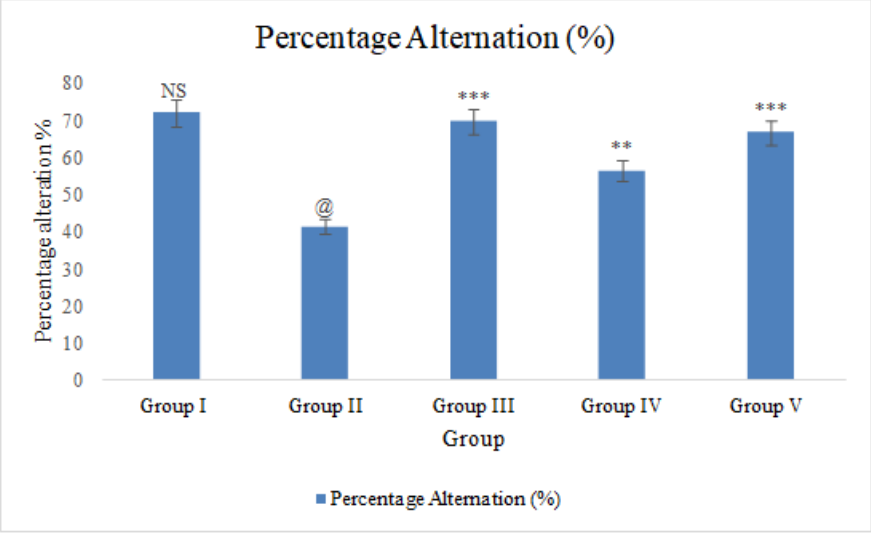


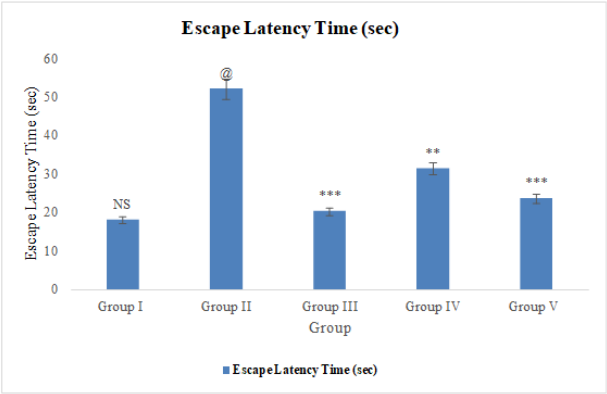
Fig. 2 : Effect of *Bacopa monnieri* extract on percentage Alteration

Morris Water Maze Test

Treatment with Bacopa monnieri extract significantly reduced escape latency time and increased time spent in target quadrant indicating improvement in spatial learning and memory.

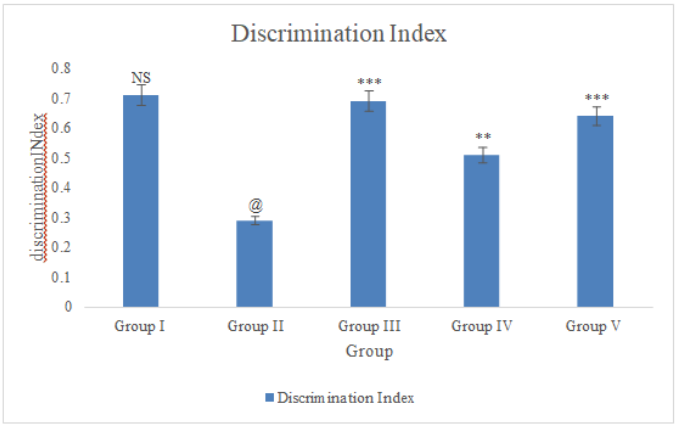
Table 2: Effect of *Bacopa monnieri* extract in Morris Water Maze Test

Group	Group	Escape Latency Time (sec)	Time in Target Quadrant (sec)
Group I	Normal Control	18.22 ± 1.13	34.41 ± 1.45
Group II	Scopolamine Control	52.34 ± 2.11***	14.26 ± 1.22***
Group III	Donepezil + Scopolamine	20.43 ± 1.28###	32.75 ± 1.17###
Group IV	Bacopa monnieri Low Dose	31.62 ± 1.46**	25.32 ± 1.31**
Group V	Bacopa monnieri High Dose	23.84 ± 1.17###	30.61 ± 1.26###



Novel Object Recognition Test

Bacopa monnieri treated groups showed significant increase in discrimination index indicating improvement in recognition memory.



Passive Avoidance Test

Administration of Bacopa monnieri extract significantly increased step-through latency compared with scopolamine control group.

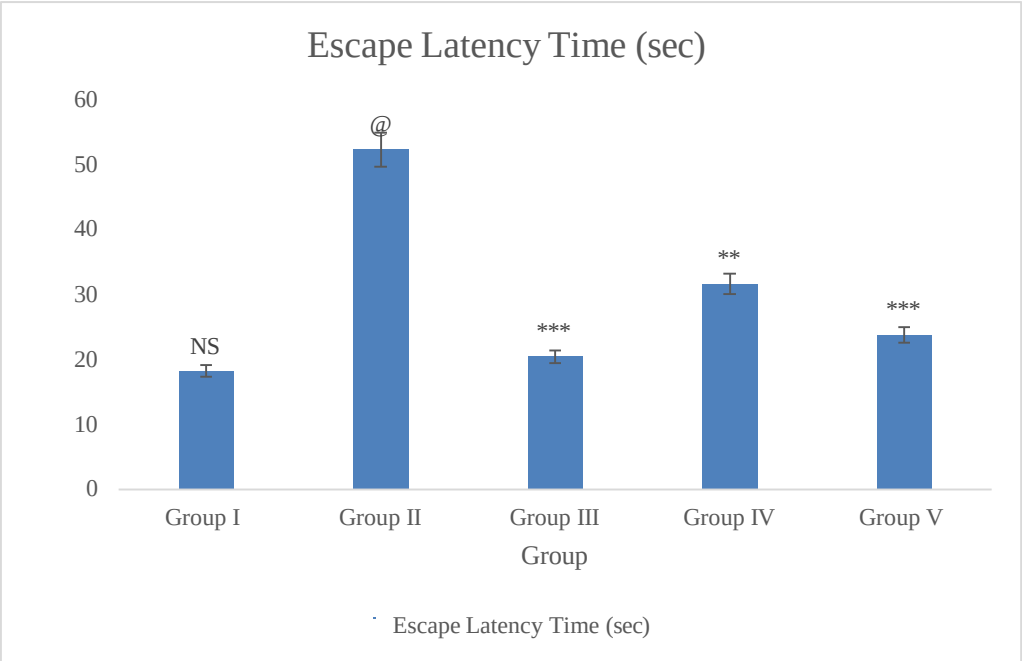


Table 3: Effect of *Bacopa monnieri* extract on Passive Avoidance Test

Group	Group	Step-through Latency (sec)
Group I	Normal Control	198.45 ± 4.32
Group II	Scopolamine Control	72.16 ± 3.21***
Group III	Donepezil + Scopolamine	185.24 ± 3.86###
Group IV	Bacopa monnieri Low Dose	141.38 ± 3.47**
Group V	Bacopa monnieri High Dose	176.42 ± 3.11###

Biochemical Estimation

Estimation of Acetylcholinesterase (AChE)

Scopolamine administration significantly increased AChE activity, whereas treatment with Bacopa monnieri extract significantly reduced AChE levels.

Table 4: Effect of *Bacopa monnieri* extract on Acetylcholinesterase (AChE) level

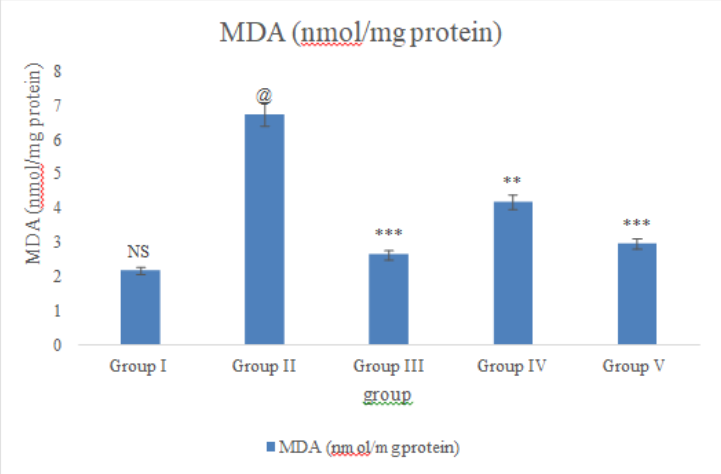
Group	Group	AChE Activity (µmol/min/mg protein)
Group I	Normal Control	12.18 ± 0.84
Group II	Scopolamine Control	28.34 ± 1.12***
Group III	Donepezil + Scopolamine	14.26 ± 0.93###
Group IV	Bacopa monnieri Low Dose	20.13 ± 1.04**
Group V	Bacopa monnieri High Dose	15.81 ± 0.96###

Estimation of Lipid Peroxidation (MDA)

MDA levels were significantly elevated in scopolamine-treated animals, while Bacopa monnieri extract reduced lipid peroxidation.

Table 5: Effect of *Bacopa monnieri* extract on MDA Level

Group	Group	MDA (nmol/mg protein)
Group I	Normal Control	2.18 ± 0.14
Group II	Scopolamine Control	6.75 ± 0.28***
Group III	Donepezil + Scopolamine	2.64 ± 0.17###
Group IV	Bacopa monnieri Low Dose	4.18 ± 0.19**
Group V	Bacopa monnieri High Dose	2.96 ± 0.16###



Estimation of Superoxide Dismutase (SOD)

Treatment with Bacopa monnieri extract significantly increased SOD activity when compared with scopolamine control group.

Table 6: Effect of *Bacopa monnieri* extract on SOD Level

Group	Group	SOD Activity (U/mg protein)
Group I	Normal Control	15.82 ± 0.65
Group II	Scopolamine Control	7.18 ± 0.42***
Group III	Donepezil + Scopolamine	14.93 ± 0.61###
Group IV	Bacopa monnieri Low Dose	11.76 ± 0.54**
Group V	Bacopa monnieri High Dose	13.84 ± 0.59###

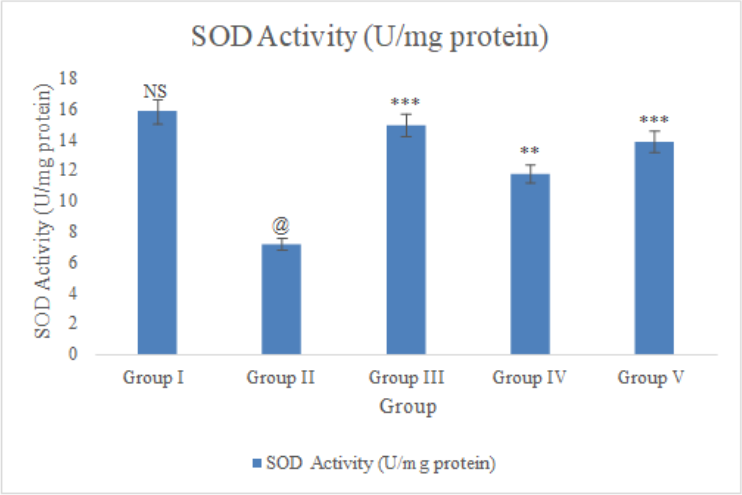


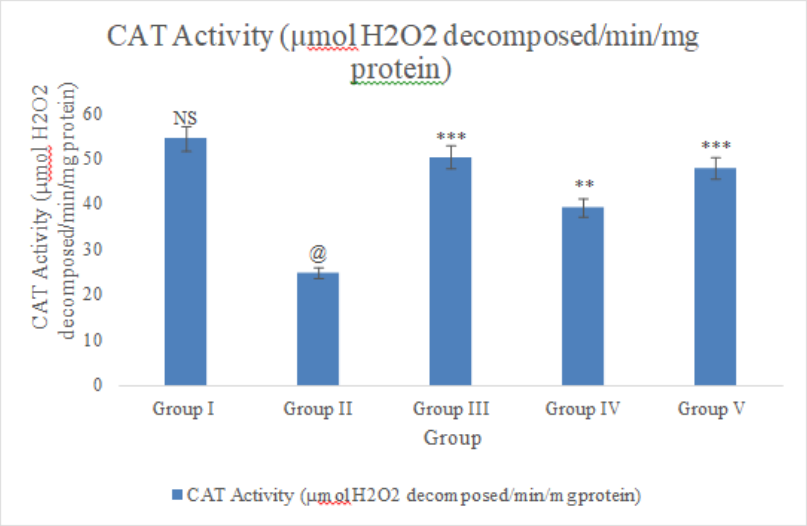
Fig 3 : Effect of *Bacopa monnieri* extract on SOD Level

Estimation of Catalase (CAT)

Catalase activity was significantly restored by Bacopa monnieri extract treatment.

Table 7: Effect of *Bacopa monnieri* extract CAT Level

Group	Group	CAT Activity (µmol H2O2 decomposed/min/mg protein)
Group I	Normal Control	54.36 ± 2.15
Group II	Scopolamine Control	24.75 ± 1.42***
Group III	Donepezil + Scopolamine	50.28 ± 1.84###
Group IV	Bacopa monnieri Low Dose	39.16 ± 1.65**
Group V	Bacopa monnieri High Dose	47.83 ± 1.73###



Estimation of Reduced Glutathione (GSH)

Bacopa monnieri extract significantly elevated reduced glutathione levels in brain tissue.

Table 8: Effect of *Bacopa monnieri* extract on GSH Level

Group	Group	GSH (µg/mg protein)
Group I	Normal Control	8.64 ± 0.42
Group II	Scopolamine Control	3.12 ± 0.24***
Group III	Donepezil + Scopolamine	7.95 ± 0.31###
Group IV	Bacopa monnieri Low Dose	5.84 ± 0.28**
Group V	Bacopa monnieri High Dose	7.28 ± 0.34###

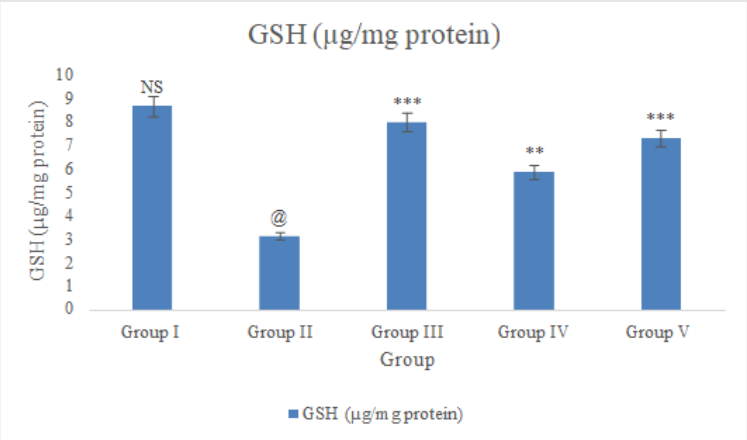


Fig 4: Effect of *Bacopa monnieri* extract on GSH Level

Histopathological Study

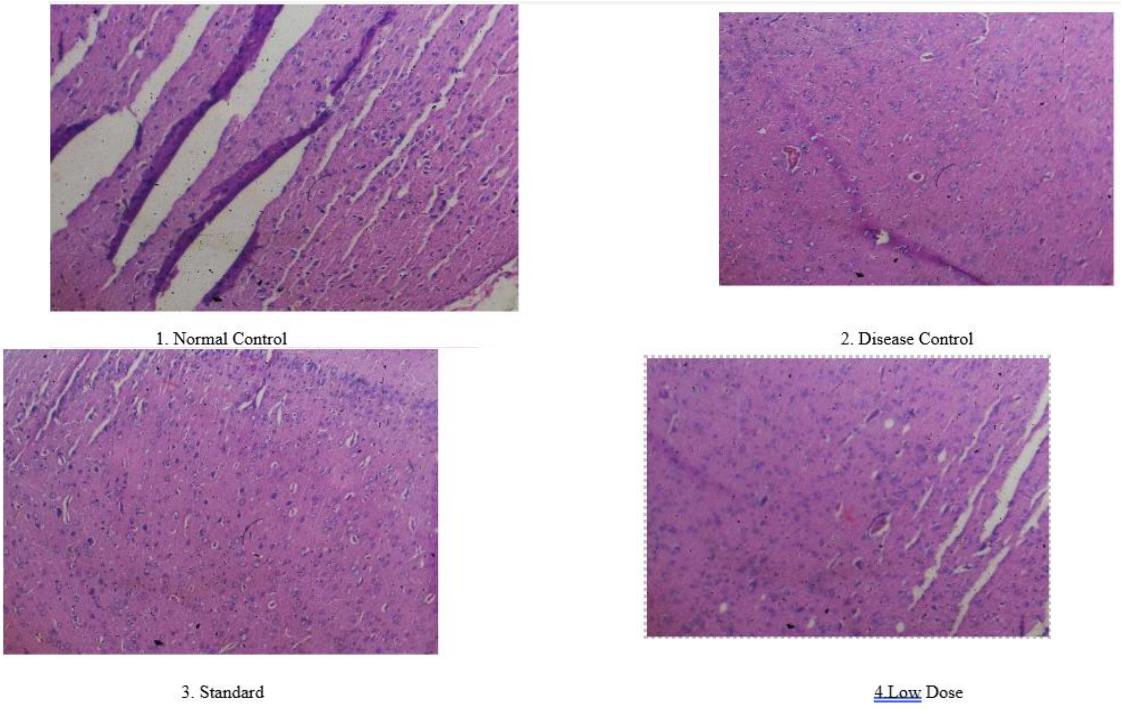


Fig 5 : Histopathological Study

IV. CONCLUSION

The present study demonstrated that the hydroalcoholic extract of Bacopa monnieri possesses significant neuroprotective and cognition-enhancing activity against scopolamine-induced memory impairment in mice. Preliminary phytochemical screening and HPLC analysis confirmed the presence of important bioactive constituents including bacosides, flavonoids, phenolic compounds, and saponins. Behavioral studies such as Y-maze, Morris water maze, Novel Object Recognition, and Passive Avoidance tests revealed marked improvement in learning, memory, and cognitive performance following treatment with the extract. Biochemical estimations showed significant reduction in acetylcholinesterase activity and lipid peroxidation along with restoration of endogenous antioxidant enzymes including SOD, CAT, and GSH. Histopathological examination further demonstrated protection of hippocampal neurons and restoration of normal neuronal architecture. The neuroprotective activity of Bacopa monnieri may be attributed to enhancement of cholinergic neurotransmission, antioxidant defense, anti-inflammatory action, and inhibition of neuronal degeneration. The high-dose extract exhibited effects comparable to the standard drug donepezil. The study scientifically validates the traditional use of Bacopa monnieri as a memory-enhancing medicinal plant. Overall, the findings suggest that Bacopa monnieri has promising therapeutic potential for the management of neurodegenerative disorders such as Alzheimer's disease.

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