RESEARCH PAPER

PHARMACOLOGICAL INVESTIGATION OF STANDARDIZED BIOACTIVE FRACTIONS OF *STRYCHNOS NUX-VOMICA* AGAINST SCOPOLAMINE INDUCED MEMORY IMPAIRMENT IN EXPERIMENTAL RATS

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Abstract

Memory impairment is a major clinical feature of neurodegenerative disorders such as Alzheimer's disease, where cholinergic dysfunction, oxidative stress, neuroinflammation, and neuronal degeneration contribute to progressive cognitive decline. Although currently available therapeutic agents provide symptomatic relief, their long-term efficacy is limited and is frequently associated with adverse effects. Consequently, there is growing interest in identifying plant-derived neuroprotective agents with multi-target pharmacological actions. *Strychnos nux-vomica* has been traditionally used in various medicinal systems for neurological disorders after appropriate detoxification. It contains several biologically active phytoconstituents that may exhibit antioxidant, anti-inflammatory, and neuroprotective properties. The present study aims to investigate the pharmacological efficacy of standardized bioactive fractions of detoxified *Strychnos nux-vomica* against scopolamine-induced memory impairment in experimental rats. Memory impairment will be induced by scopolamine administration, and cognitive performance will be evaluated using validated behavioural paradigms including the Morris Water Maze, Y-Maze, Novel Object Recognition Test, and Passive Avoidance Test. The neuroprotective potential of the standardized fractions will be assessed by estimating acetylcholinesterase activity, oxidative stress markers (malondialdehyde, superoxide dismutase, catalase, and reduced glutathione) was performed to evaluate neuronal integrity into the observed pharmacological effects.

It is anticipated that standardized bioactive fractions of detoxified *Strychnos nux-vomica* will significantly improve learning and memory, reduce oxidative stress and neuroinflammation, inhibit acetylcholinesterase activity, and protect neurons from degeneration. The findings of this study may establish *Strychnos nux-vomica* as a promising source of neuroprotective phytoconstituents and provide a scientific basis for the development of safer, standardized phytopharmaceuticals for the management of memory impairment and Alzheimer's disease

Keywords: *Strychnos nux-vomica,* Memory impairment, oxidative stress

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1. INTRODUCTION

Learning and memory are fundamental cognitive processes that enable organisms to acquire, retain, store and retrieve knowledge necessary for adaptation and survival. Memory is the ability to encode, store and retrieve information when needed.[1,2] Learning is the process of acquiring new information, skills or experiences. The traditional Ayurvedic medical system describes three basic elements of mental functions, Dhi (learning or acquisition of knowledge), Dhriti (retention of knowledge) and Smriti (recall of stored information).[3,4] Disruption of any of these systems can lead to memory loss, learning difficulties and cognitive impairment. Memory is one of the most important functions of the central nervous system and is vital for learning, decision making, problem solving and performance of daily tasks.[5,6]

Memory can be broadly classified into three types depending on the time duration of storing

information: sensory memory, short term memory, and long-term memory. There are also two types . Non-declarative (implicit) memory (learned skills, habits , conditioning , procedural activities) and declarative (explicit) memory (conscious recollection of facts and events).[7,8] Memory formation is a complicated neurobiological process involving synaptic plasticity, neurotransmitter release, gene expression, and protein synthesis. Brain regions like the hippocampus, cerebral cortex and amygdala are involved in learning and memory consolidation.[9,10] Long-term potentiation (LTP), a key cellular mechanism of memory formation, is regulated by neurotransmitter systems, including the cholinergic system, and glutamatergic receptors such as the N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors. These signaling pathways involving brain-derived neurotrophic factor (BDNF), protein kinase C (PKC) and cAMP response element-binding protein (CREB) become activated to improve neuronal transmission and promote synaptic remodeling, both of which are crucial for long-term memory.[11,12]

Memory impairment is linked with ageing and several neurological diseases including Alzheimer's disease (AD), Parkinson's disease, vascular dementia, schizophrenia, traumatic brain injury and cerebrovascular diseases.[13,14] Alzheimer's disease is the most common form of dementia and is marked by a gradual loss of behavior and thinking and memory skills. The pathological features of AD are extracellular β-amyloid plaque formation, intracellular neurofibrillary tangles of hyperphosphorylated tau protein, oxidative stress, neuroinflammation, mitochondrial dysfunction, cholinergic neuronal degeneration, and synaptic loss.[15,16] One of the main treatment approaches for AD management is acetylcholinesterase (AChE) inhibition, as cholinergic dysfunction is believed to be a significant factor in cognitive loss.[17]

Scopolamine, a non-selective antagonist at muscarinic acetylcholine receptors, is often used to reversibly impair memory in laboratory animals. It disrupts central cholinergic neurotransmission and causes learning, attention and memory problems similar to those of early Alzheimer's disease.[18,19] Therefore, the scopolamine induced amnesia model has been proposed as a reliable experimental model to evaluate potential neuroprotective and nootropic drugs. Cognitive ability in these models is often tested behaviorally with tasks such as the Morris Water Maze, Y-Maze, Novel Object Recognition Test and Passive Avoidance Test.[20,21]

Although synthetic drugs, such as donepezil, rivastigmine, galantamine, and memantine, are available, their clinical efficacy is still limited and long-term use is often associated with side effects, such as hepatotoxicity, dizziness, insomnia, gastrointestinal disorders and cardiovascular problems.[22,23] Hence, the study of medicinal plants as multi-target, safer alternatives for the treatment of cognitive problems is gaining attention. Various phytochemicals such as flavonoids, alkaloids, phenolics, tannins and terpenoids possessing antioxidant, anti-inflammatory, anti-apoptotic, and acetylcholinesterase inhibitory properties may achieve neuroprotection against cognitive decline.[24,25]

Strychnos nux-vomica after proper purification is an important therapeutic herb which has been used for a long time in ayurvedic and other indigenous medical systems. The seeds contain toxic alkaloids such as brucine and strychnine; however, detoxified preparations and well-standardized bioactive fractions have demonstrated significant pharmacological activities, including antioxidant, anti-inflammatory, analgesic, neuroprotective, and immunomodulatory effects. The presence of diverse bioactive phytoconstituents suggests that standardized fractions of Strychnos nux-vomica may affect multiple molecular pathways involved in cognitive impairment.[26,27]

2. METHODOLOGY

2.1 Collection of Plant Material:

Dried seeds of Strychnos nux-vomica were obtained from a licensed local herbal supplier. The plant material was checked by a qualified taxonomist, cleaned of any unwanted material and stored under appropriate conditions until needed again. After confirmation, the seeds were grinded into powder for research on extraction, fractionation and characterisation of phytochemicals.

2.2 Extraction & isolation of fractions:

The powdered dried seeds of Strychnos nux-vomica were extracted with a series of solvents using a Soxhlet apparatus. The progressive extraction was carried out using petroleum ether, chloroform, acetone, methanol and ethanol as solvents of increasing polarity. Each extraction cycle was continued until all the plant material was used up. The extracts were concentrated under reduced pressure using a rotary vacuum evaporator and stored in sealed containers for further analysis.

Further separation of the ethanolic extract was considered for the first biological activity screening test with the most promising therapeutic potential. The concentrated ethanolic extract was floated in distilled water and partitioned with ethyl acetate and n-butanol. The n-butanol and ethyl acetate fractions were separated, concentrated under low pressure, dried and stored for further phytochemical and biological study.

2.3 PHARMACOLOGICAL STUDY

2.3.1 Animals

The acute toxicity studies were carried out in healthy adult male and female Swiss albino mice, weighing 18–20 g and 8–10 weeks old. Healthy adult Wistar rats of either sex weighing 180-220 g (8-10 weeks old) were used for pharmacological investigations. The animals were obtained from CPCSEA registered animal facility and were housed in hygienic polypropylene cages under conventional laboratory conditions like 12 h light/dark cycle, temperature 25 ± 2 °C and relative humidity 50 ± 5%. During the trial, animals had ad libitum access to filtered drinking water and standard pellet feed. The animals were acclimatised to laboratory conditions for 7 days before the start of the trial. Each experimental group contained six animals (n = 6). All the experimental procedures were performed during light phase (08:00–16:00 h) following the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) with prior approval of Institutional Animal Ethics Committee (IAEC).

2.3.2 Oral acute toxicity Studies:

Acute oral toxicity of the standardized Strychnos nux-vomica seed extract was assessed in healthy adult female albino rats as per the Organization for Economic Co-operation and Development (OECD) Guideline No. 420 (Fixed Dose Procedure). The trial was performed at the limit dose of 2000 mg/kg bw. Animals were fasted overnight with free access to water before dosing. The extract was administered as a single oral dose in 0.5% w/v sodium carboxymethylcellulose (CMC). Careful observation of the animals was made for the first 3 h after treatment, with special attention to the changes in general behaviour and the functions of the central nervous system (CNS) and autonomic nervous system (ANS). Daily observations for 14 days and at regular intervals during the first 24 hours were made to observe signs of toxicity, morbidity, mortality and any delayed adverse effects.

2.3.3 Pharmacological Animal Testing of extracts and fractions

In order to induce memory impairment, we used scopolamine hydrobromide, a non-selective muscarinic acetylcholine receptor antagonist that is widely used to produce experimental models of amnesia. Scopolamine was dissolved in sterile normal saline (0.9% NaCl) and administered intraperitoneally (i.p.) at a dose of 3 mg/kg body weight in a dosing volume of 1 mL/kg. The drug blocks central cholinergic neurotransmission and produces short-term cognitive deficits and mimics the memory loss associated with dementia and Alzheimer's disease. Five minutes after scopolamine administration, when the cognitive impairment was sufficiently developed, behavioral tests of memory and learning were initiated. This model was used to evaluate the efficacy of standardized bioactive fractions of Strychnos nux-vomica in protecting experimental rats against scopolamine-induced learning and memory impairment.

2.3.3.1 Morris water maze test

The spatial learning and memory of experimental rats was assessed using Morris Water Maze (MWM) test. The apparatus consisted of a circular tank (60 cm diameter, 26 cm high) filled with opaque water at a temperature of 26 ± 1°C and a hidden platform submerged 1 cm below the water surface. Animals were trained from different starting points for four trials per day for five consecutive days with the pool divided into four quadrants. Each trial lasted a maximum of 120 seconds. Spatial learning was evaluated by recording the escape latency time (ELT) to locate the hidden platform**.**

- Group I (Normal Control): Received 0.1% NaCMC orally as the vehicle control.
- Group II (Disease Control): Received Scopolamine (3 mg/kg, i.p.) to induce memory impairment.
- Group III: Received ethanolic extract of *Strychnos nux-vomica* (100 mg/kg, p.o.) followed by Scopolamine (3 mg/kg, i.p.).
- Group IV: Received acetone extract (100 mg/kg, p.o.) plus Scopolamine (3 mg/kg, i.p.).
- Group V: Received methanolic extract (100 mg/kg, p.o.) plus Scopolamine (3 mg/kg, i.p.).
- Group VI: Received chloroform extract (100 mg/kg, p.o.) plus Scopolamine (3 mg/kg, i.p.).
- Group VII (Positive Control): Received Piracetam (100 mg/kg, p.o.) plus Scopolamine (3 mg/kg, i.p.).

2.3.3.2 Passive avoidance test: Light and dark model

The Passive Avoidance Test was employed to evaluate learning and memory retention in experimental rats. The apparatus consisted of two compartments, a brightly illuminated chamber and a dark chamber, separated by a sliding door, with the floor of the dark chamber connected to an electric shock generator. On the first day, each rat was allowed to explore both compartments for 5 minutes. During the training session, the rat was placed in the light chamber, and upon entering the dark chamber, a mild foot shock (2.5 mA for 1 second) was delivered. The animal remained in the dark chamber for an additional 10 seconds before

being returned to its home cage. After 24 hours, the retention test was conducted by placing the rat in the light chamber and recording the step-through latency (time taken to enter the dark chamber) and the time spent in each compartment. Increased latency to enter the dark chamber was considered an indicator of improved learning and memory retention.

- **Group I** (Normal Control, NC): Received 0.1% NaCMC solution (vehicle) orally throughout the experimental period.
- **Group II** (Disease Control, DC): Received Scopolamine (3 mg/kg, i.p.) to induce memory impairment.
- **Group III** (TC-ETOH): Received ethanolic extract of *Strychnos nux-vomica* (100 mg/kg, p.o.) followed by Scopolamine (3 mg/kg, i.p.).
- **Group IV** (TC-ACT): Received acetone extract of *Strychnos nux-vomica* (100 mg/kg, p.o.) followed by Scopolamine (3 mg/kg, i.p.).
- **Group V** (TC-MEOH): Received methanolic extract of *Strychnos nux-vomica* (100 mg/kg, p.o.) followed by Scopolamine (3 mg/kg, i.p.).
- **Group VI** (TC-$CHCl_3$): Received chloroform extract of *Strychnos nux-vomica* (100 mg/kg, p.o.) followed by Scopolamine (3 mg/kg, i.p.).
- **Group VII** (Positive Control, PC): Received Piracetam (100 mg/kg, p.o.) followed by Scopolamine (3 mg/kg, i.p.) as the standard nootropic treatment.

2.3.3.3 High Plus Maze test

The Elevated Plus Maze (EPM) was used to assess learning and memory in experimental rats. The apparatus consisted of two open arms (16 × 5 cm) and two enclosed arms (16 × 5 × 12 cm), connected by a central platform (5 × 5 cm) and elevated 25 cm above the floor. On the first day (training session), each animal was placed at the end of an open arm facing away from the central platform. The transfer latency (TL), defined as the time taken by the animal to enter a closed arm with all four paws, was recorded. If the animal failed to enter a closed arm within 90 seconds, it was gently guided into the arm and assigned a TL of 90 seconds. After remaining in the closed arm for 10 seconds, the animal was returned to its home cage. A retention test was performed 24 hours later, and the transfer latency was recorded again to evaluate memory retention.

- **Group I** (Normal Control, NC): Received 0.1% NaCMC orally as the vehicle control.
- **Group II** (Disease Control, DC): Received Scopolamine (3 mg/kg, i.p.) to induce memory impairment.
- **Group III** (TC-ETOH): Received ethanolic extract of *Strychnos nux-vomica* (100 mg/kg, p.o.) followed by Scopolamine (3 mg/kg, i.p.).
- **Group IV** (TC-ACT): Received acetone extract of *Strychnos nux-vomica* (100 mg/kg, p.o.) followed by Scopolamine (3 mg/kg, i.p.).
- **Group V** (TC-MEOH): Received methanolic extract of *Strychnos nux-vomica* (100 mg/kg, p.o.) followed by Scopolamine (3 mg/kg, i.p.).
- **Group VI** (TC-$CHCl_3$): Received chloroform extract of *Strychnos nux-vomica* (100 mg/kg, p.o.) followed by Scopolamine (3 mg/kg, i.p.).
- **Group VII** (Positive Control, PC): Received Piracetam (100 mg/kg, p.o.) as the standard drug, followed by Scopolamine (3 mg/kg, i.p.) to evaluate its protective effect against scopolamine-induced cognitive impairment.

2.3.3.4 Test of active avoidance: Pole climbing model

The Step-Down Passive Avoidance Test was used to evaluate long-term memory retention in experimental rats. Each animal was placed on a wooden platform positioned at the center of a grid floor connected to an electric shock source. The step-down latency (SDL), defined as the time taken to place all four paws on the grid, was recorded. During the training session, stepping onto the grid resulted in a 20 V AC foot shock for 15 seconds. A retention test was conducted 24 hours later without administering a shock, and increased SDL was considered indicative of improved learning and memory retention.

- **Group I** (Normal Control, NC): Received 0.1% NaCMC solution (vehicle, p.o.) throughout the study.
- **Group II** (Disease Control, DC): Received Scopolamine (3 mg/kg, i.p.) to induce memory impairment.
- **Group III** (TC-ETOH): Received ethanolic extract of *Strychnos nux-vomica* (100 mg/kg, p.o.) followed by Scopolamine (3 mg/kg, i.p.).
- **Group IV** (TC-ACT): Received acetone extract of *Strychnos nux-vomica* (100 mg/kg, p.o.) followed by Scopolamine (3 mg/kg, i.p.).
- **Group V** (TC-MEOH): Received methanolic extract of *Strychnos nux-vomica* (100 mg/kg, p.o.) followed by Scopolamine (3 mg/kg, i.p.).

- **Group VI** (TC-$CHCl_3$): Received chloroform extract of *Strychnos nux-vomica* (100 mg/kg, p.o.) followed by Scopolamine (3 mg/kg, i.p.).
- **Group VII** (Positive Control, PC): Received Piracetam (100 mg/kg, p.o.) as the standard nootropic drug, followed by Scopolamine (3 mg/kg, i.p.) to evaluate its protective effect against scopolamine-induced memory impairment.

2.3.3.5 Hole-board test

The Hole-Board Test was used to evaluate spatial learning and memory in experimental rats. The apparatus consisted of a square wooden box (50 × 50 × 50 cm) containing four holes (3 cm diameter), with one hole consistently baited with food throughout the experiment. During the acquisition trial, each rat was placed in the center of the board and allowed to explore freely for 3 minutes to locate the baited hole. A retention test was conducted 24 hours later to assess memory performance. The apparatus was thoroughly cleaned between trials to eliminate olfactory cues.

- **Group I** (Normal Control, NC): Received 0.1% NaCMC solution (vehicle, p.o.) throughout the experimental period.
- **Group II** (Disease Control, DC): Received Scopolamine (3 mg/kg, i.p.) to induce memory impairment.
- **Group III** (TC-ETOH): Received ethanolic extract of *Strychnos nux-vomica* (100 mg/kg, p.o.) followed by Scopolamine (3 mg/kg, i.p.).
- **Group IV** (TC-ACT): Received acetone extract of *Strychnos nux-vomica* (100 mg/kg, p.o.) followed by Scopolamine (3 mg/kg, i.p.).
- **Group V** (TC-MEOH): Received methanolic extract of *Strychnos nux-vomica* (100 mg/kg, p.o.) followed by Scopolamine (3 mg/kg, i.p.).
- **Group VI** (TC-$CHCl_3$): Received chloroform extract of *Strychnos nux-vomica* (100 mg/kg, p.o.) followed by Scopolamine (3 mg/kg, i.p.).
- **Group VII** (Positive Control, PC): Received Piracetam (100 mg/kg, p.o.) as the standard nootropic drug, followed by Scopolamine (3 mg/kg, i.p.) to evaluate its protective effect against scopolamine-induced cognitive impairment.

2.4 Dissection and Homogenization

At the end of the experimental period, the animals were euthanized by cervical dislocation, and the brains were immediately excised, washed with ice-cold 0.9% normal saline, blotted dry, and weighed. Brain tissues were homogenized (10% w/v) in 0.1 M phosphate buffer (pH 7.4) and centrifuged at 12,000 × g for 60 minutes at 4°C to obtain the post-nuclear supernatant for biochemical analysis. Total protein content was estimated using the Lowry method. Briefly, 100 mg of brain tissue was homogenized in 2.5 mL normal saline, followed by the addition of 2.5 mL of 10% trichloroacetic acid (TCA). After centrifugation at 2500 rpm for 20 minutes, the precipitate was dissolved in 5 mL of 0.1 N sodium hydroxide. The sample was reacted with alkaline copper reagent and Folin–Ciocalteu phenol reagent, incubated for 30 minutes, and the absorbance was measured at 578 nm using a UV-Visible spectrophotometer. Total protein concentration was expressed as g/g fresh tissue weight.

2.5 Determination of markers of oxidative stress

2.5.1 Catalase CAT:

Catalase (CAT) activity was determined in the brain homogenate by measuring the decomposition of hydrogen peroxide (H_2O_2). The reaction mixture consisted of diluted brain homogenate, 0.1 M phosphate buffer (pH 7.4), distilled water, and H_2O_2 to initiate the reaction. After incubation at 37°C, the reaction was terminated by adding potassium dichromate-acetic acid reagent. The tubes were heated in a boiling water bath for 15 minutes, cooled to room temperature, and the absorbance was measured at 570 nm using a UV-Visible spectrophotometer. Catalase activity was calculated using the molar extinction coefficient and expressed as nmol H_2O_2 decomposed/mg protein.

2.5.2 Lipid peroxidation LPO

Lipid peroxidation was estimated by measuring malondialdehyde (MDA) levels using the thiobarbituric acid reactive substances (TBARS) assay. Brain homogenate (0.1 mL) was mixed with 20% acetic acid (pH 3.5) and 0.8% thiobarbituric acid (TBA), vortexed, and heated in a boiling water bath at 95°C for 60 minutes. After cooling, n-butanol:pyridine (15:1) was added, followed by centrifugation at 2200 × g for 10 minutes. The absorbance of the clear pink supernatant was measured at 532 nm, and MDA concentration was calculated using the molar extinction coefficient and expressed as nmol MDA/mg protein.

2.5.3 Reduced Glutathione GSH

Reduced glutathione (GSH) levels were estimated using the Ellman method, based on the reaction between GSH and 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB), which produces a yellow-colored chromogen. Briefly, 0.5 mL of brain homogenate was mixed with 0.1 mL of 25% trichloroacetic acid (TCA) to precipitate proteins and centrifuged at 4000 rpm for 5 minutes. The supernatant was

mixed with 0.1 M phosphate buffer (pH 7.4) and DTNB reagent, incubated for 10 minutes, and the absorbance was measured at 412 nm. Reduced glutathione concentration was calculated using the extinction coefficient and expressed as µmol GSH/mg protein.

3. RESULT AND DISCUSSION

3.1 Acute toxicity studies of different extracts of *Strychnos nux-vomica*seedss

S. No.	Treatment	Acute Oral Toxicity	Acute Dermal Toxicity
1	*S. nux-vomica* Ethanol extract	Safe	Safe
2	*S. nux-vomica* Methanol Extract	Safe	Safe
3	*S. nux-vomica* Petroleum Ether Extract	Safe	Safe
4	*S. nux-vomica* Chloroform Extract	Safe	Safe
5	*S. nux-vomica* Acetone Extract	Safe	Safe

Table 1. Acute toxicity studies of different extracts of S. nux-vomica seeds

3.2 Effect of *Strychnos nux-vomica*extracts on the scopolamine induced spatial learning and memory deficit using Morris water maze test

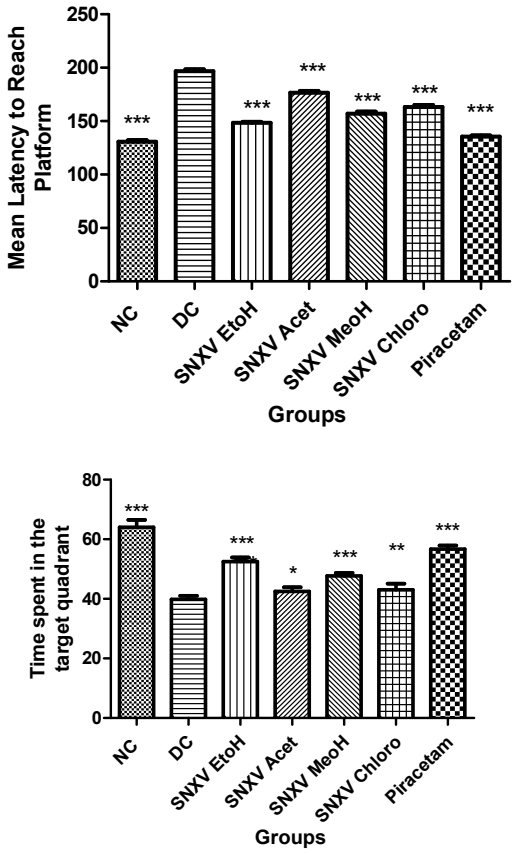

Figure 1. Effect of S. nux-vomica extracts in Morris water maze test on mean latencies and Time spent

Treatment with *S. nux-vomica* extracts significantly improved spatial learning and memory in scopolamine-induced rats, as evidenced by reduced mean escape latency compared with the disease control group. Among the extracts, the acetone and chloroform fractions showed the greatest improvement, with effects comparable to the standard drug, Piracetam.

The Morris Water Maze results demonstrated that treatment with *S. nux-vomica* extracts significantly increased the time spent in the target quadrant compared with the disease control group, indicating improved spatial memory retention. Among the tested extracts, the ethanolic and methanolic fractions showed marked enhancement in target quadrant occupancy, while the acetone and chloroform extracts also produced significant improvements. The standard drug, Piracetam, exhibited the highest increase in time spent in the target quadrant. These findings suggest that *S. nux-vomica* extracts effectively attenuated scopolamine-induced cognitive deficits and enhanced memory performance.

3.2 Target quadrant

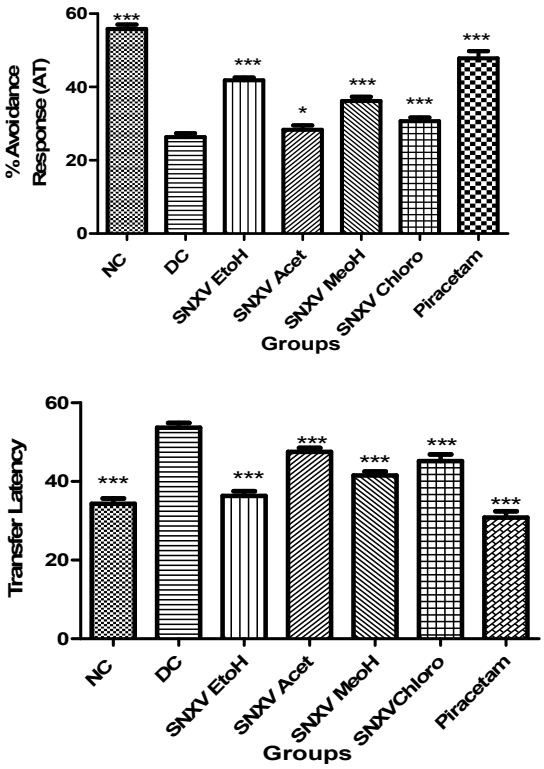

Figure 2. Effect of Strychnos nux-vomicaextracts on the transfer latency in the acquisition AT using elevated plus maze in the scopolamine induced amnesiac rats

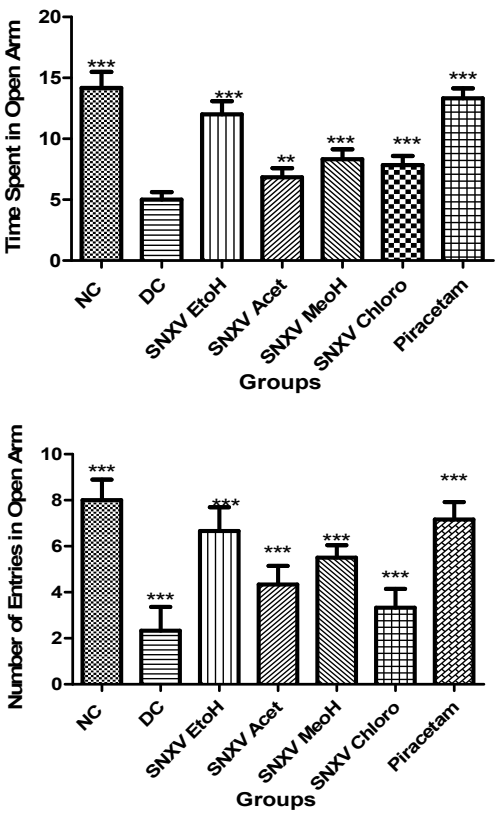

Figure 3. Effect of Strychnos nux-vomica extracts on the open arm time spent and entry in the acquisition AT using elevated plus maze in the scopolamine induced amnesiac rats

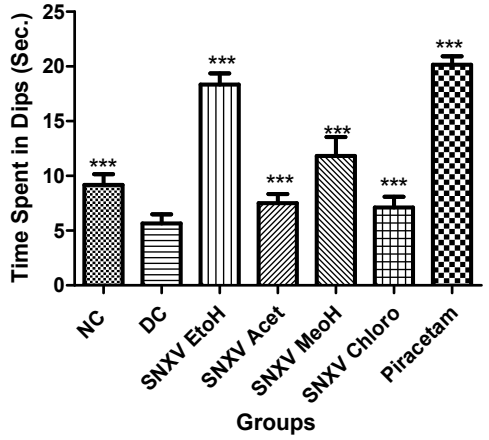

Figure 4. Effect of Strychnos nux-vomicaextracts on the time spent in the dips s of the hole board model in the scopolamine induced amnesiac rats

3.3 Response to Strychnos nux-vomica extracts of the oxidative stress markers

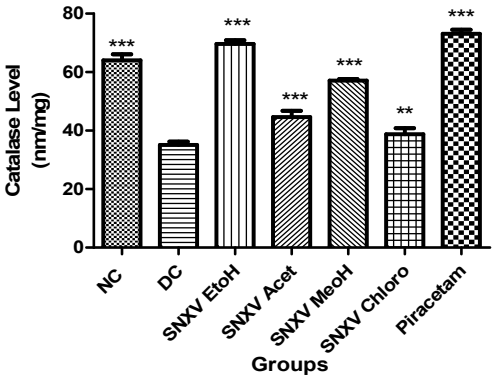

Figure 5. Effect of Strychnos nux-vomicaextracts on the markers of oxidative stress Catalase CAT level in the scopolamine induced amnesiac rats.

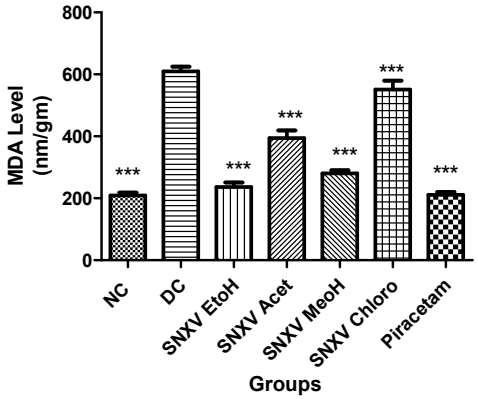

Figure 6. Effect of Strychnos nux-vomica extracts on the markers of oxidative stress Lipid peroxidation MDA level in the scopolamine induced amnesiac rats

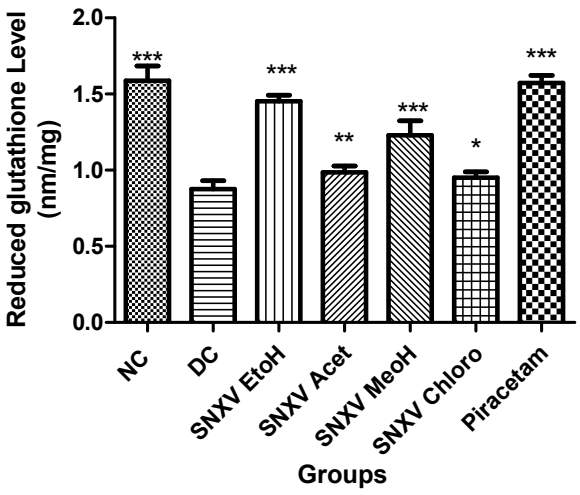

Figure 7. Effect of Strychnos nux-vomica extracts on the markers of oxidative stress Reduced Glutathione GSH level in the scopolamine induced amnesiac rats

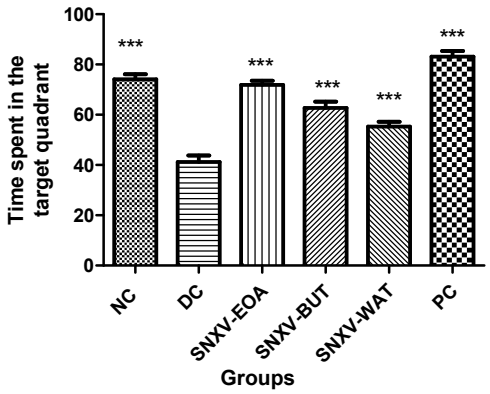

Figure 8. Effect of fraction of S. nux-vomica Ethanol extract in Morris water maze test on Time spent in the target quadrant

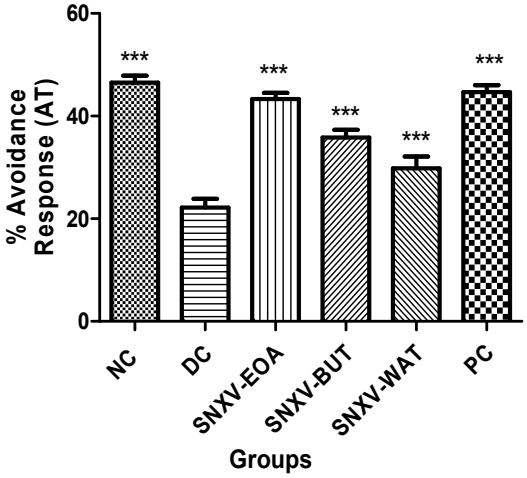

Figure 9. Effect of fraction of S. nux-vomica Ethanol extract on the % avoidance response AR in the acquisition AT of scopolamine Induced amnesiac rats using light & dark model in the passive avoidance test

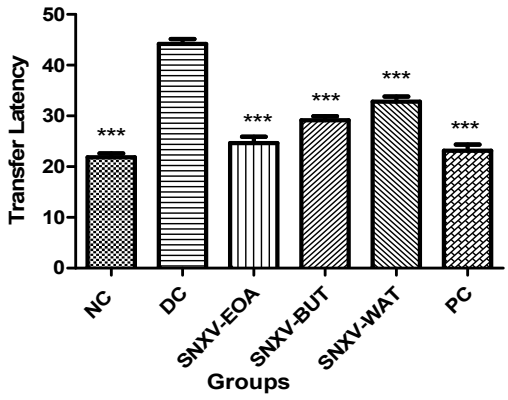

Figure 10. Effect of fraction of S. nux-vomica Ethanol extract on the transfer latency in the acquisition AT using elevated plus maze in the scopolamine induced amnesiac rats

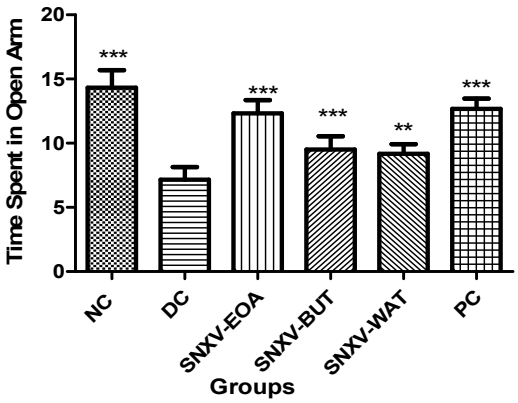

Figure 12. Effect of fraction of S. nux-vomica Ethanol extract on the time spent in the acquisition AT using elevated plus maze in the scopolamine induced amnesiac rats

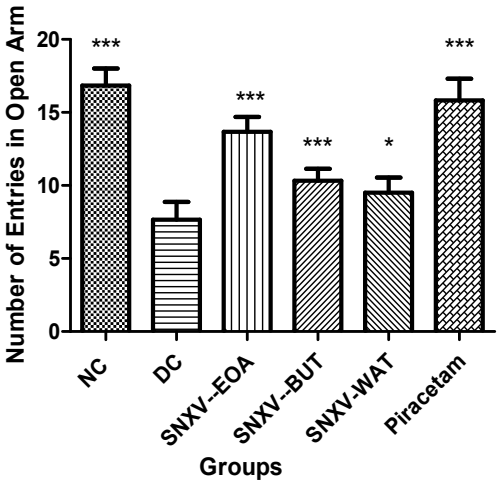

Figure 11. Effect of fraction of S. nux-vomica Ethanol extract on the open arm entry in the acquisition AT using elevated plus maze in the scopolamine induced amnesiac rats

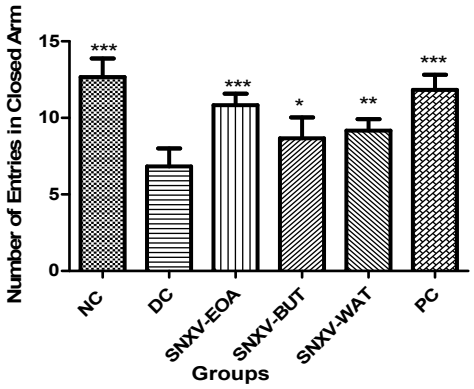

Figure 13. Effect of fraction of S. nux-vomica Ethanol extract on the entry inside closed arm in the acquisition AT using elevated plus maze in the scopolamine induced amnesiac rats

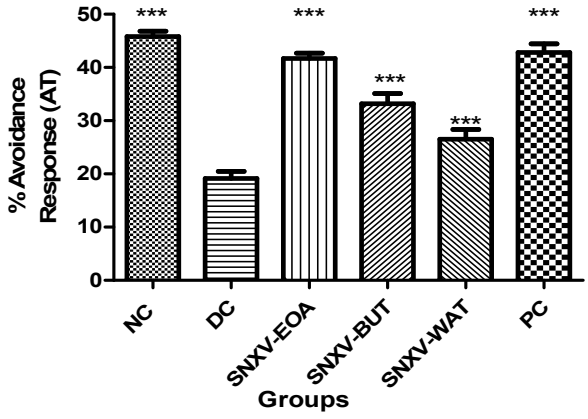

Figure 14. Effect of fraction of S. nux-vomica Ethanol extract on the % avoidance response AR in the acquisition AT of scopolamine Induced amnesiac rats using Cook's Pole climbing model in the active avoidance test

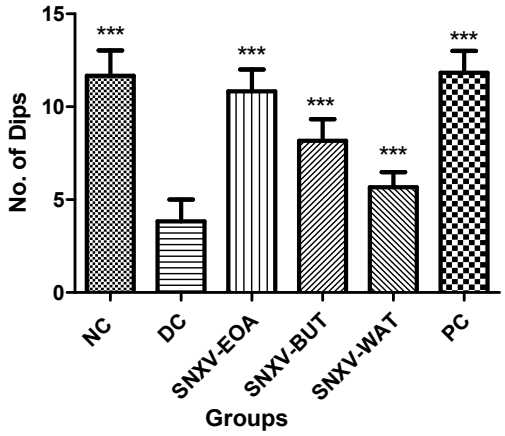

Figure 15. Effect of fraction of S. nux-vomica Ethanol extract on the number of dips made in the hole board model in the scopolamine induced amnesiac rats

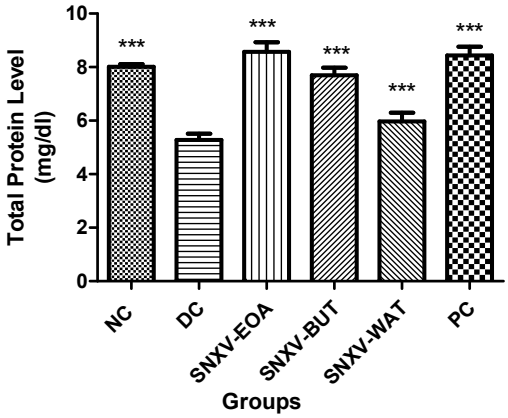

Figure 16. Effect of fraction of S. nux-vomica Ethanol extract on the total protein level in the scopolamine induced amnesiac rats

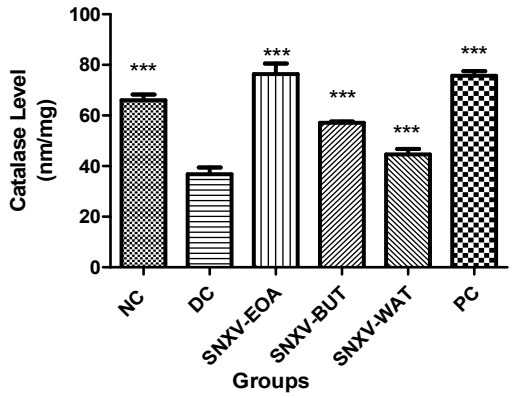

Figure 17. Effect of fraction of S. nux-vomica Ethanol extract on the markers of oxidative stress Catalase CAT level in the scopolamine induced amnesiac rats

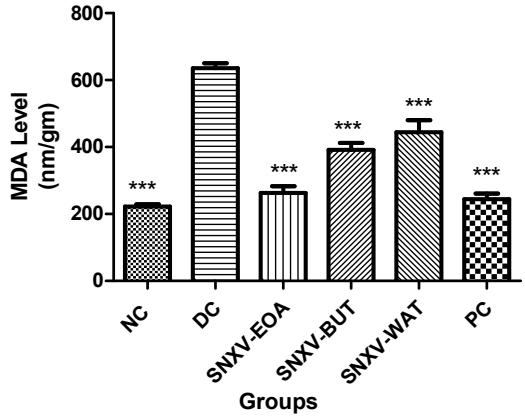

Figure 18. Effect of fraction of S. nux-vomica Ethanol extract on the markers of oxidative stress Lipid peroxidation MDA level in the scopolamine induced amnesiac rats

Discussion

The present study evaluated the nootropic and neuroprotective potential of standardized *Strychnos nux-vomica* extracts against scopolamine-induced memory impairment in experimental rats using behavioral and biochemical parameters. Scopolamine, a non-selective muscarinic receptor antagonist, significantly impaired learning and memory by disrupting central cholinergic neurotransmission, thereby producing cognitive deficits similar to those observed in Alzheimer's disease. These findings were evidenced by increased escape latency in the Morris Water Maze, prolonged transfer latency in the Elevated Plus Maze, reduced avoidance response in passive

avoidance tests, and decreased exploratory behavior in the Hole-Board Test.

Oral administration of *Strychnos nux-vomica* extracts significantly reversed scopolamine-induced cognitive impairment. Among the tested extracts, the ethanolic extract and its ethyl acetate fraction demonstrated the greatest improvement in spatial learning, memory retention, and exploratory behavior, with effects comparable to the standard nootropic drug, Piracetam. These findings indicate that the extracts possess significant memory-enhancing and anti-amnesic properties.

Biochemical investigations further supported the behavioral observations. Treatment with *Strychnos nux-vomica* significantly increased catalase (CAT) and reduced glutathione (GSH) levels while decreasing malondialdehyde (MDA), indicating attenuation of oxidative stress and lipid peroxidation. The improvement in total protein content suggested preservation of neuronal integrity and enhanced cellular function. The neuroprotective effects may be attributed to the presence of bioactive phytoconstituents such as flavonoids, phenolic compounds, tannins, glycosides, and triterpenoids, which possess antioxidant and free radical scavenging activities.

The observed cognitive improvement is likely mediated through multiple mechanisms, including enhancement of central cholinergic neurotransmission, inhibition of acetylcholinesterase activity, reduction of oxidative stress, and protection against neuronal damage. Overall, the findings demonstrate that standardized *Strychnos nux-vomica* extracts possess significant nootropic and neuroprotective potential and may serve as promising phytotherapeutic candidates for the management of memory impairment and neurodegenerative disorders such as Alzheimer's disease.

4. Conclusion

The present study demonstrated that standardized *Strychnos nux-vomica* extracts possess significant nootropic and neuroprotective activity against scopolamine-induced memory impairment in experimental rats. The extracts significantly improved learning, memory retention, and exploratory behavior in various behavioral models while restoring antioxidant status by increasing catalase (CAT), reduced glutathione (GSH), and protein levels and decreasing malondialdehyde (MDA). Among the tested fractions, the ethanolic extract and its ethyl acetate fraction exhibited the greatest efficacy. These findings suggest that *Strychnos nux-vomica* is a promising natural therapeutic candidate for the management of cognitive impairment and neurodegenerative disorders such as Alzheimer's disease.

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