Trop J Nat Prod Res, November 9(11): 5683 - 5693

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Ethanol Extract from Aerial Parts of *Waltheria indica (Sleepy Morning*) Ameliorates Scopolamine-Induced Amnesia in Wistar Albino Rats

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ARTICLE INFO

Article history:
Received 11 July 2025
Revised 05 October 2025
Accepted 10 October 2025
Published online 01 December 2025

ABSTRACT

Waltheria indica, commonly known as Sleepy Morning, is renowned for its neuroprotective bioactive constituents and long-standing use in managing neurological disorders. This study investigated the neuroprotective efficacy of its ethanol extract against scopolamine-induced amnesia in Wistar albino rats, a model that mimics Alzheimer-like cognitive decline. Thirty-six adult male Wistar rats were divided into six groups (n=6). Group 1 (control) received normal saline (10 ml/kg, orally), while Group 2 received piracetam (200 mg/kg, standard drug) plus scopolamine. Groups 3, 4, and 5 were administered 200, 400, and 800 mg/kg of W. indica extract, respectively, followed by scopolamine. Group 6 (amnesic control) was given scopolamine (1.0 mg/kg) and saline. After seven days of treatment, learning and memory were evaluated using the Morris water maze and passive avoidance tests. Rats were then fasted for twelve hours, and brain tissues were analyzed for acetylcholinesterase (AChE) activity and oxidative stress biomarkers—superoxide dismutase (SOD), catalase (CAT), and malondialdehyde (MDA). Pretreatment with W. indica extract significantly ($P < 0.05$) enhanced memory performance compared with the scopolamine group. Biochemically, the extract increased CAT and SOD activities and reduced MDA levels and AChE activity, suggesting decreased oxidative stress. These findings indicate that ethanol extract of Waltheria indica mitigates scopolamine-induced cognitive dysfunction through antioxidant mechanisms and cholinesterase inhibition.

Keywords: *Waltheria indica,* amnesia, induced scopolamine, cognitive function, stressor biomarkers..

Introduction

Neurodegeneration associated with aging is usually irreversible and impairs cognitive abilities such as learning, memory, intellectual thinking and reasoning as well as personality.[1,2] Reactive oxygen species (ROS) production is linked to declines in cognitive performance as well as changes in the levels of acetylcholine and monoamines.[3] The association between Alzheimer's disease (AD) and the cholinergic system has been well established since it was discovered that patients' brains often exhibit a decline in cholinergic activity.[4] AD is the most prevalent type of dementia and is characterised by neurological and cognitive dysfunction caused by oxidative damage to brain proteins, nucleic acids, and mitochondria.[4] Research by [5] revealed a direct correlation between neurotransmitter activity—most notably dopamine, noradrenaline, serotonin, and hypothalamic–pituitary – adrenal (HPA-A) axis activity—and the physiological abnormalities linked to neurodegenerative diseases. Additionally, there is a strong correlation between mitochondrial activity and oxidative and nitrosamine stress.[6]

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Citation: Jabba HL, Dimeji IY, Ayodeji AS, Baba ZM, Adeoye SW, Olawale, AS. Ethanol Extract from Aerial Parts of *Waltheria indica (Sleepy Morning*) Ameliorates Scopolamine-induced Amnesia in Wistar Albino Rats. Trop J Nat Prod Res. 2025; 9(11): 5683 – 5693 https://doi.org/10.26538/tjnpr/v9i11.57

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

Currently, the most widely used method for treating cholinesterase inhibitors is AD), which can inactivate the enzyme acetylcholinesterase (AchE) and increase brain acetylcholine levels.[7,8] Acetylcholinesterase inhibitors include donepezil, rivastigmine, tacrine, galatamine and memantine.[9] These medications treat symptoms but do not treat AD. In addition, they are expensive and are associated with adverse effects.[10,11] Many commonly used medications have noticeable adverse effects.[12] In 2021, aducanumab, which removes beta-amyloid plaques, was the first disease-modifying medication for Alzheimer's disease. Its major adverse effect is amyloid-related imaging abnormalities associated with edema or hemorrhage.[13]. This has encouraged scientific research on medicinal plants in search of compounds that are more effective with fewer side effects. Numerous scientific studies have demonstrated the value of medicinal herbs in improving neurological processes.[14, 15, 16] Scientific research has shown that medicinal herbs used to treat AD, such as *Ginko biloba*, *Withania somnifera*, *Waltheria americana*, and Panax ginseng, have fewer negative effects than synthetic medications currently in use.[17,18] Many human ailments are addressed via the use of plant-based materials in contemporary treatments. *Waltheria indica* (WI) Linn. (Malvaceae) is used in conventional medicine worldwide to address many diseases, including pain, inflammation, neuralgia, convulsions, diabetes, and seizures. WI is of American origin and worldwide distribution and is a widespread species in the tropics. In northeastern Nigeria, a plan's aerial sections are decocted to address memory loss (personal communication). Sedative, analgesic, and antioxidant actions are just a few of the many pharmacological activities of the entire plant and its roots, both in their raw and refined forms.[14,19,20,21]. Whole-plant *(Waltheria indica*) extracts have been reported to prevent neurotoxic damage and behavioral deficits in animal models, inhibit acetic acid-induced abdominal stretches and exhibit nootropic properties in animal models.[22] A diverse array of plants is

utilised in ethnomedicine by traditional healers in Nigeria. Among them is *Waltheria indica*, which is commonly used to manage mood disorders, anxiety, and convulsions in children. *Waltheria indica L.* belongs to the Sterculiaceae family.[23]

WI (commonly known as Sleepy Morning) has been extensively employed in conventional medicine for its neuroprotective and therapeutic properties. Despite its ethnomedicinal importance, scientific validation of its potential in mitigating neurological disorders remains limited. This study investigated the ability of *Waltheria indica* aerial parts extracted with ethanol to ameliorate cognitive and neurological impairments. Given the reported anxiolytic, anticonvulsant, and anti-inflammatory properties of these plants, exploring their neuroprotective mechanisms could provide valuable insights into alternative therapeutic strategies for managing neurodegenerative conditions. The results of this study could aid in the development of plant-based therapies for cognitive dysfunction and related disorders.

Materials and Methods

Plant Collection and Identiification

Waltheria indica's aerial part was collected from fields in Samaru-Zaria, Kaduna State, Nigeria (11°03'60.00"N; 7°41'59.99"E), on September 5, 2022, between 9:00 am and 12:00 noon. The plant materials were collected in accordance with standard pharmacognostic procedures and were identified by plant taxonomists on the basis of their morphological characteristics. After taxonomic identification, a voucher specimen (*Waltheria indica*.2022.NG. NPR-2006) was preserved in the Department of Pharmacognosy and Drug Development (DPDD) herbarium, Ahmadu Bello University, Zaria, Nigeria.

Plant extraction

First, the collected plant material was cleaned to remove sticky debris, and water was run under the tap, followed by air drying at ambient temperature. It was then oven-dried for 2 hours at the Department of Pharmacognosy and Drug Development (DPDD), Ahmadu Bello University, Zaria, Nigeria. The dried material was pulverised and sieved to obtain a fine powder. A total of 500 g of the powdered sample was extracted in a Soxhlet apparatus using 60% v/v aqueous ethanol (500 mL) as the solvent. The resulting extract was 40–60 °C fraction of petroleum ether defatted and subsequently suspended in distilled water. The concentration of the extract was determined with less pressure at 52°C via a rotary evaporator, yielding a yellowish-green residue with an extraction yield of 15.3% w/w. The final dried extract was stored under refrigeration until further use in the present study.

Animals

The research was authorised by the Ethics Review Committee of the Faculty of Basic Medical Sciences, LAUTECH (ethical code FBMS/AEC/P/078/25). Male adult Wistar albino rats with weights ranging between 185 and 205 g were acquired. They were housed in compliance with the 1985 International Ethical Standards for the Care and Use of Laboratory Animals Guideline, which was ratified by Cadmus and Daramola.[24] In addition to a 12-hour light/dark cycle with a 25 ± 20°C temperature range and a humidity level of 55–65%, the animals had unlimited availability of food and water.

Acute Toxicity

The LD_{50} (acute oral toxicity) of a plant's aerial portions was previously reported to be 875 mg/kg.[14]

Extract doses

On the basis of the established oral acute toxicity of the plant materials, three dose levels of the extract were selected for the experiments: 200, 400, and 800 milligrams per kilogram. These dose selections were guided by the established LD_{50} of the plant extract.

Treatment regimen

Thirty-six rats, weighing between 185 and 205 g, were split into six groups at random (n = 6) in accordance with the methods of [49].

Group 1: The animals in this group were given one oral dose of saline (10 ml/kg B) each day from day 1 to day 8 (control).

Group 2: This group received one oral dosage each day of saline (10 ml/kg B.W.) from day 1 to day 8 plus one oral dosage of scopolamine (1 mg/kg) on the 8th day (to confirm that amnesia was induced by scopolamine).

Group 3: The rats in this group received one oral dose of piracetam (200 mg/kg B.W.) from day 1 to day 8 plus one oral dose of scopolamine (1 mg/kg) on the 8th day (to evaluate the antiamnesic effects of piracetam in comparison with those of the test extract).

Group 4: The rats in this group received the ethanol extract *Waltheria indica* aerial parts (200 mg/kg) orally, followed by one oral dose of scopolamine (1 mg/kg) on the 8th day.

Group 5: The rats in this group were orally administered an ethanol extract *of the* aerial parts of W. indica (400 mg/kg), followed by one oral dose of scopolamine (1 mg/kg) on the 8th day.

Group 6: The animals in this group received *Waltheria indica* extracts at a dose of 800 mg/kg plus one oral dose of scopolamine (1 mg/kg) on the 8th day.[14]

The animals in their respective groups were given normal saline, extract doses (200, 400, and 800 mg/kg), piracetam (200 mg/kg), or scopolamine (1.0 mg/kg) 45 minutes prior to the behavioral experiments, as described previously.

Elevated plus maze (EPM)

Animal models of memory and learning have been evaluated via the EPM test as described by [48], and the transfer latency (TL) has been employed as a metric in this regard. The animal was positioned with its back on the center platform in one of the arms that was open to the maze.[25] The initial transfer latency (ITL), or the duration required for an animal to leave its arms open to closed, is measured by a trained researcher.[25] Every trial has a 90 second time limit. The initial transfer latency (ITL) was measured at 90 seconds if the animal did not complete the assignment in the allotted period. The time was recorded when the animal fully entered the closed arm of the maze and all four paws crossed the line that separated it from the center platform. After finishing the task, the creatures were returned to their cages. Using the same process used for the ITL,[25] the retention transfers latency (RTL) was measured after twenty-four hours. Learning on the 1st trial day and memory retention on the 2nd trial day were evaluated via transfer latency. The inflection ratio (IR) was then calculated via the following formula, which was adopted by[25]:

IR = \frac{L_) – L_1}{L_1}……equation 1

IR = inflection ratio

L_O = ITL in seconds

L_1 = RTL in seconds

Successful retention was measured by a drop in TL on repeated maze exposure.[26]

A longer TL interval to arrive at the enclosing arm on the 2nd trial suggested poor retention. The equipment was carefully rinsed with 70% ethanol prior to any evaluation of the animals.

Y – Maze experiment

The 3 arms that make up the Y-maze are A, B, and C. All 3 arms are the same size and are tilted 120 degrees from one another. Rat spatial working memory and short-term memory can be evaluated via the maze and spontaneous alteration tests, respectively.[27] Rats are permitted to traverse all three arms of the maze since they are inherently curious and like exploring new areas. The rats were placed in one arm in accordance with the order (for example, C B A, B C C), and after a five-minute session, the number of arm entrances was noted. See Figure 1.

As soon as the rat's back legs were completely inside one arm, the entry was deemed to be an alternation (repeated entries by the rat into the three distinct arms). To prevent olfactory signals, the arena was cleaned between trials with 70% ethanol. Additionally, the starting position of the arms within the same group of rats was altered to prevent bias in arm placement. Among the metrics that were measured were the quantity of arm entry, returns with the same arm and returns with the opposite arm. The following formula was used to calculate the % alternation performance:

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

(%Alternation} = {Number of alternations} \ {Total arm entries} - 2} \times 100) -…….. equation 2

Y-Maze spontaneous alternation test

An animal's natural curiosity for exploration is the basis for a behavioral test known as the Y-maze spontaneous alternation. Rats, of course, usually investigate a different section of the maze than the one they have already traversed. This task involves the septum, hippocampus, prefrontal cortex and basal forebrain. Three identical arms are positioned 120 degrees apart in a Y-shaped maze that serves as the testing area. The maze's center is presented to the animal, and it is given unrestricted access to each arm. A rat is said to have made an alternation (the right response) if it selects a different arm from the one it came from; returning to the first arm is seen as a mistake.[28,29] See Figures 2 a and b.

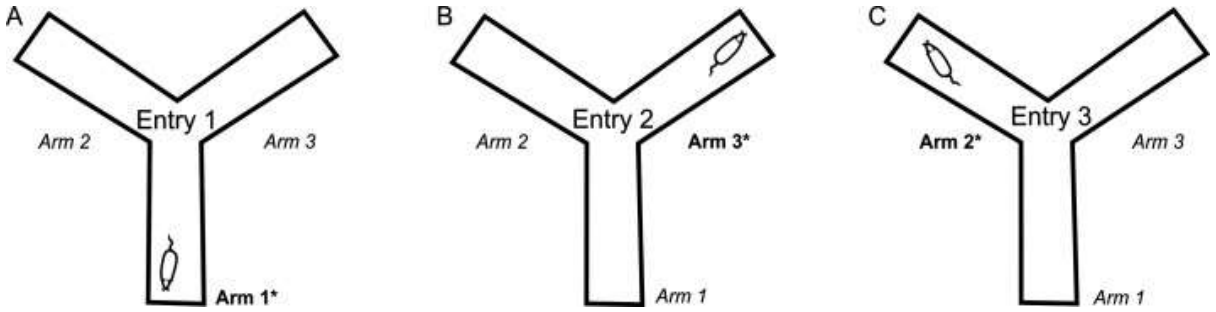
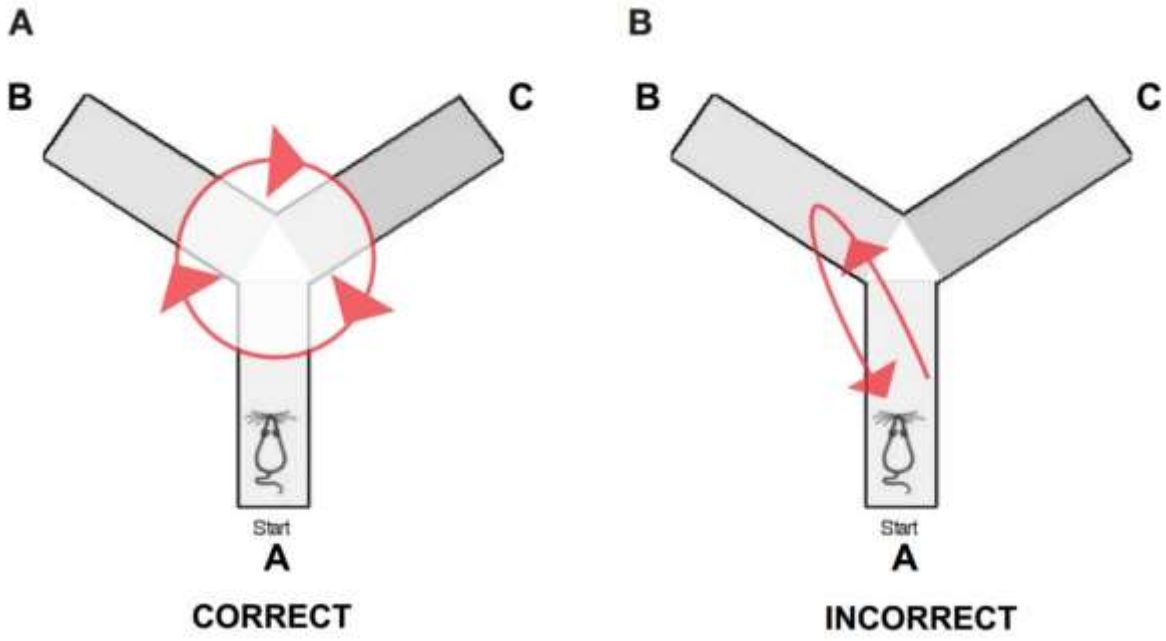


Figure 1: Y – Maze.[14]



Figures 2: a and b Y-maze spontaneous alternation test.[29]

Novel object recognition test (NORT)

The approach used in this investigation to conduct the new object recognition test (NORT) was taken from an earlier description by.[30] and reported by.[31.] Three sections of the test were placed in an open field box measuring 50 by 50 by 40 cm. Following extract dosages and drug treatments, the animals were permitted to roam in the open field for 5 minutes during the habituation phase. Two identical items, blue cubes that were four to four centimetres in size, were positioned in each of the two corners of the open field box during the acquisition phase (T1), with the objects being ten centimetres from the walls. The rats were positioned in the middle of the open field and given five minutes to investigate the two items before being returned to their own cages. The second test, T2 (choice), was administered 24 hours after T1. The rats were re-exposed to the two objects (the new, N, and the familiar, F) during the T2 phase. The unusual object was a red cone, which had a distinct size, shape, and color. The amount of time the rat spent using a stop watch looking at each object during T1 and T2 was recorded. The discrimination index (DI) was determined via the formula below:

(DI = \frac{T_{novel} - T_{familiar}}{T_{novel} + T_{familiar}}) -------equation 3.30

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

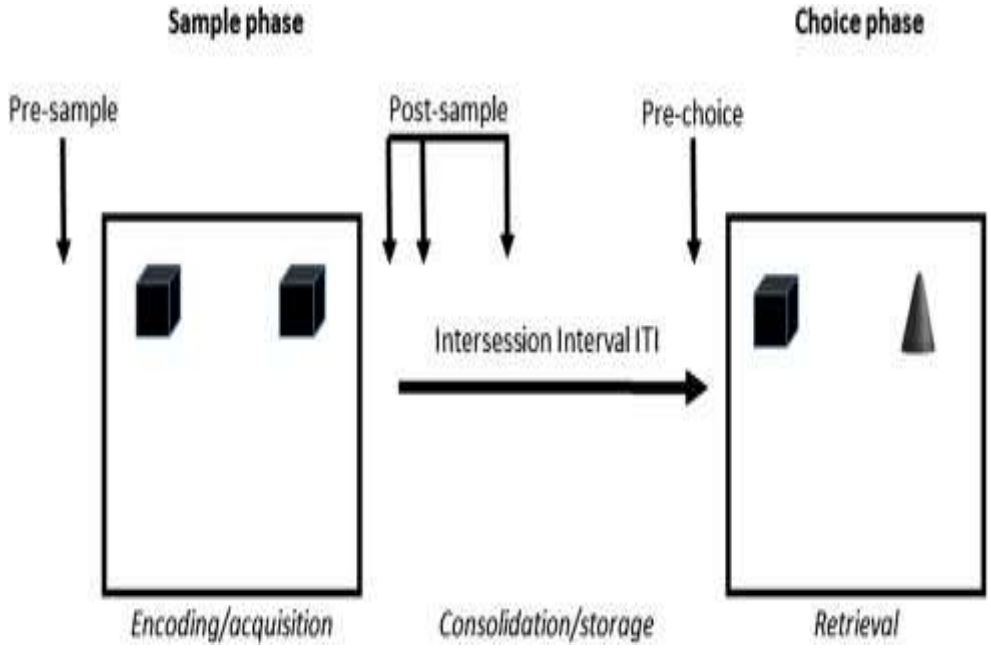


Figure 3: Novel object test.[30]

TN is the amount of time spent on the new item. The time spent with the familiar object equals the TF. The interest of the rats in the new object was then assessed via the DI (% of time spent on a new thing split by the duration of time on both the new and familiar objects). See Figure 3.

Evaluation of lipid peroxidation biomarkers (malondialdehyde)
Brain tissue sampling/preparation
The rats were fasted for twelve hours[32-37] at the conclusion of the behavioral testing. The animals were sacrificed following light anaesthesia with isoflurane, and 0.5–1.0 mL blood samples were obtained via orbital puncture.[33] Each blood sample was split into two parts. The larger part was allowed to clot in clean, dry plain bottles before being centrifuged for 10 min at 3000 rpm to extract the serum.[38-40] The smaller piece was then placed in ethylenediaminetetraacetic acid (EDTA) for the superoxide dismutase assay. For the MDA assay, the serum was frozen at -20°C. "Following the collection of blood samples, the animals were decapitated, and their entire brains were quickly removed under conventional operating procedures and thoroughly cleansed with ice-cold saline". The brain tissue was homogenised immediately to create a 10% (w/v) homogenate in a cold solution with 300 mM sucrose and 50 mM Tris-HCl (pH 7.4).[36] After that, for ten minutes, the homogenate was centrifuged at 4°C and 3000 rpm. To perform the acetylcholinesterase (AChE) test, the supernatant was separated.

Evaluation of acetylcholinesterase (AChE)
The colorimetric technique outlined by.[41] and modified by Patel.[42] was used to evaluate the activity of AChE. The rate at which acetylthiocholine iodide (ATCI) is hydrolysed to produce thiocholine, which then interacts with 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) to make a yellow 5-thio-2-nitrobenzoate anion, was used to quantify AChE activity. To obtain AChE-containing supernatants, brain phosphate buffer was used to create homogenates (pH 7.4), which were subsequently centrifuged. DTNB, ATCI, and phosphate buffer (pH 8.0) were used in combination in the assay. To determine AChE activity, the sample was incubated with the reaction mixture at 25–37°C for a predetermined amount of time. The absorbance changes were measured at 412 nm at regular intervals via a spectrophotometer. Beer–Lambert's law was then used to express the activity in µmol of substrate hydrolysed per minute per mg of protein. This method is widely used in neurobiological and pharmacological studies to assess AChE inhibition in Alzheimer's disease models and in neurotoxicity studies.

Superoxide dismutase (SOD) assay
The technique outlined by Misra and Fridovich[43] was employed to conduct the superoxide dismutase (SOD) assay.

Assays of catalase (CAT)
The activity of CAT was estimated via a standard procedure. Five millilitres of phosphate buffer (pH 7.0) were combined with one millilitre of brain homogenate from each treated group. Four millilitres of hydrogen peroxide (H_2O_2) solution (800 µmol) was then added. In addition, the mixture was gently spun at ambient temperature. A portion of the reaction—approximately 1 milliliter—was then taken via a spectrophotometer and added to 2 mL of dichromate/acetic reagent. Umukoro *et al*. reported that the process was converted into catalase activity after the absorbance changes were measured at 570 nM.[44]

Assay for total glutathione (GSH)
Ellman's reagent-based approach was used to perform the assay for total glutathione (GSH).[45]

Statistical analysis
The means ± standard deviations are used to display the results. Analysis of variance in one direction (ANOVA) was followed by Tukey's test to measure intergroup variation. For statistical analysis, significance values of $p < 0.05$ were employed. The statistical program used was Graph Pad Prism 8.0 (San Diego, California, USA).

Results and Discussion

Elevated plus maze
For many years, plants have been utilised as medicinal substances to cure a range of ailments, offering a readily available and more affordable alternative to orthodox medicines. The therapeutic value of plants is attributed to bioactive compounds such as alkaloids, flavonoids, tannins, phenols, and saponins. Previous studies have shown that *Waltheria indica* contains these compounds, suggesting that the biological potential of plants might be due to the following phyto-constituents[46]: flavonoids, alkaloids, terpenoids, tannins, saponins, and cardiac glycosides. Specifically, the use of compounds such as epicatechin, kaempferol derivatives, tiliroside, and quercetin in the EPM paradigm suggests that an ethanol infusion of *Waltheria indica* may be an effective treatment for cognitive impairment, particularly for improving scopolamine-induced deficits. In the EPM paradigm, the

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

addition of transfer rotational latency (TL) is associated with memory impairment, whereas a decrease in TL indicates improved cognitive function.[48,49,50]

The administration of scopolamine (1 mg/kg) has been reported to increase the initial transportation rotational latency (ITL) and retention transportation rotational latency (RTL), causing cognitive damage.[51] The scopolamine-treated group had an ITL of 56.25±5.30 seconds and an RTL of 53.42±6.00 seconds, together with an inflection ratio of 0.05 ±0.01. This impairment is consistent with previous findings that scopolamine disrupts cholinergic neurotransmission, notably leading to deficiencies in memory and learning.[52-55] The results are depicted in Table 1.

Piracetam (200 mg/kg), a standard nootropic agent, notably (P< 0.05) simultaneously reduced both ITL and RTL (21.02 ± 2.02 and 18.02±0.51 s, respectively) and increased IR (0.17±0.01), demonstrating its efficacy in reversing cognitive impairment. Similarly, high-dose *Waltheria indica* infusion (800 mg/kg) resulted in comparable improvements (ITL: 23.06±1.40 second, RTL: 19.65±1.15 second, IR: 0.17±0.01), indicating a potential nootropic effect. This observation supports the findings of a previous phytochemical analysis of the cognitive impairments caused by scopolamine and the dose-dependent cognitive-enhancing effect of the *Waltheria indica* infusion, which was revealed with a single dose.[56] Compared with scopolamine alone, the 800 mg/kg extract of *W. indica* notably (P < 0.05) reduced ITL (24.92±0.72 s) and RTL (22.69±0.95 s), suggesting a protective effect against cholinergic derangement. The moderate dose (400 mg/kg) also improved performance, although not notably (ITL: 40.05±3.05 second, RTL: 36.44±1.72 second, IR: 0.12± .04), suggesting that it was less effective than the 800 mg/kg dose. These findings suggest that *Waltheria indica* extract may improve cholinergic neurotransmission equivalent to piracetam, possibly through the transition of acetylcholinesterase activity.[57,58]

Taken together, these findings indicate that *Waltheria indica* possesses cognitive properties comparable to those of piracetam, especially at higher doses, such as 800 mg/kg. The extract may exert its effects through several nerve pathways, including the antioxidant pathway, cholinergic transition, and neuroprotection.

The effects of *Waltheria indica* extract on percentage alteration (cognitive function), a crucial measure of spatial functional memory in rats, are illustrated in Table 2.

Table 1: Effects of *Waltheria indica* aerial parts extracted on transfer latency and performance in the elevated plus maze paradigm

Treatment/dose (mg/kg)	Initial transfer latency(ITL)	Retention transfer latency(RTL)	Inflexion Ratio (IR)
Normal control (vehicle)	47.69 ± 0.05	42.85 ± 0.51	0.10 ± 0.03
Scopolamine, 1 mg/kg + Saline (amnesic control)	56.25 ± 5.30	53.42 ± 6.00	0.05 ± 0.01
Piracetam + scopolamine,1 mg/kg (standard drug)	23.85 ± 0.85[a,b]	22.05 ± 1.10[b]	0.20 ± 0.08
Extract, 200 mg/kg + scopolamine, 1 mg/kg	43.88 ± 3.00	40.01 ± 2.00	0.08 ± 0.03
Extract, 400 mg/kg + scopolamine (1 mg/kg)	40.05 ± 3.05	36.44 ± 1.72	0.12 ±0.04
Extract, 800 mg/kg + scopolamine, (1 mg/kg)	24.92 ± 0.72[a,b]	22.69 ± 0.95[b]	0.08 ±0.02

The values represent the means ± standard deviations (n = 6), with a = P < 0.05 compared with the control group (normal saline) and b = P < 0.05 compared with the amnesic control group (scopolamine-treated).

Table 2: Effects of *Waltheria indica* aerial parts extract on percentage alteration in rats

Treatment/Dose (mg/kg)	8th day (%)	9th day (%)
Normal control (vehicle)	22.53 ± 1.12	25.85 ± 0.92
Piracetam, 200 mg/kg	52.94 ± 7.54[a,b]	50.22 ± 8.05 [a,b]
Piracetam,(200 mg/kg) +scopolamine (1.0 mg/kg)	40.02 ± 1.87[a,b]	39.25 ± 2.02 [a,b]
Extract,(200 mg/kg) +scopolamine (1.0 mg/kg)	24.90 ± 8.95	23.44 ± 7.95
Extract, (400 mg/kg) +scopolamine (1.0 mg/kg)	26.05 ± 4.87	28.43 ± 5.53
Extract, (800 mg/kg) +scopolamine 1.0 mg/kg	40.52 ± 4.35 [b]	39.57 ± 5.04 [b]

The values are expressed as the means ± SDs (n = 6), with a = P < 0.05 compared with the normal control group and b = P < 0.05 compared with the rats treated with scopolamine.

The percentage alternation measures the ability of a rodent to examine a new environment and remember a previously visited place, which is crucial in any cognitive performance test similar to the Y-maze.[59,60,61] The normal restraint group showed strong percentage variations on both the 8th and 9th days, indicating baseline cognitive function. In contrast, scopolamine administration (1.0 mg/kg) led to a noteworthy (P< 0.05) decrease in percentage alternation, as demonstrated in the 200 mg/kg + scopolamine group (24.90±8.95% on day 8 and 23.44± 0.95% on day 9). This decline is consistent with earlier studies showing scopolamine-induced cognitive impairment due to its antagonistic effects on the muscarinic cholinergic receptor.[62]

Notably, piracetam (200 mg/kg), a well-established nootropic agent (P <0.05), increased the percentage of alternations (52.94 ± .54% on day 8 and 50.22 ± 8.05% on day 9), corresponding to better functional memory. A high dose of *Waltheria indica* extract (800 mg/kg) produced equivalent cognitive enhancements with piracetam (42.08±4.95% on day 8 and 45.20±5.85% on day 9), suggesting that the extract has memory-enhancing properties comparable to those of piracetam. These results verify previous studies on *the* neuroprotective properties of *Waltheria indica*, probably due to its anti-inflammatory and antioxidant properties.[63,64,65]

Moreover, the mixture of piracetam and scopolamine led to a moderate increase in the % alternation (40.02±1.87% over day 8 and 39.25±2.02% on day 9), although this increase was not statistically significant, demonstrating the ability of piracetam to reduce scopolamine-induced cognitive decline. Similarly, *Waltheria indica* extract (800 mg/kg) combined with scopolamine had a notably (P<0.05) greater percentage of alternation (40.52 ± 4.35% on day 8 and 39.57 ± 5.04% on day 9) than did the lower extract doses (400 and 200 mg/kg). These findings imply that W. indica may be able to counteract cholinergic disruptions, especially at relatively high dosages, perhaps by inhibiting acetylcholinesterase and promoting neurotrophic transitions.[66]

Consequently, these findings support *W. Indica's cognitive* enhancement capac*ity*, especially at higher doses, which possess an efficacy comparable to that of piracetam. The beneficial effects of the extract are likely attributed to a phytochemical component, possibly flavonoids and alkaloids, which have been reported to increase neurotransmission and lower oxidative stress.

The impacts of the extract of *W. indica* aerial parts on spatial active memory via the Y-maze project displayed a noteworthy (P < 0.05) change in cognitive function in the treated groups (P< 0.05). The total number of arms that a rodent enters is a crucial indicator of exploratory behavior and active memory performance. The scopolamine-treated group had a greater number of arm movements on the 8th and 9th days, suggesting hyperactivity and impaired active memory. Scopolamine can cause cognitive impairment by antagonising the muscarinic acetylcholine receptor, which has a noteworthy effect on memory formation and retrieval.[67,68] In contrast, compared with previous analyses, the administration of piracetam (200 mg/kg) notably (P< 0.05) reduced the number of arm entries, indicating the neuroprotective and cognitive-enhancing properties of piracetam.[69,70] *Waltheria* indica at the 800 mg/kg dose led to a noteworthy (P< 0.05) decrease in arm entries compared with those of piracetam, confirming its nootropic effects. In particular, the combination of piracetam and scopolamine resulted in the same decrease in arm entries, which reinforced the protective effect of piracetam in preventing the memory damage caused by scopolamine. A dose-dependent pattern of behavior was observed in the groups that were treated with the plant extract in combination with scopolamine. The 200 mg/kg scopolamine group, however, did not notably differ from the scopolamine-alone group, indicating insufficient neuroprotection at the current dose. However, the 400 mg/kg and 800 mg/kg scopolamine groups presented a gradual decrease in arm entry, together with a noteworthy increase (P< 0.05) at the maximum dosage (800 mg/kg). These findings suggest that *Waltheria indica* extract ameliorated the memory impairment caused by scopolamine in a dose-dependent manner. These studies support *W. Indica's* cognitive enhancement potential, possibly via cholinergic transition or antioxidant mechanisms, as detected in alternative medicinal products with neuroprotective properties.[71,72,73] The results are illustrated in Table 3.

Table 3: Effects of *Waltheria indica* aerial parts extracted on the number of arm entries

Treatments (mg/kg)	(day 8th)	(day 9th)
Normal control (vehicle)	19.25 ± 1.06	17.66 ± 1.62
Scopolamine (1 mg/kg)	24.90 ± 2.14	25.05 ± 1.04
Piracetam(200 mg/kg + scopolamine(1 mg/kg)	8.54 ± 1.66 [a]	7.20 ± 2.04[a,b]
Extract (200 mg/kg) + scopolamine(1 mg/kg)	13.65 ± 1.70	19.01 ± 1.82
Extract (400 mg/kg) + scopolamine (1 mg/kg)	14.37 ± 0.94	14.00 ± 2.00
Extract (800 mg/kg) + scopolamine (1 mg/kg)	11.85 ± 1.35[b]	10.53 ± 1.40[b]

For n = 6, all values are means ± SDs. One-way analysis of variance (ANOVA) and Dunnet's post hoc multiple test was used to assess P values. Comparisons of the amnesic and extract groups and the amnesic and normal control groups were performed as follows: a = P < 0.05, b = P< 0.05.

The evaluation of memory retention and appreciation capacity via the novel object understanding test (NORT) also provides insights into the cognitive benefits of *Waltheria indica* extract. The data of this investigation show the noteworthy (P< 0.05) impact of *Waltheria indica's* effect on extended memory, as defined by the bias index (DI) and the investigation interval. Scopolamine-induced amnesia led to a noteworthy (P< 0.05) decrease in the DI (32.90±0.67) compared with that of normal controls, confirming the role of scopolamine in impaired cognitive function via cholinergic framework unsettling.[62,74] The decreased number of fresh objects in the scopolamine-treated group compared with the normal group (53.03±1.50) indicates a decrease in recognition memory, which is consistent with earlier conclusions on the amnestic effects of scopolamine.[67,75,76,77]

Waltheria indica extract markedly (P < 0.05) increased the DI in a dose-dependent manner, with 800 mg/kg extract resulting in a DI of 54.95±1.08, which was close to the normal range. These findings indicate that the biological compounds in *W. indica* may be able to counteract the cholinergic dysfunction caused by scopolamine, possibly through antioxidant and anti-inflammatory mechanisms.[78,79] In particular, together with *W. indica* administration, the survey duration of the novel object (A1) during the trial phase increased. This indicates enhanced identification memory. The 800 mg/kg group (16.21±0.80 s) presented a high increase in awareness, confirming the memory-enhancing potential of the extract. Piracetam, a well-known nootropic agent, clearly influences DI (70.02±1.75) and fresh object discovery (23.63±1.21), as expected because of its favourable neurotransmission and synaptic malleability.[80] The results are illustrated in Table 4.

One important factor in neurodegenerative diseases is oxidative stress, including amnesia, which disrupts cell homeostasis through excessive lipid peroxidation, protein oxidation, and DNA damage.[3,81,82,83] The current study measured the effects of *Waltheria indica* extract on

oxidative stress biomarkers in scopolamine-induced amnestic rats by measuring the MDA, AChE, SOD, GSH, and CAT levels.

Scopolamine administration markedly increased the levels of MDA, a key indicator of lipid peroxidation, compared with those in the control group (28.01± 1.35 vs. 9.10± 1.03/mg protein, p<0.05). This elevation implies increased oxidative stress and membrane damage, which are hallmarks of neurotoxicity and cognitive impairment.[62,84,85] In addition, after the administration of 200, 400, or 800 mg/kg *W. indica* extract, the highest dose (800 mg/kg) resulted in the greatest decrease of 17.22± 1.48/mg protein (p< 0.05). The antioxidant properties of the extract, as previously observed in medicinal plants rich in flavonoid and phenolic resin, may contribute to its protective effect.[86,87]

Notably, the AChE level in the scopolamine-treated rats was greater than that in the control rats (678.95 ± 20.50 vs. 562.30 ± 21.05/mg protein, respectively; P < 0.05). Excessive AChE activity accelerates acetylcholine depletion, leading primarily to cognitive impairment.[88] *W. Indica* administration at all the tested doses notably (P< 0.05) reduced AChE levels and had promising cholinesterase inhibitory effects, which may enhance memory function. The high dose of 800 mg/kg produced

Table 4: Effects of Waltheria indica aerial extract on long-term memory in rats subjected to behavioral NORT

Treatment (Mg/kg)	Exploration time (in seconds)				
	Sample phase (phase 1)		Test phase (phase 2)		DI (%)
	Identical objects		Novel objects	Familiar objects	
	A1	A2	A1	A2	
Normal control(saline)	20.01 ±1.21	22.50 ±0.85	12.92 ±0.80	10.49 ±1.00	53.03 ±1.50
Amnesic control. (scopol. Treated, 1 mg/kg i.p)	13.02 ±0.48	12.85 ±0.98	5.93 ±0.77	13.20 ±0.75	32.90 ± 0.67
W.indica extract (200 mg/kg)+Scop. (1 mg/kg)	18.05 ± 1.20	17.83 ± 0.94	12.50 ± 0.89	11.22 ±1.02	54.01 ± 0.95
W.indica extract (400 mg/kg)+Scop. (1 mg/kg)	18.00 ± 0.75	18.45 ± 0.91	14.56 ± 0.87b	11.48 ± 0.74	53.92 ± 0.88
W.indica extract (800 mg/kg) +Scop. (1 mg/kg)	17.95 ± 0.81	19.05 ± 0.75	16.21 ± 0.80c	11.32 ± 0.57	54.95 ± 1.08
Piracetam (200 mg/kg)	16.98 ± 1.03	18.06 ± 1.05	23.63 ± 1.21c	10.92 ± 0.94	70.02 ± 1.75

For n = 6, all values are means ± SDs. One-way analysis of variance (ANOVA) and Dunnet's post hoc multiple test were used to assess P values. Comparisons of amnesic vs extract doses and amnesic vs normal control groups were performed as follows: a = P < 0.05, b= P<0.05 and c = P< 0.05.

an effect on AChE levels comparable to those in piracetam-treated rats (603.48 ± 40.24/mg protein).

Additionally, measures have been taken to protect against enzymatic antioxidants. Compared with the normal control group, the scopolamine-only (amnestic group) group presented noteworthy (P< 0.05) decreases in SOD, GSH, and CAT activities, which was consistent with an earlier report of oxidative stress-induced cognitive decline.[89] Notably, treatment with the extract (P < 0.05) restored the levels of these antioxidant enzymes in a dose-dependent manner, with 800 mg/kg extract having the most distinct effect. The increased levels of SOD, GSH, and CAT indicate an improved scavenging effect and reinforce the neuroprotective function of *W. indica*. Compared with normal conditions, piracetam (200 mg/kg) has more potent antioxidant effects, with an SOD of 12.42 ± 1.46/mg protein, a GSH of 47.00 ± 1.56/mg protein, and a CAT of 33.01 ± 1.31/mg protein, which approach those of normal rats. This finding is in line with the second antioxidative and neuroprotective mechanism of piracetam;[90] these mechanisms are depicted in Table 5. *Waltheria indica* extract in general is effective as a curative agent against cognitive impairment and has been demonstrated to lower oxidative stress, decrease AChE activity, and increase the levels of antioxidant enzymes[91].

Table 5: Effects of *W. indica* extract on oxidative stress biomarkers in rats

Treatment(mg/kg)	MDA (µ/mg protein)	AChE (µ/mg of protein)	SOD (µ/mg of protein)	GSH (µ/mg of protein)	CAT(µ/mg protein)
Normal control (normal saline)	9.10 ± 1.03	562.30±21.05	14.25 ±1.20	46.00 ±1.25	31.05 ± 2.00
Amnesic control (scopolamine 1 mg/kg i.p)	28.01 ±1.35[a]	678.95 ±20.50[a]	4.90 ± 1.83[a]	15.05 ±1.01[a]	6.95 ± 1.20[b]
W. indica extract (200 mg/kg)+scop. (1 mg/kg)	18.95 ±1.20[b]	595.41 ±15.60[b]	6.95 ± 1.02[b]	33.15 ±1.35[b]	21.95 ± 1.35[b]
W. indica extract (400 mg/kg)+scop (1 mg/kg)	18.05 ±1.50[b]	602.24 ±17.22[b]	7.98±0.95[b]	33.98 ±1.05[b]	23.20 ± 1.25[b]

W. indica extract (800 mg/kg)+scop (1 mg/kg)	17.22 ±1.48[b]	605.50 ±18.35[b]	8.15 ±1.12[b]	34.15 ±1.45[b]	23.58 ± 1.55
Piracetam (200 mg/kg *p.o*)+scop. (1 mg/kg)	11.42 ±1.5[c]	603.48 ±40.24[c]	12.42 ±1.46[c]	47.00 ±1.56[c]	33.01 ± 1.31[c]

All values are presented as the means ± standard deviations (n = 6); a denotes a noteworthy difference from the normal control at $P < 0.05$, b denotes a noteworthy difference from the amnesic control at $P < 0.05$, and c denotes a noteworthy difference from pitacetam at $P < 0.05$.

Conclusion

The ethanol extract of Waltheria indica aerial parts has strong neuroprotective potential, especially in relation to scopolamine-induced amnesia in Wistar albino rats. It increased cholinergic function, decreased oxidative stress, and markedly improved long-term memory. Antioxidative and anti-inflammatory processes are indicated by the dose-dependent restoration of antioxidant enzyme activity and decreased lipid peroxidation. Given that *W. indica* is equally effective as piracetam is, W. indica may be a natural cognitive enhancer. These results encourage further investigations to identify bioactive substances and elucidate their molecular processes. Additionally, the plant extract exhibited strong antioxidant and anticholinesterase qualities, supporting its involvement in memory enhancement. W. indica may offer a safe, reasonably priced alternative treatment for cognitive decline, particularly in neurodegenerative diseases such as Alzheimer's disease, if its efficacy is validated in clinical trials.

Conflict of Interest

The authors declare no conflicts of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

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