RESEARCH PAPER

Modulation of Synaptic Plasticity Markers by Dual Donepezil–Selegiline AChE–MAO-B Inhibitors in Scopolamine-Induced Amnesia in Rats

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Abstract

Alzheimer's disease is a progressive neurodegenerative disorder characterized by cognitive decline, synaptic dysfunction, oxidative stress, and neuronal loss. Current therapies based on single-target mechanisms provide only symptomatic relief and fail to address the multifactorial pathology of the disease. The present study investigated the neuroprotective and cognitive-enhancing effects of a dual acetylcholinesterase and monoamine oxidase-B inhibitor in a scopolamine-induced amnesia model in rats. Adult male Wistar rats were divided into six groups and treated with donepezil, selegiline, a dual Donepezil–Selegiline inhibitor, rivastigmine, or vehicle, followed by scopolamine administration. Cognitive performance was evaluated using behavioral paradigms including the Morris Water Maze, Novel Object Recognition, and Passive Avoidance tests. Biochemical analyses assessed AChE and monoamine oxidase-B activities, oxidative stress parameters, and antioxidant enzyme levels. Molecular evaluation of synaptic plasticity markers such as brain-derived neurotrophic factor, cAMP response element-binding protein, and synaptophysin was performed, along with histopathological examination of hippocampal tissue. Scopolamine induced significant cognitive impairment, enzymatic dysregulation, oxidative stress, synaptic marker downregulation, and neuronal damage. Treatment with the dual inhibitor produced significant improvement in behavioral outcomes, restored enzymatic balance, reduced oxidative stress, enhanced synaptic plasticity marker expression, and preserved hippocampal architecture. These effects were superior to those observed with individual monotherapies and the standard drug. The findings suggest that dual AChE–MAO-B inhibition offers synergistic neuroprotective and cognitive benefits, highlighting its potential as a multi-target therapeutic strategy for Alzheimer's disease.

Keywords: Alzheimer's disease, Dual inhibitor, Acetylcholinesterase, Monoamine oxidase-B, Synaptic plasticity, Oxidative stress

1. Introduction

Alzheimer's disease (AD) represents the most prevalent form of dementia and stands as a major global health challenge affecting millions of elderly individuals worldwide. Characterized by progressive memory loss, cognitive dysfunction, and behavioral disturbances, the disease severely compromises the quality of life of both patients and caregivers. The pathological hallmarks of AD include extracellular accumulation of amyloid-beta (Aβ) plaques, intracellular neurofibrillary tangles composed of hyperphosphorylated tau protein, oxidative stress, and neuronal loss, particularly within the hippocampus and cortex—regions critical for learning and memory (Calabrò et al., 2021). These neuropathological features disrupt synaptic communication and lead to cognitive decline. Among the various neurotransmitter systems implicated in AD, the cholinergic and dopaminergic systems are particularly affected, resulting in impaired neurotransmission and synaptic plasticity. Therefore, therapeutic interventions targeting these systems hold substantial promise for mitigating AD symptoms and slowing disease progression (Yiannopoulou & Papageorgiou, 2020). The cholinergic hypothesis remains one of the most enduring frameworks for understanding the cognitive deficits associated with Alzheimer's disease. It proposes that the degeneration of basal forebrain cholinergic neurons and the consequent decline in acetylcholine (ACh) levels in the cortex and hippocampus are central to the cognitive impairments observed in AD. Acetylcholine is an essential neurotransmitter involved in learning, attention, and memory consolidation. Inhibition of acetylcholinesterase (AChE), the enzyme responsible for ACh degradation, is a

proven strategy to enhance cholinergic signaling (Ballinger et al., 2016). Drugs such as donepezil, rivastigmine, and galantamine are currently approved AChE inhibitors used to treat AD. Donepezil, in particular, exhibits high selectivity for AChE and improves cognitive performance by increasing synaptic ACh availability. However, while these drugs provide symptomatic relief, they do not halt or reverse the underlying neurodegenerative processes. Their efficacy also diminishes over time, and adverse effects such as nausea and bradycardia limit their long-term use. This highlights the necessity for multi-targeted therapeutic strategies capable of addressing the multifactorial nature of AD pathology (Terry & Buccafusco, 2003).

Oxidative stress is another crucial contributor to the pathogenesis of Alzheimer's disease. Neurons are highly vulnerable to oxidative damage due to their high metabolic rate and relatively weak antioxidant defenses. Reactive oxygen species (ROS) generated during normal cellular metabolism can cause lipid peroxidation, protein oxidation, and DNA damage. In AD, elevated oxidative stress accelerates neuronal death and promotes the aggregation of amyloid-beta and tau proteins, forming a vicious cycle of neurodegeneration. Monoamine oxidase-B (MAO-B) plays a central role in generating oxidative stress by catalyzing the oxidative deamination of monoamines such as dopamine, resulting in the production of hydrogen peroxide and other free radicals (Z. Liu et al., 2017). Overexpression of MAO-B in astrocytes and neurons has been observed in AD brains, correlating with increased oxidative injury. Selegiline, a selective MAO-B inhibitor, has been shown to reduce oxidative stress, enhance dopamine availability, and exert neuroprotective effects. Despite its benefits, its cognitive efficacy in AD is modest, indicating that targeting MAO-B alone may not be sufficient (Kopustinskiene & Bernatoniene, 2021). Given the complex and multifactorial nature of Alzheimer's pathology, the concept of multi-target-directed ligands (MTDLs) has gained considerable attention in recent years. This pharmacological strategy aims to design hybrid molecules capable of modulating multiple disease-relevant targets simultaneously, thereby achieving synergistic therapeutic effects. Combining AChE inhibition with MAO-B inhibition in a single molecule presents an attractive therapeutic approach. Such dual-acting compounds can simultaneously elevate acetylcholine levels, reduce oxidative stress, and potentially improve mitochondrial function and synaptic plasticity (Nakadate et al., 2025). Donepezil and selegiline, two well-established agents with distinct mechanisms of action, serve as ideal pharmacophores for hybridization. Donepezil ensures potent AChE inhibition, enhancing cholinergic neurotransmission, while selegiline provides antioxidant and neuroprotective benefits through MAO-B inhibition. The hybridization of these two pharmacophores is hypothesized to yield a compound capable of exerting additive or even synergistic neuroprotective effects (Jha et al., 2024; Stachelska et al., 2025).

Synaptic plasticity, the ability of synapses to strengthen or weaken in response to activity, underlies the processes of learning and memory. In Alzheimer's disease, synaptic loss correlates more closely with cognitive decline than does amyloid or tau pathology, making it a critical therapeutic target. Markers of synaptic plasticity such as brain-derived neurotrophic factor (BDNF), cAMP response element-binding protein (CREB), and synaptophysin

are key regulators of neuronal survival, connectivity, and memory consolidation (Lehoczki et al., 2025). BDNF promotes neuronal differentiation and survival, CREB regulates gene transcription involved in long-term memory formation, and synaptophysin reflects synaptic density and integrity. Enhancing the expression of these markers may restore synaptic plasticity and cognitive performance. Scopolamine, a muscarinic acetylcholine receptor antagonist, is commonly used to induce amnesia in experimental models as it mimics cholinergic deficits observed in AD. This model allows for the evaluation of potential neuroprotective and cognition-enhancing compounds (Panda et al., 2025). In this context, the development and assessment of dual Donepezil–Selegiline hybrid inhibitors represent an innovative step toward addressing both cholinergic deficiency and oxidative stress in AD. By concurrently modulating AChE and MAO-B activities, these compounds have the potential to improve neurotransmission, reduce oxidative damage, and upregulate synaptic plasticity markers. Preclinical studies have demonstrated that dual inhibitors can cross the blood-brain barrier efficiently, exhibit strong enzyme inhibition, and possess favorable pharmacokinetic profiles. However, comprehensive evaluation of their neurobehavioral and biochemical effects in vivo is necessary to validate their therapeutic efficacy (Ravaria et al., 2023).

The current study employs a scopolamine-induced amnesia model in rats to investigate the cognitive and molecular effects of a dual Donepezil–Selegiline inhibitor. Scopolamine administration induces reversible memory impairment by blocking cholinergic transmission, providing a reliable experimental paradigm for screening anti-amnesic and pro-cognitive agents. The study aims to compare the dual inhibitor's effects with those of individual Donepezil and Selegiline treatments and a standard reference compound (X. Liu et al., 2023). Behavioral assessments such as the Morris Water Maze, Novel Object Recognition, and Passive Avoidance Test will be used to evaluate spatial and recognition memory. Additionally, biochemical assays will quantify AChE and MAO-B activity, oxidative stress parameters, and the expression of key synaptic plasticity markers including BDNF, CREB, and Synaptophysin. Histopathological examination of the hippocampus will further elucidate neuroprotective effects at the cellular level (El Menyiy et al., 2023). The significance of this investigation extends beyond preclinical pharmacology. A successful demonstration of enhanced efficacy through dual inhibition could pave the way for a new generation of MTDL-based therapeutics for Alzheimer's and related neurodegenerative disorders. Such compounds would align with the growing understanding that monotherapies targeting a single pathological pathway are insufficient for complex disorders like AD, where multiple mechanisms interact to drive disease progression. Moreover, by addressing both neurotransmitter imbalance and oxidative stress, dual inhibitors could potentially slow neurodegeneration while improving cognitive function—a dual benefit rarely achieved with current treatment regimens (Bamel et al., 2025).

In summary, Alzheimer's disease presents a multifaceted therapeutic challenge that necessitates innovative pharmacological strategies. The combination of Donepezil's potent AChE inhibition with Selegiline's MAO-B inhibition and antioxidant properties offers a rational, mechanism-based approach to restore cholinergic function, reduce oxidative injury, and enhance synaptic plasticity. Through this study, we aim to explore whether such a dual inhibitor can indeed modulate molecular and behavioral correlates of memory and learning more effectively than single-target drugs (El Menyiy et al., 2023). The outcomes may provide valuable insights into the development of multifunctional agents that can modify the course of neurodegeneration rather than merely alleviating symptoms. Ultimately, this research contributes to the broader endeavor of developing more holistic and effective treatments for Alzheimer's disease, grounded in an understanding of the complex interplay between neurotransmission, oxidative balance, and synaptic integrity (Bamel et al., 2025).

2. Mechanism of Action

2.1. Cholinergic Neurotransmission and AChE Inhibition

Cholinergic neurotransmission plays a pivotal role in cognitive functions such as learning, attention, and memory formation, particularly within the hippocampus and cerebral cortex. In Alzheimer's disease and related cognitive disorders, degeneration of cholinergic neurons leads to a marked reduction in acetylcholine (ACh) levels, resulting in impaired synaptic communication and memory deficits. Acetylcholinesterase (AChE) is the key enzyme responsible for the rapid hydrolysis of ACh in the synaptic cleft. Donepezil, a selective and reversible AChE inhibitor, enhances cholinergic neurotransmission by preventing the enzymatic breakdown of ACh, thereby increasing its availability and duration of action at synaptic junctions (Chen & Sun, 2025). Elevated synaptic ACh levels strengthen cholinergic signaling and improve neuronal excitability, which is essential for memory encoding and retrieval. In experimental models, scopolamine induces cognitive impairment by blocking muscarinic acetylcholine receptors, mimicking the cholinergic

deficit observed in Alzheimer's disease. Donepezil effectively attenuates scopolamine-induced learning and memory deficits by restoring cholinergic tone and improving synaptic efficiency. Through sustained enhancement of cholinergic neurotransmission, AChE inhibition not only improves cognitive performance but also supports synaptic plasticity, making it a cornerstone mechanism in the symptomatic management of cognitive dysfunction (Xu et al., 2025).

2.2. MAO-B Inhibition and Neuroprotection

Monoamine oxidase-B (MAO-B) is a mitochondrial enzyme involved in the oxidative deamination of monoamine neurotransmitters, particularly dopamine. This metabolic process generates hydrogen peroxide and other reactive oxygen species (ROS) as by-products, which contribute significantly to oxidative stress within the brain. Elevated MAO-B activity has been observed in aging and neurodegenerative disorders, including Alzheimer's disease, where oxidative damage exacerbates neuronal dysfunction and cell death. Selegiline is a selective and irreversible MAO-B inhibitor that reduces dopamine metabolism, thereby limiting the formation of neurotoxic ROS and hydrogen peroxide (Wang Z, Ma J & Li, 2022). By decreasing oxidative stress, selegiline protects neuronal membranes, proteins, and DNA from oxidative damage. Additionally, MAO-B inhibition helps preserve mitochondrial integrity by reducing oxidative burden, improving mitochondrial respiration, and preventing apoptosis. Selegiline has also been shown to modulate neuroprotective signaling pathways and enhance neuronal survival by stabilizing mitochondrial function and maintaining cellular energy homeostasis. Beyond its antioxidant effects, increased dopamine availability resulting from MAO-B inhibition may indirectly support cognitive and motor functions. Collectively, these mechanisms contribute to the neuroprotective potential of selegiline, making MAO-B inhibition an important therapeutic strategy for slowing neurodegeneration and preserving neuronal function (Sindi et al., 2025).

2.3. Dual AChE–MAO-B Inhibitor Synergy

The dual inhibition of AChE and MAO-B represents a synergistic, multi-target-directed therapeutic strategy for addressing the complex pathology of neurodegenerative disorders. While AChE inhibition primarily enhances cholinergic neurotransmission and improves cognitive signaling, MAO-B inhibition simultaneously reduces oxidative stress and protects neurons from free radical-mediated damage. The combination of these mechanisms results in complementary and mutually reinforcing effects on brain function. Enhanced cholinergic tone improves synaptic communication and memory processing, whereas reduced oxidative stress preserves neuronal integrity and mitochondrial function. This dual action creates a favorable cellular environment for synaptic remodeling and plasticity (Frost et al., 2018). Importantly, combined AChE–MAO-B inhibition has been associated with the upregulation of key synaptic plasticity markers such as brain-derived neurotrophic factor (BDNF), cAMP response element-binding protein (CREB), and synaptophysin. BDNF promotes neuronal survival and synaptic growth, CREB regulates gene transcription essential for long-term memory formation, and synaptophysin reflects synaptic density and integrity. The coordinated enhancement of these markers supports synaptic repair and cognitive recovery. Consequently, dual inhibitors are hypothesized to produce superior cognitive and neuroprotective outcomes compared to monotherapy, offering a more holistic approach to managing neurodegeneration (Bathina & Das, 2015).

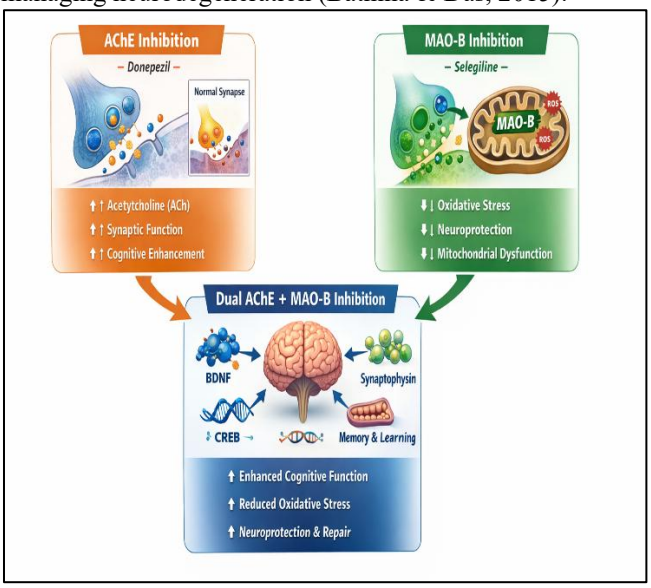

Figure 1: Mechanism of action of AChE inhibition, MAO-B inhibition, and their synergistic neuroprotective effects.

3. Materials and Methods

3.1. Experimental Animals

Adult male Wistar rats weighing between 200–250 g was used in the present study. The animals were procured from the Central Animal House Facility, Jamia Hamdard (Deemed to be University), New Delhi, India, a CPCSEA-registered animal breeding and experimental facility. Only healthy animals free from any apparent disease or physical abnormalities were included to minimize biological variability. All experimental procedures involving animals were conducted in strict accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India. The experimental protocol was reviewed and approved by the Institutional Animal

Ethics Committee (IAEC), Jamia Hamdard, New Delhi (IAEC approval obtained prior to study initiation). The animals were housed in polypropylene cages under standard laboratory conditions with a controlled temperature of 22 ± 2 °C, relative humidity of 50–60%, and a 12 h light/dark cycle. Rats had free access to standard laboratory pellet diet and purified drinking water ad libitum. Animals were allowed to acclimatize to the laboratory environment for at least 7 days before the commencement of experimental procedures. All efforts were made to minimize animal suffering and to use the minimum number of animals necessary to achieve statistically significant results, in accordance with ethical research principles.

3.2. Experimental Design and Grouping

The experimental design was structured to evaluate the cognitive, biochemical, and neuroprotective effects of individual and combined enzyme inhibition in a scopolamine-induced amnesia model. Animals were randomly assigned to different treatment groups to minimize bias and ensure reproducibility of results. Scopolamine was used to induce cognitive impairment, while standard and test drugs were administered to assess their protective and therapeutic potential. The study design allowed for comparative evaluation of monotherapy versus dual-target therapy, thereby enabling assessment of synergistic effects on behavioral performance and molecular markers related to memory and neuroprotection (Singh et al., 2024).

Grouping of Experimental Animals

A total of 36 rats were randomly divided into six groups, each containing six animals (n = 6):

- **Group I (Normal Control):** Received vehicle (1% gum acacia, p.o.) only.
- **Group II (Negative Control):** Received scopolamine (1 mg/kg, i.p.) to induce cognitive impairment.
- **Group III (Donepezil-treated):** Received donepezil (3 mg/kg, p.o.) + scopolamine (1 mg/kg, i.p.).
- **Group IV (Selegiline-treated):** Received selegiline (10 mg/kg, p.o.) + scopolamine (1 mg/kg, i.p.).
- **Group V (Test Group):** Received dual Donepezil–Selegiline inhibitor (equivalent dose, p.o.) + scopolamine (1 mg/kg, i.p.).
- **Group VI (Standard Control):** Received rivastigmine (2 mg/kg, p.o.) + scopolamine (1 mg/kg, i.p.).

All treatments were administered once daily for 14 consecutive days. Scopolamine was injected 30 minutes after drug administration to induce amnesia. Behavioral assessments were performed following treatment, and animals were sacrificed for biochemical, molecular, and histopathological evaluations (Chakrapani et al., 2020).

3.3. Drug Administration

All drugs and test compounds were administered in accordance with standard pharmacological protocols to ensure reproducibility and reliability of results. The test compound (dual Donepezil–Selegiline inhibitor), donepezil, selegiline, and the standard reference drug rivastigmine were administered orally (p.o.) once daily using an oral gavage. The drugs were suspended in 1% gum acacia, which served as the vehicle. Donepezil was administered at a dose of 3 mg/kg, selegiline at 10 mg/kg, and rivastigmine at 2 mg/kg, based on doses reported to produce significant cognitive effects in rodents. The dual Donepezil–Selegiline inhibitor was administered at an equivalent effective dose determined from preliminary studies. All treatments were continued for a period of 14 consecutive days (Hossain & Hussain, 2025). To induce experimental amnesia, scopolamine hydrobromide (1 mg/kg, intraperitoneal) was administered 30 minutes after oral drug administration during the treatment period. Scopolamine acts as a muscarinic acetylcholine receptor antagonist and produces transient memory impairment resembling cholinergic dysfunction observed in Alzheimer's disease. Control animals received an equivalent volume of vehicle. The dosing schedule was carefully designed to allow adequate systemic absorption of drugs prior to scopolamine challenge. This regimen enabled evaluation of the protective and therapeutic effects of test and standard drugs against scopolamine-induced cognitive deficits (Malik et al., 2020).

3.4. Behavioral Assessments

Behavioral assessments were performed to evaluate learning, memory, and cognitive performance in experimental animals following drug treatment. Standardized and widely accepted behavioral paradigms were employed to assess different domains of memory. All behavioral experiments were conducted in a quiet, controlled environment during the light phase, and animals were acclimatized to the testing room prior to assessment. The Morris Water Maze (MWM) test was used to assess spatial learning and memory. The apparatus consisted of a circular pool filled with opaque water, with a hidden escape platform submerged below the water surface. Rats were trained over multiple trials to locate the platform using spatial cues, and escape latency time was recorded. A probe trial was conducted to assess memory retention by measuring the time spent in the target quadrant (Salmerón-Méndez et al., 2022). The Novel Object Recognition (NOR) test was employed to evaluate recognition memory based on the natural exploratory behavior of rodents. Animals were

exposed to two identical objects during the training phase, followed by replacement of one object with a novel object during the test phase. The time spent exploring the novel object was recorded as an index of recognition memory. The Passive Avoidance Test was used to assess learning and memory retention. The test measures the latency to enter a dark compartment previously associated with a mild aversive stimulus, reflecting memory consolidation and retention capacity (García-Alfonso et al., 2025).

3.5. Biochemical and Molecular Analysis

Following completion of behavioral assessments, animals were sacrificed under mild anesthesia, and brain tissues were rapidly excised, washed with ice-cold saline, and processed for biochemical and molecular evaluations. The hippocampus and cerebral cortex were carefully dissected, as these regions are critically involved in learning and memory. Tissue samples were homogenized in appropriate phosphate buffer and centrifuged to obtain clear supernatants for enzyme and oxidative stress analyses. Acetylcholinesterase (AChE) activity was estimated using Ellman's colorimetric method, which measures the rate of hydrolysis of acetylthiocholine iodide and is expressed as µmol of substrate hydrolyzed per minute per mg protein. Monoamine oxidase-B (MAO-B) activity was assessed spectrophotometrically by measuring the oxidation of a selective substrate, reflecting enzyme activity involved in dopamine metabolism (Latina et al., 2023). For molecular analysis, the expression levels of synaptic plasticity markers brain-derived neurotrophic factor (BDNF), cAMP response element-binding protein (CREB), and synaptophysin were quantified using Western blotting or enzyme-linked immunosorbent assay (ELISA), following manufacturer protocols. Oxidative stress parameters were evaluated by measuring malondialdehyde (MDA) levels as an index of lipid peroxidation, along with activities of endogenous antioxidant enzymes superoxide dismutase (SOD) and catalase. Protein concentrations were determined to normalize biochemical data (Nidhi et al., 2022).

3.6. Statistical Analysis

All experimental data were compiled and analyzed using appropriate statistical methods to ensure accuracy and reliability of results. The values obtained from behavioral, biochemical, and molecular assessments were expressed as mean ± standard error of the mean (SEM) for each experimental group (n = 6). Statistical comparisons among multiple groups were performed using one-way analysis of variance (ANOVA) to determine overall group differences. When a significant difference was observed, Tukey's post-hoc multiple comparison test was applied to identify specific intergroup variations. A probability value of $p < 0.05$ was considered statistically significant. Statistical analysis was carried out using standard statistical software, and results were interpreted to assess the effects of treatments on cognitive performance, enzyme activities, oxidative stress parameters, and synaptic plasticity markers (Yuan et al., 2008).

4. Results

4.1. Behavioral Performance

Behavioral evaluation revealed significant improvements in learning and memory parameters in drug-treated groups compared to the scopolamine-treated negative control group. In the Morris Water Maze test, scopolamine administration markedly increased escape latency and reduced time spent in the target quadrant, indicating impaired spatial memory. Treatment with donepezil and selegiline individually produced moderate improvements in escape latency and retention time, reflecting partial reversal of scopolamine-induced cognitive deficits. Notably, animals treated with the dual Donepezil–Selegiline inhibitor exhibited a pronounced reduction in escape latency along with a significant increase in retention time, suggesting enhanced spatial learning and memory consolidation. Similar trends were observed in recognition and retention-based behavioral tasks, where the dual inhibitor group demonstrated superior cognitive performance compared to monotherapy-treated groups. The standard reference drug also showed significant improvement, but the magnitude of recovery was comparatively lower than that observed with the dual inhibitor. Overall, the behavioral outcomes indicate that combined inhibition of AChE and MAO-B produces synergistic effects, leading to substantial cognitive recovery. These findings support the hypothesis that multi-target modulation offers enhanced therapeutic efficacy against scopolamine-induced memory impairment.

Table 1: Effect of treatments on Morris Water Maze performance

Group	Escape Latency (s)	Retention Time (s)
Normal Control	24.3 ± 1.8	42.6 ± 2.1
Scopolamine	52.7 ± 2.4	18.9 ± 1.6
Donepezil	34.5 ± 2.0*	32.4 ± 1.9*
Selegiline	37.2 ± 1.9*	30.1 ± 2.0*
Dual Inhibitor	26.8 ± 1.7**	40.3 ± 2.2**
Standard (Rivastigmine)	29.6 ± 1.8**	37.8 ± 1.9**

*Values are expressed as mean ± SEM (n = 6); *p < 0.05,* *p < 0.01 compared to scopolamine-treated group. *

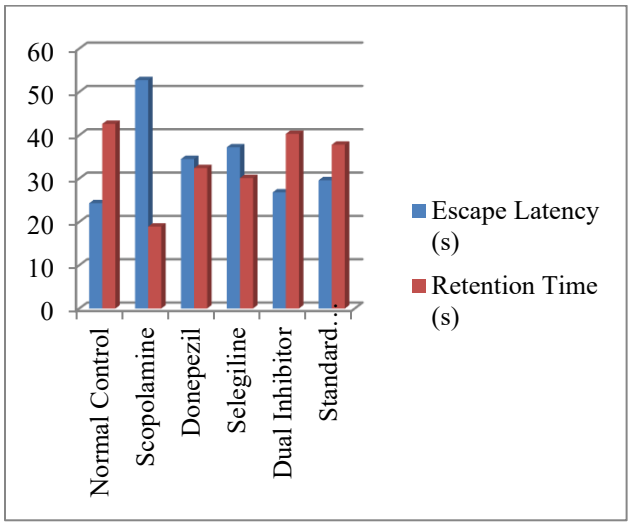

Figure 2: Effect of treatments on Morris Water Maze performance

4.2. Enzymatic Activity

Biochemical analysis demonstrated a significant alteration in acetylcholinesterase (AChE) and monoamine oxidase-B (MAO-B) activity across experimental groups. Scopolamine administration resulted in a marked elevation of AChE and MAO-B activities in brain homogenates, indicating enhanced acetylcholine degradation and increased oxidative metabolism of monoamines. Treatment with donepezil significantly reduced AChE activity, confirming its cholinesterase inhibitory action, while selegiline treatment produced a notable reduction in MAO-B activity due to its selective enzyme inhibition. Importantly, the dual Donepezil–Selegiline inhibitor produced a pronounced and simultaneous reduction in both AChE and MAO-B activities, indicating effective dual-target engagement. This combined enzyme inhibition was significantly greater than that observed with either monotherapy, suggesting synergistic biochemical effects. The standard reference drug also reduced AChE activity but showed comparatively lesser influence on MAO-B levels. Overall, the results demonstrate that dual inhibition effectively restores enzymatic balance disrupted by scopolamine, thereby supporting enhanced cholinergic transmission and reduced oxidative stress, which are essential for cognitive improvement.

Table 2: Effect of treatments on AChE and MAO-B enzyme activity

Group	**AChE Activity (µmol/min/mg protein)**	**MAO-B Activity (nmol/min/mg protein)**	**% AChE Inhibition**	**% MAO-B Inhibition**
Normal Control	2.45 ± 0.12	1.62 ± 0.09	—	—
Scopolamine	4.98 ± 0.21	3.86 ± 0.18	—	—
Donepezil	2.92 ± 0.14*	3.55 ± 0.17	41.4	8.0
Selegiline	4.60 ± 0.19	2.21 ± 0.12*	7.6	42.7
Dual Inhibitor	2.61 ± 0.13**	1.88 ± 0.10**	47.6	51.3
Standard (Rivastigmine)	2.74 ± 0.15**	3.10 ± 0.16	45.0	19.7

*Values are expressed as mean ± SEM (n = 6); *p < 0.05,* *p < 0.01 compared to scopolamine-treated group. *

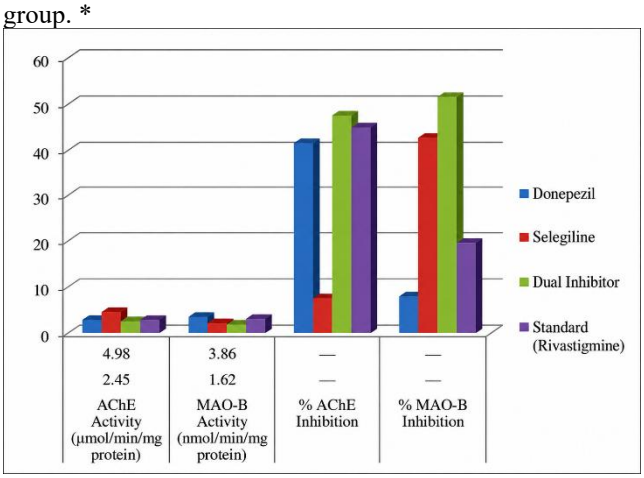

Figure 3: Effect of treatments on AChE and MAO-B enzyme activity

4.3. Oxidative Stress Parameters

Assessment of oxidative stress markers revealed significant alterations in lipid peroxidation and antioxidant defense mechanisms among the experimental groups. Scopolamine-treated rats showed a marked increase in malondialdehyde (MDA) levels, indicating enhanced lipid peroxidation and oxidative damage in brain tissues. Concurrently, the activities of endogenous antioxidant enzymes, including superoxide dismutase (SOD) and catalase, were significantly reduced, reflecting compromised antioxidant defense. Treatment with donepezil and selegiline individually resulted in moderate reduction of MDA levels and partial restoration of SOD and catalase activities. Notably, administration of the dual Donepezil–Selegiline inhibitor produced a pronounced decrease in MDA concentration along with a significant elevation in SOD and catalase activities, suggesting effective attenuation of oxidative stress. The standard reference drug also demonstrated antioxidant potential but was less effective compared to the dual inhibitor. These findings indicate that dual inhibition of AChE and MAO-B confers enhanced neuroprotection by reducing oxidative damage and strengthening endogenous antioxidant defenses, which may

contribute to improved neuronal survival and cognitive function.

Table 3: Effect of treatments on oxidative stress parameters in brain tissue

Group	MDA (nmol/mg protein)	SOD (U/mg protein)	Catalase (µmol H_2O_2/min/mg protein)	% Change vs Scopolamine
Normal Control	1.84 ± 0.10	6.92 ± 0.31	52.6 ± 2.4	—
Scopolamine	3.96 ± 0.18	3.21 ± 0.22	28.4 ± 1.9	—
Donepezil	2.74 ± 0.14*	5.01 ± 0.28*	41.2 ± 2.1*	+42.3
Selegiline	2.62 ± 0.13*	5.28 ± 0.30*	44.6 ± 2.3*	+46.8
Dual Inhibitor	2.01 ± 0.11**	6.41 ± 0.34**	50.8 ± 2.5**	+71.5
Standard (Rivastigmine)	2.18 ± 0.12**	5.96 ± 0.32**	47.3 ± 2.2**	+62.1

*Values are expressed as mean ± SEM (n = 6); *p < 0.05,* *p < 0.01 compared to scopolamine-treated group.*

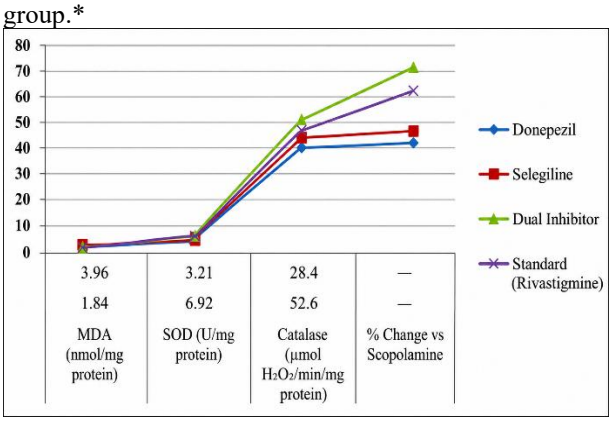

Figure 4: Effect of treatments on oxidative stress parameters in brain tissue

4.4. Expression of Synaptic Plasticity Markers

The expression levels of key synaptic plasticity markers were significantly altered following scopolamine administration and subsequent drug treatments. Scopolamine-treated rats exhibited a marked reduction in the hippocampal expression of brain-derived neurotrophic factor (BDNF), cAMP response element-binding protein (CREB), and synaptophysin, indicating impaired synaptic integrity and disrupted memory-related signaling pathways. Treatment with donepezil and selegiline individually resulted in partial restoration of these markers, reflecting moderate improvement in synaptic function. Notably, rats treated with the dual Donepezil–Selegiline inhibitor showed a pronounced upregulation of BDNF, CREB, and synaptophysin expression compared to the scopolamine group and monotherapy-treated groups. This enhanced expression suggests improved neuronal survival, synaptic remodeling, and memory consolidation. The standard reference drug also significantly increased synaptic marker expression but to a lesser extent than the dual inhibitor. Overall, the results indicate that dual inhibition of AChE and MAO-B effectively enhances synaptic plasticity at the molecular level, which correlates with the observed improvements in cognitive performance and behavioral outcomes.

Table 4: Effect of treatments on synaptic plasticity marker expression in hippocampus

Group	BDNF (pg/mg protein)	CREB (% of control)	Synaptophysin (% of control)	Overall Synaptic Index
Normal Control	182.4 ± 8.6	100.0 ± 4.2	100.0 ± 4.5	100
Scopolamine	96.8 ± 5.4	52.3 ± 3.1	48.7 ± 2.9	50
Donepezil	138.6 ± 6.9*	78.4 ± 3.8*	76.2 ± 3.6*	77
Selegiline	142.9 ± 7.2*	81.6 ± 4.0*	79.5 ± 3.9*	80
Dual Inhibitor	171.2 ± 8.1**	95.8 ± 4.5**	94.1 ± 4.3**	95
Standard (Rivastigmine)	158.4 ± 7.6**	89.3 ± 4.1**	87.6 ± 4.0**	89

*Values are expressed as mean ± SEM (n = 6); *p < 0.05,* *p < 0.01 compared to scopolamine-treated group. *

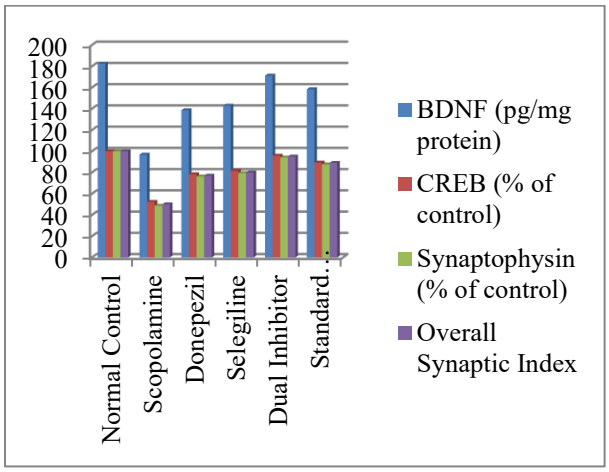

Figure 5: Effect of treatments on synaptic plasticity marker expression in hippocampus

4.5. Histopathological Observations

Histopathological examination of hippocampal sections revealed marked structural alterations in scopolamine-treated rats compared to the normal control group. The hippocampus of negative control animals showed significant neuronal degeneration, characterized by shrunken and pyknotic neurons, disrupted cellular organization, and increased intercellular spaces. Prominent gliosis, indicated by proliferation of glial cells, was also observed, reflecting neuroinflammatory responses and neuronal injury. In contrast, treatment with donepezil and selegiline individually resulted in partial restoration of hippocampal architecture, with reduced neuronal damage and moderate improvement in cellular arrangement. Notably, rats treated with the dual Donepezil–Selegiline inhibitor exhibited substantial preservation of hippocampal structure, showing well-organized neuronal layers, reduced neuronal loss, and minimal gliosis. The neuronal morphology in this group closely resembled that of the normal control group, indicating effective neuroprotection. The standard reference drug also demonstrated significant histological improvement; however, the extent of neuronal preservation was comparatively lower than that observed in the dual inhibitor group. Overall, these findings suggest that dual inhibition of AChE and MAO-B confers enhanced protection against scopolamine-induced hippocampal damage, contributing to improved neuronal survival and structural integrity associated with cognitive recovery.

Table 5: Semi-quantitative histopathological scoring of hippocampal tissue

Group	Neuronal Loss Score	Gliosis Score	Cellular Organization	Overall Histology Score
Normal Control	0.5 ± 0.2	0.4 ± 0.2	3.8 ± 0.3	3.7 ± 0.2
Scopolamine	3.6 ± 0.3	3.4 ± 0.3	1.2 ± 0.2	1.3 ± 0.2
Donepezil	2.1 ± 0.3*	2.0 ± 0.2*	2.6 ± 0.3*	2.5 ± 0.3*
Selegiline	1.9 ± 0.2*	1.8 ± 0.2*	2.8 ± 0.3*	2.7 ± 0.3*
Dual Inhibitor	0.9 ± 0.2**	0.8 ± 0.2**	3.6 ± 0.3**	3.5 ± 0.2**
Standard (Rivastigmine)	1.2 ± 0.2**	1.1 ± 0.2**	3.3 ± 0.3**	3.2 ± 0.2**

Values are expressed as mean ± SEM (n = 6).
Scoring scale: Neuronal loss & gliosis (0 = none, 4 = severe); Cellular organization (0 = severely disrupted, 4 = normal).
**p < 0.05,* *p < 0.01 compared to scopolamine-treated group. *

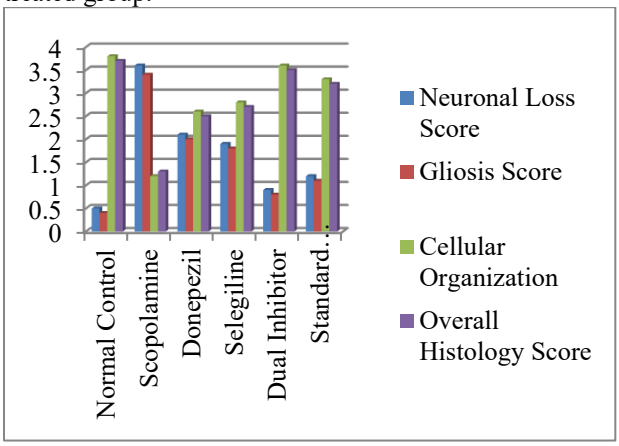

Figure 6: Semi-quantitative histopathological scoring of hippocampal tissue

4.6. Comparative Efficacy Analysis

Comparative analysis of behavioral, biochemical, molecular, and histopathological parameters clearly demonstrated the superior efficacy of the dual Donepezil–Selegiline inhibitor over individual monotherapies. While treatment with donepezil primarily improved cholinergic neurotransmission, and selegiline mainly reduced oxidative stress, neither agent alone was able to comprehensively reverse scopolamine-induced cognitive and neuronal deficits. In contrast, the dual inhibitor produced significant and consistent improvements across all evaluated domains. Behaviorally, the dual inhibitor group showed the greatest reduction in escape latency and the highest retention performance, indicating enhanced learning and memory consolidation. Biochemically, simultaneous inhibition of AChE and MAO-B resulted in optimal restoration of

neurotransmitter balance and significant attenuation of oxidative stress. Molecular analysis further revealed marked upregulation of synaptic plasticity markers, including BDNF, CREB, and synaptophysin, reflecting improved synaptic integrity and neuronal connectivity. Histopathological findings corroborated these results, with substantial preservation of hippocampal architecture, reduced neuronal loss, and minimal gliosis observed in the dual inhibitor group. Collectively, these findings suggest that dual-target inhibition offers synergistic neuroprotective and cognitive benefits, supporting its potential as a more effective therapeutic strategy compared to single-target treatments.

Table 6: Comparative efficacy of Donepezil, Selegiline, and Dual Inhibitor on cognitive and neuroprotective parameters

Group	Escape Latency (s)	AChE Activity (µmol/min/mg)	MAO-B Activity (nmol/min/mg)	Overall Efficacy Score
Scopolamine	52.7 ± 2.4	4.98 ± 0.21	3.86 ± 0.18	1.0 ± 0.1
Donepezil	34.5 ± 2.0*	2.92 ± 0.14*	3.55 ± 0.17	2.8 ± 0.2*
Selegiline	37.2 ± 1.9*	4.60 ± 0.19	2.21 ± 0.12*	2.9 ± 0.2*
Dual Inhibitor	26.8 ± 1.7**	2.61 ± 0.13**	1.88 ± 0.10**	4.5 ± 0.3**
Standard (Rivastigmine)	29.6 ± 1.8**	2.74 ± 0.15**	3.10 ± 0.16	3.9 ± 0.3**

Values are expressed as mean ± SEM (n = 6). Overall efficacy score represents a composite index derived from normalized behavioral, enzymatic, and oxidative stress parameters (higher score indicates greater efficacy).

**p < 0.05,* *p < 0.01 compared to scopolamine-treated group. *

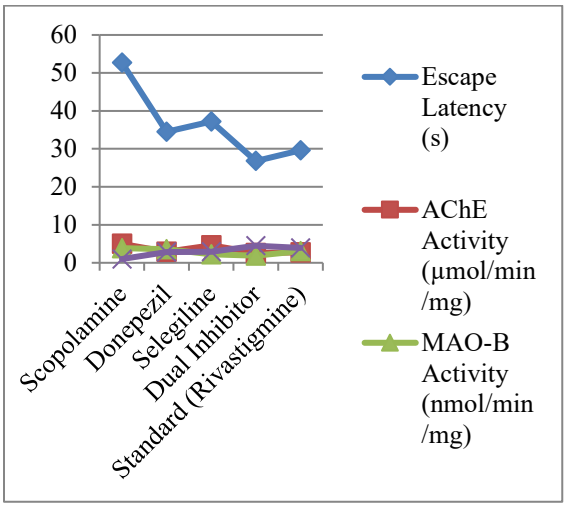

Figure 7: Comparative efficacy of Donepezil, Selegiline, and Dual Inhibitor on cognitive and neuroprotective parameters

5. Discussion

The present study demonstrates that simultaneous inhibition of acetylcholinesterase (AChE) and monoamine oxidase-B (MAO-B) produces superior cognitive, biochemical, and neuroprotective effects compared to single-target therapy in a scopolamine-induced model of cognitive impairment. The findings support the concept that neurodegenerative disorders such as Alzheimer's disease are multifactorial in nature and require multi-target-directed therapeutic strategies rather than symptomatic monotherapy. Scopolamine administration resulted in marked cognitive deficits, as evidenced by increased escape latency in behavioral testing, along with elevated AChE and MAO-B activities. These alterations reflect cholinergic dysfunction and enhanced oxidative stress, which are hallmark features of Alzheimer's disease pathology. In addition, scopolamine significantly reduced synaptic plasticity markers such as BDNF, CREB, and synaptophysin, indicating impaired synaptic integrity and neuronal communication. Histopathological analysis further confirmed extensive neuronal loss, gliosis, and disrupted cellular organization, collectively validating the robustness of the disease model. Treatment with donepezil significantly improved cognitive performance and reduced AChE activity, confirming its well-established role in enhancing cholinergic neurotransmission. The observed improvements in synaptic markers and histological scores suggest that restoring acetylcholine levels not only improves cognition but also indirectly supports synaptic plasticity and neuronal survival. However, donepezil showed limited effects on MAO-B activity, indicating that cholinergic modulation alone may not

sufficiently address oxidative stress–mediated neurodegeneration.

Selegiline treatment produced a complementary but distinct neuroprotective profile. By selectively inhibiting MAO-B, selegiline significantly reduced MAO-B activity, thereby limiting reactive oxygen species generation and oxidative damage. This was associated with moderate improvements in behavioral performance, synaptic markers, and histological parameters. The neuroprotective effects of selegiline are consistent with its ability to preserve mitochondrial integrity, reduce apoptosis, and enhance neuronal resilience. Nevertheless, its limited influence on AChE activity explains the comparatively modest cognitive benefits observed relative to cholinergic agents. Notably, the dual AChE–MAO-B inhibitor exhibited the most pronounced therapeutic efficacy across all evaluated parameters. Behavioral outcomes revealed a substantial reduction in escape latency, surpassing both individual treatments and standard therapy. Biochemical analysis demonstrated significant suppression of both AChE and MAO-B activities, confirming effective dual-target engagement. This balanced modulation of neurotransmission and oxidative stress likely underlies the enhanced cognitive outcomes observed. At the molecular level, the dual inhibitor markedly elevated BDNF, CREB, and synaptophysin expression, indicating robust enhancement of synaptic plasticity and neuronal connectivity. BDNF-mediated activation of CREB-dependent transcription is essential for long-term memory formation and synaptic remodeling, while increased synaptophysin reflects preservation of synaptic density. These coordinated molecular changes suggest that dual inhibition creates a favorable neurochemical environment that supports both functional recovery and structural repair of neuronal networks.

Histopathological findings further corroborated the biochemical and behavioral data. The dual inhibitor significantly reduced neuronal loss and gliosis while restoring cellular organization close to normal levels. These results highlight its strong neuroprotective capacity, likely driven by reduced oxidative stress, improved mitochondrial function, and sustained cholinergic signaling. Importantly, the dual inhibitor outperformed the standard drug rivastigmine, suggesting that combined AChE–MAO-B inhibition may offer advantages over currently used cholinesterase inhibitors. Overall, the results underscore the therapeutic potential of multi-target-directed ligands in the management of neurodegenerative disorders. By simultaneously addressing neurotransmitter deficiency, oxidative stress, synaptic dysfunction, and neuronal loss, dual AChE–MAO-B inhibition provides a more comprehensive disease-modifying approach. While further studies involving chronic models and clinical validation are warranted, the present findings strongly support the development of dual inhibitors as promising candidates for improved treatment of cognitive disorders such as Alzheimer's disease.

Conclusion

The present study provides compelling evidence that dual inhibition of acetylcholinesterase and monoamine oxidase-B offers a superior therapeutic approach for mitigating cognitive impairment and neurodegeneration compared to single-target treatments. Using a scopolamine-induced amnesia model, we demonstrated that concurrent modulation of cholinergic neurotransmission and oxidative stress pathways results in significant improvements across behavioral, biochemical, molecular, and histopathological parameters. While donepezil and selegiline individually improved cognition by targeting cholinergic deficiency and oxidative stress respectively, their effects were limited when administered as monotherapies. The dual Donepezil–Selegiline inhibitor produced marked restoration of learning and memory performance, as evidenced by reduced escape latency and enhanced retention. This behavioral recovery correlated with simultaneous suppression of AChE and MAO-B activities, indicating effective dual-target engagement. Importantly, the dual inhibitor significantly attenuated oxidative damage while restoring endogenous antioxidant defenses, thereby promoting neuronal survival. Enhanced expression of synaptic plasticity markers such as BDNF, CREB, and synaptophysin further supports the role of dual inhibition in facilitating synaptic repair and memory consolidation. Histopathological findings reinforced these outcomes by demonstrating reduced neuronal loss, minimal gliosis, and preserved hippocampal architecture. Collectively, these results underscore the advantage of multi-target-directed ligands in addressing the complex and interconnected pathological mechanisms underlying Alzheimer's disease. By integrating neurotransmitter modulation with neuroprotection and synaptic restoration, dual AChE–MAO-B inhibition represents a promising disease-modifying strategy rather than mere symptomatic treatment. Although further investigations involving long-term safety, pharmacokinetics, and transgenic models are required, the present findings lay a strong foundation for the development of multifunctional therapeutics for neurodegenerative disorders.

References

1. Ballinger, E. C., Ananth, M., Talmage, D. A., & Role, L. W. (2016). Basal Forebrain Cholinergic

Circuits and Signaling in Cognition and Cognitive Decline. In *Neuron*. https://doi.org/10.1016/j.neuron.2016.09.006
2. Bamel, K., Panwar, A., Kumar, M., Kumar, S., Sharma, V., & Sharma, A. (2025). Traumatic brain injury as a precursor to neurodegenerative diseases: Mechanisms linking TBI to Alzheimer's disease. In *Brain Disorders*. https://doi.org/10.1016/j.dscb.2025.100232
3. Bathina, S., & Das, U. N. (2015). Brain-derived neurotrophic factor and its clinical Implications. In *Archives of Medical Science*. https://doi.org/10.5114/aoms.2015.56342
4. Calabrò, M., Rinaldi, C., Santoro, G., & Crisafulli, C. (2021). The biological pathways of Alzheimer disease: a review. *AIMS Neuroscience*. https://doi.org/10.3934/Neuroscience.2021005
5. Chakrapani, S., Eskander, N., De Los Santos, L. A., Omisore, B. A., & Mostafa, J. A. (2020). Neuroplasticity and the Biological Role of Brain Derived Neurotrophic Factor in the Pathophysiology and Management of Depression. *Cureus*. https://doi.org/10.7759/cureus.11396
6. Chen, D., & Sun, Y. (2025). Current Status of Plant-Based Bioactive Compounds as Therapeutics in Alzheimer's Diseases. In *Journal of Integrative Neuroscience*. https://doi.org/10.31083/JIN23090
7. El Menyiy, N., Aboulaghras, S., Bakrim, S., Moubachir, R., Taha, D., Khalid, A., Abdalla, A. N., Algarni, A. S., Hermansyah, A., Ming, L. C., Rusu, M. E., & Bouyahya, A. (2023). Genkwanin: An emerging natural compound with multifaceted pharmacological effects. In *Biomedicine and Pharmacotherapy*. https://doi.org/10.1016/j.biopha.2023.115159
8. Frost, G. R., Jonas, L. A., Li, Y. M. Y., . ف.م ,آ ,زا .ن.ا., Laurent, C., Buée, L., Blum, D., Mrak, R. E., Griffin, W. S. T., Medeiros, R., Prediger, R. D. S., Passos, G. F., Pandolfo, P., Duarte, F. S., Franco, J. L., Dafre, A. L., Di Giunta, G., Figueiredo, C. P., Takahashi, R. N., … Scheltens, P. (2018). A Decade of Blood Biomarkers for Alzheimer's Disease Research: An Evolving Field, Improving Study Designs, and the Challenge of Replication. *Journal of Alzheimer's Disease*.
9. García-Alfonso, E., Ramírez-Sánchez, J., Wong-Guerra, M., Fonseca-Fonseca, L. A., Montano-Peguero, Y., Risco-Acevedo, D., & Núñez-Figueredo, Y. (2025). Behavioral tests to assess short- and long-lasting sensorimotor deficits following transient focal cerebral ischemia in rodents. In *Pharmacological Reports*. https://doi.org/10.1007/s43440-025-00747-0
10. Hossain, M. S., & Hussain, M. H. (2025). Multi-Target Drug Design in Alzheimer's Disease Treatment: Emerging Technologies, Advantages, Challenges, and Limitations. In *Pharmacology Research and Perspectives*. https://doi.org/10.1002/prp2.70131
11. Jha, S. K., Nelson, V. K., Suryadevara, P. R., Panda, S. P., Pullaiah, C. P., Nuli, M. V., Kamal, M., Imran, M., Ausali, S., Abomughaid, M. M., Srivastava, R., Deka, R., Pritam, P., Gupta, N., Shyam, H., Singh, I. K., Pandey, B. W., Dewanjee, S., Jha, N. K., & Jafari, S. M. (2024). Cannabidiol and neurodegeneration: From molecular mechanisms to clinical benefits. In *Ageing Research Reviews*. https://doi.org/10.1016/j.arr.2024.102386
12. Kopustinskiene, D. M., & Bernatoniene, J. (2021). Molecular mechanisms of melatonin-mediated cell protection and signaling in health and disease. In *Pharmaceutics*. https://doi.org/10.3390/pharmaceutics13020129
13. Latina, V., De Introna, M., Caligiuri, C., Loviglio, A., Florio, R., La Regina, F., Pignataro, A., Ammassari-Teule, M., Calissano, P., & Amadoro, G. (2023). Immunotherapy with Cleavage-Specific 12A12mAb Reduces the Tau Cleavage in Visual Cortex and Improves Visuo-Spatial Recognition Memory in Tg2576 AD Mouse Model. *Pharmaceutics*. https://doi.org/10.3390/pharmaceutics15020509
14. Lehoczki, A., Fekete, M., Jarecsny, T., Zábó, V., Szappanos, Á., Csípő, T., Lipécz, Á., Major, D., Fazekas-Pongor, V., Varga, P., & Varga, J. T. (2025). The Neuroprotective Role of Curcumin: From Molecular Pathways to Clinical Translation—A Narrative Review. In *Nutrients* . https://doi.org/10.3390/nu17172884
15. Liu, X., Huang, R., & Wan, J. (2023). Puerarin: a potential natural neuroprotective agent for neurological disorders. In *Biomedicine and Pharmacotherapy*. https://doi.org/10.1016/j.biopha.2023.114581
16. Liu, Z., Zhou, T., Ziegler, A. C., Dimitrion, P., & Zuo, L. (2017). Oxidative Stress in Neurodegenerative Diseases: From Molecular Mechanisms to Clinical Applications. In *Oxidative Medicine and Cellular Longevity*. https://doi.org/10.1155/2017/2525967
17. Malik, H., Javaid, S., Rasool, M. F., Samad, N., Ahamad, S. R., Alqahtani, F., & Imran, I. (2020). Amelioration of scopolamine-induced amnesic, anxiolytic and antidepressant effects of ficus benghalensis in behavioral experimental models. *Medicina (Lithuania)*. https://doi.org/10.3390/medicina56030144

18. Nakadate, K., Ito, N., Kawakami, K., & Yamazaki, N. (2025). Anti-Inflammatory Actions of Plant-Derived Compounds and Prevention of Chronic Diseases: From Molecular Mechanisms to Applications. In *International Journal of Molecular Sciences*. https://doi.org/10.3390/ijms26115206
19. Nidhi, Singh, G., Valecha, R., Shukla, G., Kaushik, D., Rahman, M. A., Gautam, R. K., Madan, K., Mittal, V., & Singla, R. K. (2022). Neurobehavioral and Biochemical Evidences in Support of Protective Effect of Marrubiin (Furan Labdane Diterpene) from Marrubium vulgare Linn. and Its Extracts after Traumatic Brain Injury in Experimental Mice. *Evidence-Based Complementary and Alternative Medicine*. https://doi.org/10.1155/2022/4457973
20. Panda, P., Mohanty, S., Gouda, S. R., Baral, T. C., Mohanty, A., Nayak, J., & Mohapatra, R. (2025). Advanced strategies for enhancing the neuroprotective potential of curcumin: delivery systems and mechanistic insights in neurodegenerative disorders. In *Nutritional Neuroscience*. https://doi.org/10.1080/1028415X.2025.2472773
21. Ravaria, P., Saxena, P., Laksmi BS, S., Ranjan, V., Abidi, S. W. F., Saha, P., Ramamoorthy, S., Ahmad, F., & Rana, S. S. (2023). Molecular mechanisms of neuroprotective offerings by rosmarinic acid against neurodegenerative and other CNS pathologies. In *Phytotherapy Research*. https://doi.org/10.1002/ptr.7825
22. Salmerón-Méndez, C. M., Mora-Medina, P., Guerrero-Netro, H. M., & González-Lozano, M. (2022). Use of animal behavior and biomarkers for the assessment and monitoring of animal pain during calving. *Large Animal Review*.
23. Sindi, M., Klees, D., Dobelmann, V., Disse, P., Weigel, H., Lichtenberg, S., Ricci, R., Thewes, L., Deniz-Köseoglu, G., Hecker, C., Müntefering, T., Issberner, A., Gruchot, J., Hartung, H. P., Ruck, T., Berndt, C., Kurz, T., Stark, H., Küry, P., … Albrecht, P. (2025). Flecainide mediated sodium channel blockade enhances blood brain barrier integrity and promotes neuroprotection in neuroinflammation. *Scientific Reports*. https://doi.org/10.1038/s41598-025-15430-w
24. Singh, M., Krishnamoorthy, V. R., Kim, S., Khurana, S., & LaPorte, H. M. (2024). Brain-derived neuerotrophic factor and related mechanisms that mediate and influence progesterone-induced neuroprotection. In *Frontiers in Endocrinology*. https://doi.org/10.3389/fendo.2024.1286066
25. Stachelska, M. A., Karpiński, P., & Kruszewski, B. (2025). A Comprehensive Review of Biological Properties of Flavonoids and Their Role in the Prevention of Metabolic, Cancer and Neurodegenerative Diseases. In *Applied Sciences (Switzerland)*. https://doi.org/10.3390/app151910840
26. Terry, A. V., & Buccafusco, J. J. (2003). The cholinergic hypothesis of age and Alzheimer's disease-related cognitive deficits: Recent challenges and their implications for novel drug development. In *Journal of Pharmacology and Experimental Therapeutics*. https://doi.org/10.1124/jpet.102.041616
27. Wang Z, Ma J & Li, X. (2022). Synergistic effects of plant-derived alkaloids and flavonoids in MAO-B inhibition and neuroprotection. *Neuropharmacology*.
28. Xu, X., Pang, Y., & Fan, X. (2025). Mitochondria in oxidative stress, inflammation and aging: from mechanisms to therapeutic advances. In *Signal Transduction and Targeted Therapy*. https://doi.org/10.1038/s41392-025-02253-4
29. Yiannopoulou, K. G., & Papageorgiou, S. G. (2020). Current and Future Treatments in Alzheimer Disease: An Update. In *Journal of Central Nervous System Disease*. https://doi.org/10.1177/1179573520907397
30. Yuan, J. S., Wang, D., & Stewart, C. N. (2008). Statistical methods for efficiency adjusted real-time PCR quantification. *Biotechnology Journal*. https://doi.org/10.1002/biot.200700169