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Neuroprotective Potential of Diosmetin and Polmacoxib against Scopolamine-Induced Alzheimer's Disease in Rats

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Abstract

Background: Alzheimer's disease involves amyloid-β accumulation, oxidative stress, neuroinflammation, and cognitive decline. Scopolamine-induced Alzheimer's disease mimics these pathological changes in experimental models. Diosmetin and Polmacoxib possess antioxidant, anti-inflammatory, and neuroprotective properties. This study evaluated their individual and combined effects on Scopolamine-induced behavioural and neurochemical impairments in rats. **Aim:** This study aimed to investigate the potential of Diosmetin and Polmacoxib to ameliorate scopolamine-induced cognitive deficits, oxidative stress, and cholinergic dysfunction in rats, thereby evaluating their efficacy as a therapeutic candidate for Alzheimer's disease. **Methods:** Alzheimer's disease was induced in rats by scopolamine (1 mg/kg, p.o. consecutively for 14 days). Pretreatment with Diosmetin, a flavonoid (100 and 200 mg/kg p.o.) and Polmacoxib, a COX-II-specific inhibitor (7 and 15 mg/kg, p.o.) and their combination were given for 14 days. Behavioural assessments (Y-maze, EPM, NORT and Morris water maze test) were performed on days 1st, 7th and 14th followed by biochemical parameters such as oxidative stress indices (CAT, GSH, SOD, and LPO), acetylcholinesterase level, proinflammatory cytokines (TNF-α and IL-1β), and β-amyloid expression, with histopathological evaluations of the hippocampus. **Results:** Scopolamine induced significant cognitive impairment, oxidative stress, neuroinflammation, and β-amyloid elevation in rats. Diosmetin and Polmacoxib improved learning and memory, restored antioxidant defences, reduced lipid peroxidation, and modulated AChE, inflammatory, and amyloidogenic markers. Histopathology confirmed neuronal preservation. Combination therapy produced superior neuroprotection compared with individual treatments, suggesting synergistic antioxidant, anti-inflammatory, anti-amyloidogenic, and neurotrophic effects. These findings support Diosmetin–Polmacoxib co-administration as a potential strategy against Alzheimer's disease-related cognitive dysfunction. **Conclusion:** Diosmetin and Polmacoxib combination therapy offers good neuroprotection against scopolamine-induced Alzheimer's disease by preserving cognitive function, reducing oxidative stress, and maintaining neurochemical balances. This combination represents a promising multimodal therapeutic approach for the management of Alzheimer's disease.

Major Finding: Combination therapy enhanced neuroprotection through synergistic effects.

Keywords: Anti-amyloidogenic, Alzheimer's Disease, Diosmetin, Neuroinflammation, Oxidative Stress, Polmacoxib

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1. Introduction

One of the leading causes of dementia is Alzheimer's Disease (AD), which involves the continuous deterioration of learning, various executive functions, as well as memory. Pathological features of Alzheimer's disease include extracellular β-amyloid aggregation and intracellular tau protein hyperphosphorylation leading to neurofibrillary tangles, synaptic dysfunction, neuroinflammation, and oxidative stress. Together, these pathophysiological cascades culminate in neuronal death and cognitive impairment[1,2].

Despite decades of research, therapeutic strategies remain largely symptomatic. Acetylcholinesterase inhibitors and NMDA receptor antagonists offer modest clinical benefit but fail to halt disease progression. Thus,

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there is an urgent need to explore multi-target agents capable of modulating oxidative stress, inflammation, and amyloid pathology simultaneously[3]. Scopolamine is an antagonist of muscarinic acetylcholine receptors and has been widely employed as an experimental model of AD, as it induces memory impairment, cholinergic dysfunction, oxidative damage and Aβ accumulation[4,5].

Diosmetin (5,7,3′-trihydroxy-4′-methoxyflavone), a naturally occurring flavonoid, shows remarkable antioxidant, anti-inflammatory, anti-apoptotic, and neurotrophic activities. The literature suggests it enhances cognitive function in experimental dementia models by reducing oxidative stress and modulating signalling pathways[6,7].

Polmacoxib, originally developed as a selective COX-2 inhibitor for osteoarthritis, is being repurposed for Alzheimer's disease because it has dual actions, including anti-inflammatory and carbonic anhydrase inhibitory effects[8]. Carbonic anhydrase inhibitors improve cerebral blood flow, regulate mitochondrial metabolism, restore pH homeostasis, and indirectly attenuate neuroinflammation[9]. COX-2 inhibition helps to suppress pro-inflammatory pathways and neuroinflammation linked to microglial activation and neuronal damage. Its central nervous system mechanisms will offer potential neuroprotection by reducing amyloid-beta burdens and improving metabolic regulation in neurodegenerative conditions like Alzheimer's disease. Methazolamide and acetazolamide are the two primary, FDA-approved Carbonic Anhydrase Inhibitors (CAIs) being heavily researched and repurposed for neurodegenerative diseases like Alzheimer's disease[8,9].

Drug repurposing refers to the process of utilising established drugs for novel clinical application with known safety profiles This method facilitates faster clinical translation, cost, and risk compared to *de novo* drug discovery. The current study aimed to evaluate the neuroprotective potential of Diosmetin and Polmacoxib individually and in combination against scopolamine-induced Alzheimer-like pathology in rats, with a focus on β-amyloid deposition, proinflammatory markers (TNF-α and IL-1β) and antioxidant defense mechanisms[10].

Diosmetin provides antioxidant, anti-inflammatory, and neuroprotective effects, whereas Polmacoxib targets COX-2/carbonic anhydrase-associated inflammatory and oxidative pathways. It will decrease amyloid-beta burdens and restore metabolic regulation in neurodegenerative conditions. We hypothesise that combining a dual COX-2/carbonic anhydrase inhibitor with a neurotrophic flavonoid yields a synergistic therapeutic outcome in Alzheimer's disease pathology by simultaneously addressing neurovascular dysfunction, chronic inflammation, and synaptic decay. We predict that this combination will significantly reduce amyloid plaque burden and restore cognitive deficits to a greater degree than can be accounted for by the additive effects of the individual components alone.

Therefore, the objective of the present study was to evaluate the synergistic effect of Diosmetin and Polmacoxib in a scopolamine-induced cognitive deficit model of AD.

2. Materials and Methods

2.1 Animals

In the present study, Male Wistar rats weighing 230–250 g were used and maintained in a controlled environment (25 ± 2°C, 12 h light/dark, 50 ± 5% humidity) with food and water provided *ad libitum* and acclimatised before experiments. Each group consisted of six animals (n=6). All experimental studies were performed between 08:00 and 16:00 h in accordance with the guidelines of the Committee for the Control and Supervision of Experiments on Animals (CCSEA), New Delhi, India. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC/2023/01) of M.V.P.S. College of Pharmacy, Nashik.

2.2 Drugs and Reagents

Scopolamine hydrobromide was procured from Sigma–Aldrich Pvt. Ltd., India and was dissolved in saline (0.9% NaCl). Piracetam was obtained from Vital Laboratories Pvt. Ltd., Gujarat and was dissolved in Tween 80. Diosmetin purchased from Yucca Enterprises, Mumbai was prepared in DMSO, whereas Polmacoxib was purchased from Sigma–Aldrich Pvt. Ltd., India and was initially dissolved in a small amount of DMSO and subsequently diluted with 0.5% CMC as a vehicle for oral administration in rats.

The dose of Diosmetin 100 mg/kg, p.o. and 200 mg/kg, p.o.[11] and Polmacoxib 7 mg/kg, p.o., 15 mg/kg, p.o.[12,13] were administered 30 min before the treatment with scopolamine for 14 days.

2.3 Experimental Design

Animals were randomly allocated into nine groups (GR) (n=6), and treatment was administered daily for 14 days as follows:

GR I (Control) received normal saline p.o.

GR II (SCP) received scopolamine (1 mg/kg, i.p.)

GR III (DM-100) received diosmetin (100 mg/kg, p.o.) along with scopolamine (1 mg/kg, p.o.)

GR IV (DM-200) received diosmetin (200 mg/kg, p.o.) along with scopolamine (1 mg/kg, p.o.)

GRV (PM-7) received Polmacoxib (7 mg/kg, p.o.) along with scopolamine (1 mg/kg, p.o.)

GRVI (PM-15) received Polmacoxib (15 mg/kg, p.o.) along with scopolamine (1 mg/kg, p.o.)

GR VII (DM-100+PM-7) received Diosmetin (100 mg/kg, p.o.) + Polmacoxib (7 mg/kg, p.o.) along with scopolamine (1 mg/kg, p.o.)

GR VIII (DM-200+PM-15) received Diosmetin (200 mg/kg, p.o.) + Polmacoxib (15 mg/kg, p.o.) along with scopolamine (1 mg/kg, p.o.)

GR IX (PC-50) received Piracetam 50 mg/kg p.o along with scopolamine (1mg/kg, p.o)

3. Behavioural Tests

3.1 Y-maze Test

The Y-maze, used to assess spatial working memory, consisted of three arms (each 50 × 10 cm) at 120° angles. The experimental animals were positioned near one arm and permitted to move easily throughout the apparatus for a period of 5 min. Changes in activity were subsequently recorded, and the alternation percentage was estimated as an index of working memory[14,15].

3.2 Elevated Plus-Maze Test (EPM)

EPM was employed to determine memory and spatial learning. The apparatus consisted of two arms in open and closed positions, measuring 50 × 10 cm in length and 40 cm in height. All arms were connected to the central platform measuring 10 × 10 cm, and the entire setup was elevated 50 cm above the ground. An individual rat was kept near the end of an open arm facing a closed arm and permitted to freely move within the apparatus for 5 min. The duration spent and no. of entries into each arm were documented. An arm entry was recorded when the animal placed all four paws into an arm. Cognitively impaired animals spent less time exploring the open arms, while animals with improved memory explored them more freely[16-18].

3.3 Object Recognition Test (ORT)

Rats were acclimatised to the empty open-field arena for 5 min one day before experimental procedures. During the training session, two similar objects (A1 and A2) were placed in opposite corners of the arena, and the rats were allowed to explore them freely for a period of 5 min. After a retention interval of 4 h, one of the familiar objects was substituted with a new object (B). The animals were then reintroduced into the arena and permitted to explore both objects for another 5 min. Between each trial using 70% ethanol, the apparatus was cleaned properly to eliminate olfactory cues. The discrimination index was calculated to evaluate recognition memory by comparing the exploration time of the novel object with that of the familiar object[19-21].

3.4 Morris Water Maze Test (MWM)

The MWM consists of a circular pool (150 × 45 cm, water depth 30 cm) divided into four quadrants. For acquisition trials, rats were kept in different quadrants for each trial and allowed 120 s to locate a visible platform (1 cm above water) and remained on it for 20 s. Animals failing to find the platform were guided to it. On days 7th and 14th the mean escape latency to reach the hidden platform was recorded as the first and second retention latencies, respectively[22-24].

4. Biochemical Estimations

4.1 Dissection and Homogenisation

Following behavioural experimentation, animals were humanely killed using a CO_2 euthanasia chamber; medical-grade carbon dioxide gas was supplied at a 20-30% flow rate according to the AVMA Guidelines 2020 for Euthanasia of animals. This gradual fill rate minimises distress and avoids sudden hypercapnia shock. Flow of Gas was maintained until 1 min after cessation of respiratory movement to ensure death, followed by a secondary physical confirmation method (*e.g.* cervical

dislocation) before brain extraction; brains were excised, followed by rinsing with saline and weighing. Brain tissue homogenate (10% w/v) was prepared using 0.1 M phosphate buffer maintained at pH 7.4. The post-nuclear fraction for catalase estimation was separated by centrifuging the homogenate at 1,000×g for 20 min, whereas fractions for the remaining enzymes were collected after centrifugation at 12,000×g for 60 min at 4°C. Enzymatic assays were carried out using an Elico BL200 spectrophotometer[25].

4.2 Estimation of Catalase Level (CAT)

Catalase activity was determined using Luck's method, in which the decomposition of H_2O_2 was determined at 240 nm using a UV spectrophotometer. Enzyme activity was quantified as micromoles of H_2O_2 degraded per min per mg of protein[26].

4.3 Estimation of Superoxide Dismutase Activity (SOD)

Superoxide Dismutase (SOD) activity was estimated using Kono's method, in which SOD inhibits the reduction of Nitro Blue Tetrazolium (NBT), and optical density was recorded at 560 nm. The results were expressed as the percentage inhibition of NBT reduction[27].

4.4 Lipid Peroxidation Assay (LPO)

Quantitative assessment of LPO was performed using Wills' method. The malondialdehyde (MDA) concentration produced was measured by reacting it with Thiobarbituric Acid (TBA), and the absorbance was measured at 532 nm[28].

4.5 Estimation of Reduced Glutathione Level (GSH)

Reduced Glutathione (GSH) levels were estimated by Ellman's method. Briefly, 0.75 ml of tissue homogenate was mixed with 4% sulphosalicylic acid to precipitate protein, followed by centrifugation for 15 min at 1200×g at 4°C. Subsequently, an aliquot of 0.5 ml of the obtained supernatant was mixed with 4.5 ml of 0.01 M DTNB solution prepared in 0.1 M phosphate buffer (pH 8.0). The absorbance of the reaction mixture was recorded at 412 nm, and the GSH level was represented as µM GSH per mg of protein[29].

4.6 Determination of Nitric Oxide Level (NO)

NO was quantified calorimetrically from homogenates of brain tissues as per the Griess method. In this procedure, nitrate present in the sample was converted to nitrites, which subsequently reacted with the Griess reagent (1.5% sulphanilamide in 1 mol/L HCl containing 0.15% N-(1-naphthyl) ethylenediamine dihydrochloride) to give an intense purple azo dye. The absorbance of the formed chromophore was determined at 540 nm[30].

4.7 AChE Level Estimation

The cholinergic marker enzyme, acetylcholinesterase (AChE), was estimated in whole-brain homogenates using Ellman's method. Ellman's reagent, 5,5′-dithiobis-(2-nitrobenzoate) (DTNB), was employed for the assay. For analysis, the homogenate was first allowed to react with 2.7 mL phosphate buffer and 0.1 mL DTNB solution for 5 min. Then, 0.1mL of freshly prepared acetylthiocholine iodide (pH 8) was added, and the absorbance was read at 412 nm[31].

4.8 Estimation of β-amyloid

Brain Aβ1 levels were measured using a rat ELISA kit (YH Bioresearch, Shanghai, China). Brain samples were homogenised using 0.1 M PBS, pH 7.4, followed by centrifugation at 5,000 rpm. Supernatants were assayed following the kit instructions, with standards run in parallel. Absorbance was measured at 450 nm using a Multiskan GO spectrophotometer (Thermo Scientific). All samples were analysed in triplicate[32,33].

4.9 Estimation of TNF-α and IL-6

The concentrations of the pro-inflammatory cytokines TNF-α and IL-6 were estimated in brain tissue utilising commercially procured rat ELISA kits. In brief, 100 µL of standards and test samples were transferred into antibody-coated 96-well plates and allowed to incubate. After washing, biotin-conjugated detection antibody and streptavidin-HRP were added, followed by TMB substrate to develop the colour. The enzymatic reaction was stopped by the addition of stop solution, after which the absorbance was measured at 450 nm using a microplate reader. Cytokine content was determined from the standard calibration curve and expressed as pg/mg protein[33,34].

5. Histopathological Analysis

The hippocampal regions from both control and treated animals were excised and sectioned into small pieces and fixed in Bouin's fixative for 24 h. Subsequently, the tissues were processed, followed by paraffin embedding and sliced into 4–6 µm thick slices. The obtained tissue sections were subjected to hematoxylin and eosin (H&E) staining. and finally examined and visualised under a light microscope fitted with a camera at 10× magnification[34].

6. Statistical Analysis

All data were expressed as mean ± SEM. Statistical analysis was carried out using two-way ANOVA followed by Tukey's post hoc test with GraphPad Prism

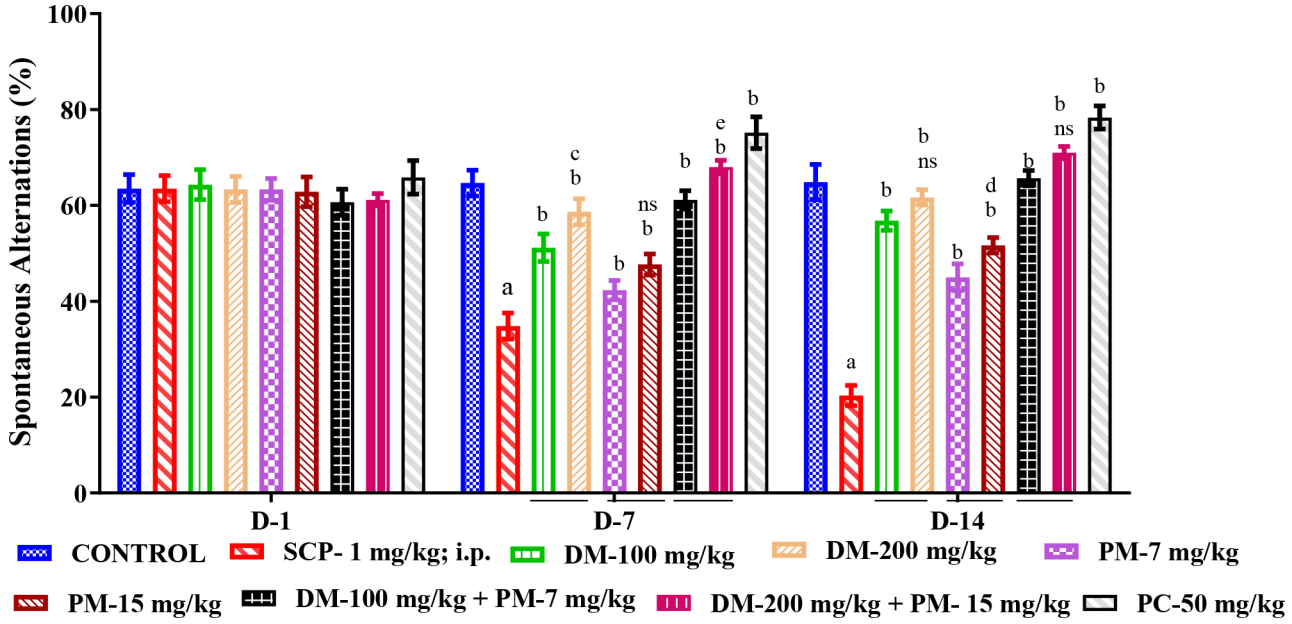


Figure 1. Results are expressed as mean± SEM (n=6) and Data were analysed by two-way ANOVA with Tukey's post hoc test *p<0.05, **p<0.01, ***p<0.001, [ns]non significant, [a]p<0.001 compared with untreated group, [b]p<0.001 compared with SCP group, [c]p<0.001 compared with DM (100mg/kg), [d]p<0.001 compared with PM (7mg/kg), [e]p<0.001 compared with DM 100 mg/kg + PM 7 mg/kg.

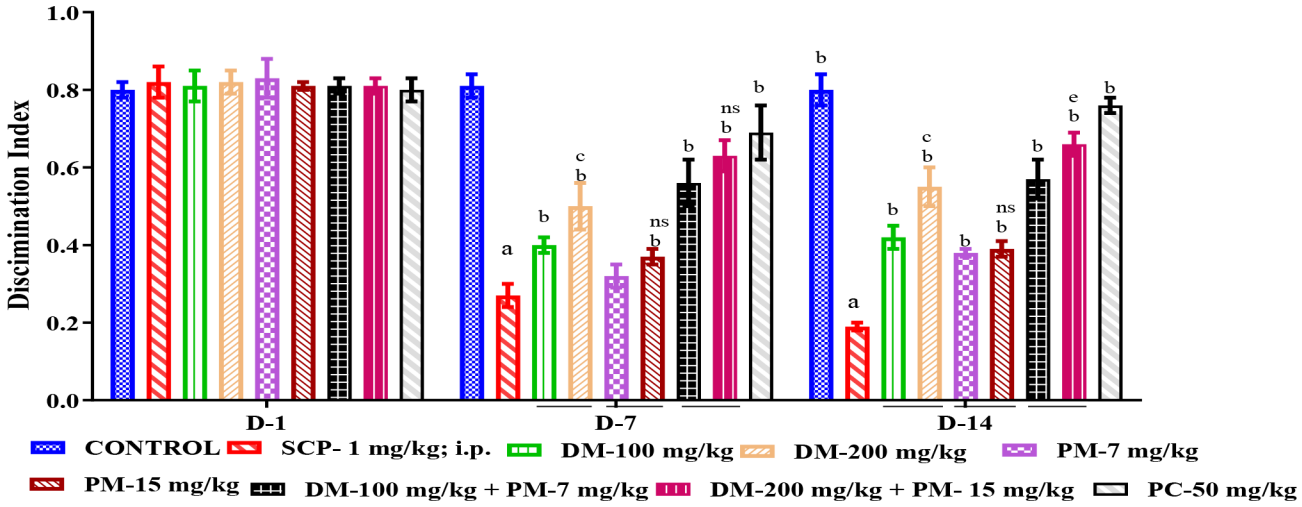


Figure 2. Results are expressed as mean± SEM (n=6) and Data were analysed by two-way ANOVA with Tukey's post hoc test *p<0.05, **p<0.01, ***p<0.001, [ns]non significant, [a]p<0.001 compared with untreated group, [b]p<0.001 compared with SCP group, [c]p<0.001 compared with DM (100mg/kg), [d]p<0.001 compared with PM (7mg/kg), [e]p<0.001 compared with DM 100 mg/kg + PM 7 mg/kg.

10 software. Differences were considered statistically significant at *p<0.05, **p<0.01, and ***p<0.001, while ns indicated non-significant differences

7. Results

7.1 Behavioural Parameters

7.1.1 Y-maze Test

In the Y-maze test, spontaneous alternation behaviour remained unaltered on Day 1 but was significantly reduced by Day 14 (p<0.001), confirming impairment in short-term working memory. Treatments were administered individually or in combination and markedly improved performance in a dose-dependent manner, with the high-dose combination (DM 200 mg/kg + PM 15 mg/kg) demonstrating the most robust improvement, equivalent to the standard drug piracetam (p<0.001) (Figure 1).

7.2 Novel Object Recognition Test

Similarly, in the Novel Object Recognition Test (NORT), scopolamine produced a significant decline

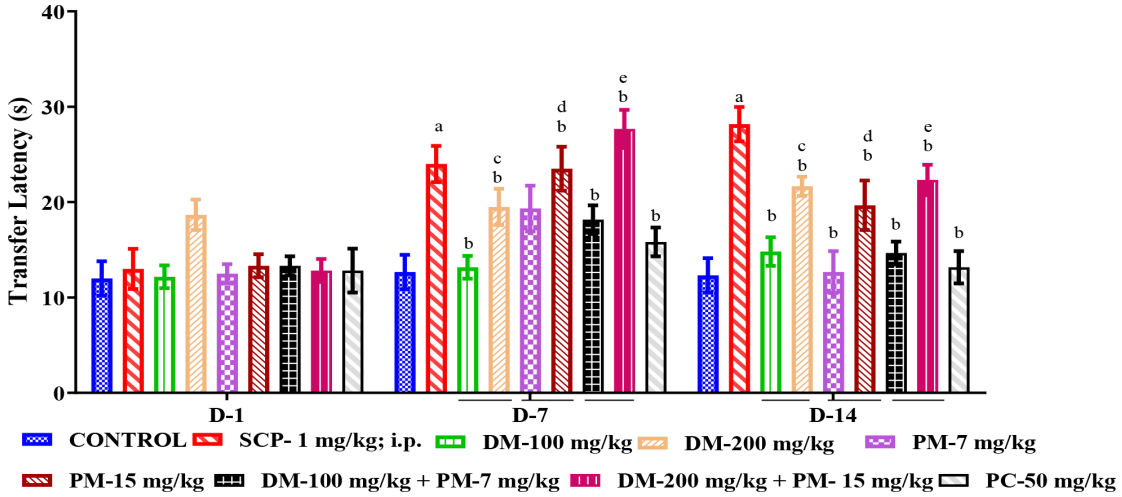


Figure 3. Results are expressed as mean± SEM (n=6) and analysed by Two-way Anova followed by Tukey's post hoc test *p<0.05, **p<0.01, ***p<0.001, [ns]non significant, [a]p<0.001 compared with untreated group, [b]p<0.001 compared with SCP group, [c]p<0.001 compared with DM (100mg/kg), [d]p<0.001 compared with PM (7mg/kg), [e]p<0.001 compared with DM 100 mg/kg + PM 7 mg/kg.

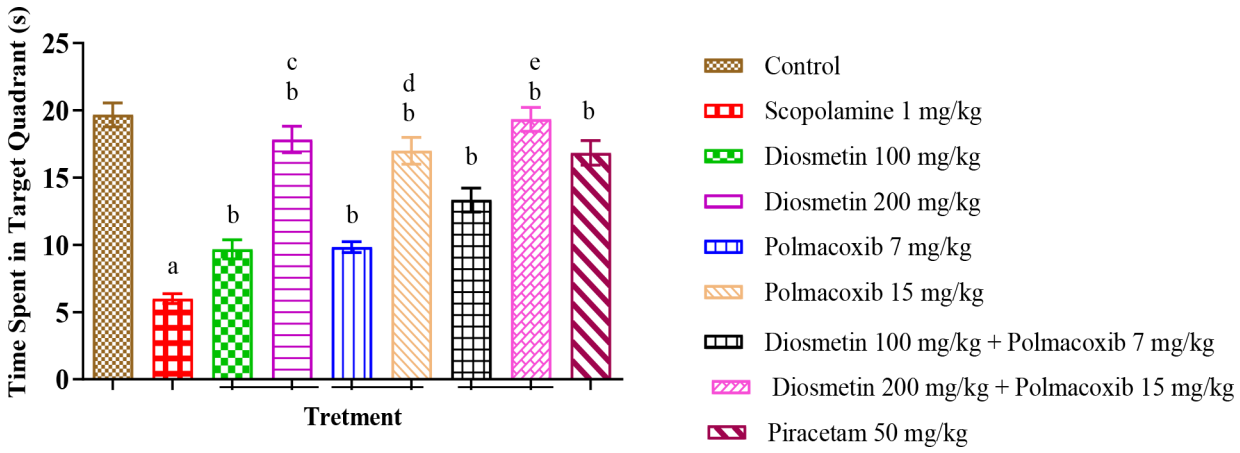


Figure 4. Results are expressed as mean± SEM (n=6) and analysed by One-way Anova followed by Tukey's post hoc test *p<0.05, **p<0.01, ***p<0.001, [ns]non significant, [a]p<0.001 compared with untreated group, [b]p<0.001 compared with SCP group, [c]p<0.001 compared with DM (100mg/kg), [d]p<0.001 compared with PM (7mg/kg), [e]p<0.001 compared with DM 100 mg/kg + PM 7 mg/kg.

in the discrimination index ($p<0.001$), indicating compromised recognition memory. Treatments significantly restored recognition performance, and their combination exhibited the greatest effect, which persisted through Day 14 (Figure 2).

7.3 Elevated Plus Maze Model

In the Elevated Plus Maze (EPM), scopolamine markedly increased transfer latency ($p<0.001$), suggesting deficits in learning and memory retention. Both Diosmetin and Polmacoxib treatments effectively reduced latency, with the high-dose combination showing maximal improvement, closely approximating piracetam-treated animals ($p<0.001$) (Figure 3).

7.4 Morris Water Maze Test

7.4.1 Time Spent in Target Quadrant

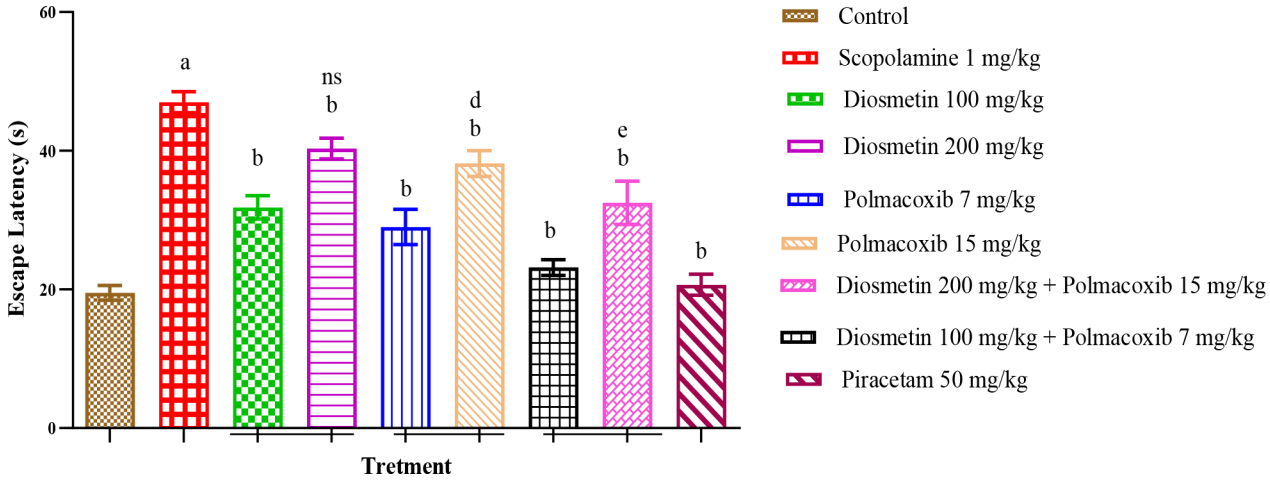


Figure 5. Results are expressed as mean± SEM (n=6) and analysed by One-way Anova followed by Tukey's post hoc test *p<0.05, **p<0.01, ***p<0.001, ^{ns}non significant, ^{a}p<0.001 compared with untreated group, ^{b}p<0.001 compared with SCP group, ^{c}p<0.001 compared with DM (100mg/kg), ^{d}p<0.001 compared with PM (7mg/kg), ^{e}p<0.001 compared with DM 100 mg/kg + PM 7 mg/kg.

Table 1. Effect of Diosmetin and Polmacoxib alone and in combination on biochemical parameters

Gr.	Treatments	Catalase activity (µMole of H_2O_2 decomposed / mg protein/min)	GSH Level (µMole/mg protein)	SOD Level (% inhibition of reduction of NBT)	LPO activity (nMole of MDA/ mg protein)	NO Level (µM/ml of Homogenate)
G1	Control	14.30±0.7	7.44±0.1	85.47±2.6	6.48±0.4	16.70±2.1
G2	Scopolamine (1mg/kg, i.p.)	2.61±0.4#	3.23±0.3#	52.95±4.1#	24.26±2.9#	52.22±2.0#
G3	Diosmetin- 100 mg/kg, p. o	3.61±0.6*$	4.43±0.6*$	62.99±6.7***$	20.72±1.5*$	42.66±3.8***$
G4	Diosmetin- 200 mg/kg, p. o	3.90±0.2**@	4.74±0.7***@	71.88±2.7***	18.26±1.5***@	37.10±3.5***@
G5	Polmacoxib- 7 mg/kg, p. o	3.73±0.4*$	4.43±0.7*$	66.46±4.0***	20.11±2.1*$	40.04±3.5***
G6	Polmacoxib- 15 mg/kg, p. o	3.94±0.4**@	5.30±0.3***	69.90±3.3***	16.07±1.4***@	36.09±2.1***@
G7	Diosmetin- 100 mg/kg, p.o + Polmacoxib- 7 mg/kg, p. o	4.83±1.0***	5.59±0.8***	71.50±2.5***	13.32±2.8***	34.70±2.5***
G8	Diosmetin- 200 mg/kg, p.o + Polmacoxib- 15 mg/kg. p. o	6.37±0.1***	5.96±0.3***	75.40±4.2***	11.35±1.8***	27.70±3.1***
G9	Piracetam- 50 mg/kg, p. o	8.13±0.2***	6.34±0.7***	82.14±6.2***	10.33±0.9***	23.61±2.1***

7.4.2 Escape Latency

In the Morris Water Maze (MWM), scopolamine significantly prolonged escape latency and reduced the time spent in the target quadrant ($p<0.001$), confirming spatial learning impairment. Administration of Diosmetin and Polmacoxib, particularly in combination, significantly shortened escape latency and increased time spent in the target quadrant, indicating additive enhancement of spatial learning and memory consolidation (Figures 4 and 5).

7.5 Biochemical Parameters

7.5.1 Effect on Oxidative Stress Indices

Scopolamine administration significantly induced oxidative stress, as indicated by decreased catalase, GSH, and SOD levels along with increased LPO and NO compared to the control group. Treatment with Diosmetin and Polmacoxib significantly improved antioxidant enzyme levels and reduced oxidative markers in a dose-dependent manner. The combination treatment produced greater protective effects than individual treatments, suggesting a possible combined antioxidant and anti-inflammatory action. The results were comparable to the standard drug piracetam, indicating that Diosmetin and Polmacoxib may offer promising neuroprotective potential against scopolamine-induced oxidative damage (Table 1).

Scopolamine significantly increased AChE activity and β-amyloid levels, indicating cholinergic dysfunction and amyloid pathology. Diosmetin and Polmacoxib treatment reduced both parameters in a

Table 2. Effect of diosmetin and polmacoxib alone and in combination on biochemical parameters- AChE and β-amyloid level

Groups	Treatment	AChE level (Moles of substrate hydrolysed/min/g tissue × 10^ -6)	β- amyloid level (ng/ml homogenate)
G1	Control	0.15±0.01	6.19±0.60
G2	Scopolamine- 1mg/kg; i.p.	0.61±0.05#	15.84±1.88#
G3	Diosmetin- 100 mg/kg, p. o	0.53±0.03*$	12.46±2.20*$
G4	Diosmetin- 200 mg/kg, p. o	0.48±0.04***@	11.33±1.28***@
G5	Polmacoxib- 7 mg/kg, p. o	0.53±0.06*$	11.74±2.25**$
G6	Polmacoxib- 15 mg/kg, p. o	0.50±0.04***@	10.02±1.74***
G7	Diosmetin-100 mg/kg, p.o + Polmacoxib- 7 mg/kg, p. o	0.32±0.02***	7.78±1.41***
G8	Diosmetin- 200 mg/kg, p.o + Polmacoxib- 15 mg/kg, p. o	0.27±0.04***	7.18±1.39***
G9	Piracetam- 50 mg/kg, p. o	0.21±0.05***	6.83±0.72***

Table 3. Effect of diosmetin and polmacoxib alone and in combination on pro-inflammatory cytokine Levels - TNF-α and IL-1 β

Groups	Treatment	TNF-α level (pg/mL)	IL-1 β level (pg/mL)
G1	Control	406.02±39.2	147.53±36.6
G2	Scopolamine- 1mg/kg; i.p.	756.60±75.9#	804.03±92.6#
G3	Diosmetin- 100 mg/kg, p. o	629.82±77.4*	652.07±104.9*
G4	Diosmetin- 200 mg/kg, p. o	598.48±76.3**	606.68±76.6**@
G5	Polmacoxib- 7 mg/kg,p.o	630.00±70.5*	650.13±74.0*
G6	Polmacoxib- 15 mg/kg, p. o	571.33±73.4***	582.93±64.8***
G7	Diosmetin-100 mg/kg, p.o + Polmacoxib- 7 mg/kg, p. o	529.25±47.0***	567.30±95.8***
G8	Diosmetin- 200 mg/kg, p.o + Polmacoxib- 15 mg/kg, p. o	512.57±58.4***	444.82±78.0***
G9	Piracetam- 50 mg/kg, p. o	494.82±57.0***	362.98±49.9***

dose-dependent manner, with combination therapy showing greater improvement comparable to piracetam. These results suggest additive neuroprotective effects through modulation of cholinergic function and amyloid accumulation (Table 2).

Scopolamine significantly elevated pro-inflammatory cytokines (TNF-α and IL-1β), indicating neuroinflammation. Treatment with Diosmetin and Polmacoxib reduced cytokine levels in a dose-dependent manner, with combination therapy showing greater suppression comparable to piracetam. These findings suggest anti-inflammatory and neuroprotective effects of the treatments against scopolamine-induced neuroinflammation (Table 3).

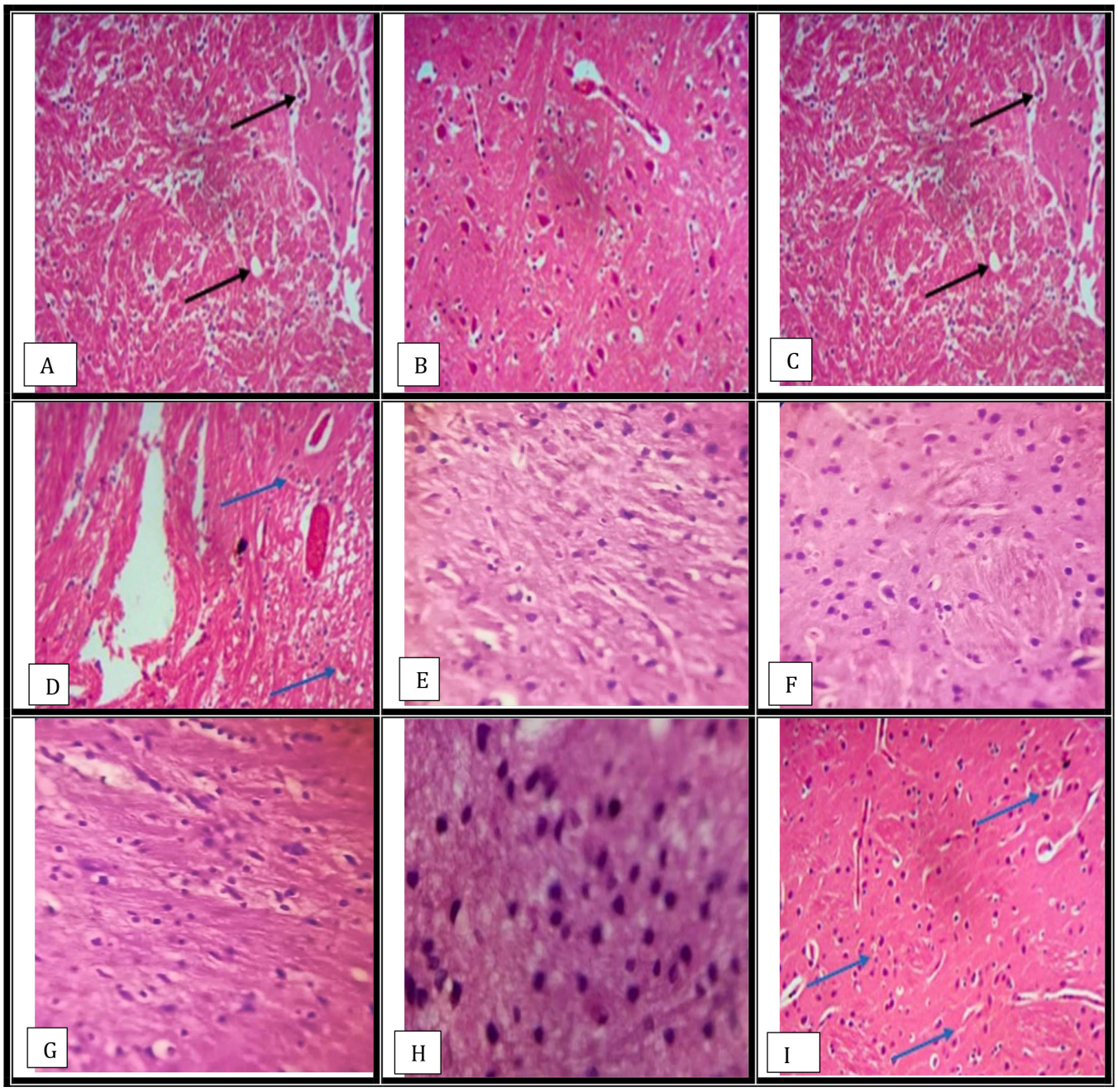


Figure 6. **(A).** Control: normal neuronal architecture, **(B).** Scopolamine: severe neurodegeneration and vacuolation, **(C, D).** Scopolamine + Diosmetin (100 and 200 mg/kg): dose-dependent neuroprotection, **(E, F)**. Scopolamine + Polmacoxib (7 mg/kg and 15 mg/kg): partial restoration of morphology, **(G, H).** Diosmetin + Polmacoxib combinations: synergistic protection, near-normal histoarchitecture, **(I).** Piracetam: marked neuroprotection.

7.6 Histopathological Evaluation

Histopathological analysis showed that scopolamine caused severe neuronal degeneration and vacuolation compared to the normal architecture observed in the control group. Treatment with Diosmetin and Polmacoxib improved neuronal morphology in a dose-dependent manner, indicating neuroprotective effects. The combination treatment produced greater protection with near-normal histoarchitecture, suggesting additive action. The effects were comparable to the standard drug piracetam, confirming significant protection against scopolamine-induced neuronal damage (Figure 6).

8. Discussion

The present study illustrates that Diosmetin and Polmacoxib confer significant neuroprotective effects against scopolamine-induced cognitive deficits and neurodegeneration in rats. Scopolamine administration effectively recapitulated several hallmark features of Alzheimer's Disease (AD), including cholinergic dysfunction and heightened oxidative stress. These pathological alterations collectively contribute to synaptic dysfunction, neuronal loss, and memory impairment, thereby validating the utility of this model to assess potential therapeutic interventions[35].

Diosmetin, a naturally occurring flavonoid, exhibited pronounced neuroprotective effects primarily through its antioxidant and neurotrophic properties. Its polyphenolic structure enables efficient free radical scavenging, stabilisation of neuronal membranes, and upregulation of endogenous antioxidant defence, including catalase, superoxide dismutase, and glutathione[36-38]. Moreover, diosmetin significantly decreased the pro-inflammatory cytokine levels TNF-α and IL-1β in brain tissue, indicating its potential to mitigate neuroinflammation and thereby ameliorate scopolamine-induced cognitive impairment.

Polmacoxib, a selective COX-2 inhibitor with additional carbonic anhydrase modulatory activity, mitigated neuroinflammation and amyloidogenesis. By suppressing COX-2-mediated pathways, Polmacoxib reduced TNF-α levels, thereby attenuating inflammatory cascades that promote neuronal damage. Importantly, Polmacoxib also decreased β-amyloid accumulation, suggesting interference with amyloidogenic processing and reduction of toxic Aβ oligomers, which are central to AD pathology[39,40].

Remarkably, the combination of Diosmetin and Polmacoxib produced additive neuroprotective effects, surpassing the efficacy of either agent alone. This complementary interaction likely arises from Diosmetin's capacity to reduce oxidative damage and decrease pro-inflammatory cytokines like TNF-α and IL-1 beta, coupled with Polmacoxib's ability to suppress inflammatory pathways[41,42]. Collectively, these mechanisms may contribute to preserving neuronal integrity and enhaning cognitive function

Behavioural assessments, including Y-maze, NORT test, elevated plus maze paradigms and Morris water maze tests, consistently confirmed that combination therapy yielded superior improvements in memory and spatial learning. Biochemical analyses further exhibited restoration of antioxidant enzyme activities, reduction in lipid peroxidation, normalisation of acetylcholinesterase activity, and decreased β-amyloid levels. Histopathological analysis supported these findings, showing near-normal hippocampal and cortical architecture with minimal gliosis in combination-treated animals, surpassing the effects observed with monotherapy and even standard Piracetam treatment.

These findings underscore the therapeutic potential of multi-target pharmacological strategies in AD. The combination demonstrated synergistic mechanisms supporting each other. The findings of this study validate the hypothesised synergistic interaction between Diosmetin, neurotrophic flavonoids and Polmacoxib, a selective COX-2/Carbonic Anhydrase (CA) inhibitor, in mitigating AD pathology. The dual-pronged strategy addressed the neurovascular, inflammatory, and neurotrophic axes simultaneously. The combination demonstrated a potential approach for simultaneously targeting oxidative stress, neuroinflammation, amyloid pathology, and neurotrophic deficits. Such integrative interventions may offer superior neuroprotection compared to single-target therapies, emphasising the importance of multi-faceted treatment strategies in managing complex neurodegenerative disorders like Alzheimer's disease.

9. Conclusion

Diosmetin and Polmacoxib revealed significant neuroprotective potential in a scopolamine-induced

Alzheimer's model in rats. Their effects were facilitated *via* attenuation of oxidative stress, suppression of neuroinflammation, and reduction of β-amyloid deposition. Combination therapy offered significantly greater synergistic benefit than monotherapy, indicating that these agents could be developed as complementary strategies for AD management

10. References

1. Long JM, Holtzman DM. Alzheimer disease: An update on pathobiology and treatment strategies. Cell. 2019; 179(2):312-339. https://doi.org/10.1016/j.cell.2019.09.001 PMid:31564456 PMCid:PMC6778042
2. DeTure MA, Dickson DW. The neuropathological diagnosis of Alzheimer's disease. Mol Neurodegener. 2019; 14:32. https://doi.org/10.1186/s13024-019-0333-5 PMid:31375134 PMCid:PMC6679484
3. Mehta NV, Kapadia A, Khambete M, Abhyankar A. The evolution of AChE inhibitors in Alzheimer's disease: From single-target to multi-target ligands. ChemBioChem. 2025; 26(17): e202500053. https://doi.org/10.1002/cbic.202500053 PMid:40720180
4. Sugandhi VV, Gadhave DG, Ugale AR, Kulkarni N, Nangare SN, Patil HP, *et al*. Advances in Alzheimer's therapy: Exploring neuropathological mechanisms to revolutionize the future therapeutic landscape. Ageing Res Rev. 2025; 109:102775. https://doi.org/10.1016/j.arr.2025.102775 PMid:40403980
5. Yadang FSA, Nguezeye Y, Kom CW, Betote PHD, Mamat A, Tchokouaha LRY, *et al*. Scopolamine-induced memory impairment in mice: neuroprotective effects of *Carissa edulis* aqueous extract. Int J Alzheimers Dis. 2020; 2020:6372059. https://doi.org/10.1155/2020/6372059 PMid:32934845 PMCid:PMC7479457
6. Yang Y, Gong XB, Huang LG, Wang ZX, Wan RZ, Zhang P, *et al*. Diosmetin exerts anti-oxidative, anti-inflammatory and anti-apoptotic effects in mice. Oncotarget. 2017; 8(19):30723-30733. https://doi.org/10.18632/oncotarget.15413 PMid:28430612 PMCid:PMC5458162
7. Singh B, Semwal BC, Singh S. Recent advances in diosmetin: Pharmacological, pharmacokinetic and toxicological profile. Curr Top Med Chem. 2025; 25(2):1-20. https://doi.org/10.2174/0115680266355377250213054844 PMid:39966369
8. Chandra R. COX-2 selectivity and beyond: exploring polmacoxib role in knee osteoarthritis treatment. Int J Pharm Sci. 2025; 3(4):2157-2165.
9. Canepa E, Parodi-Rullan R, Vazquez-Torres R, Gamallo-Lana B, Guzman-Hernandez R, Lemon NL, *et al*. FDA-approved carbonic anhydrase inhibitors reduce amyloid β pathology and improve cognition, by ameliorating cerebrovascular health and glial fitness. Alzheimers Dement. 2023; 19(11):5048-5073. https://doi.org/10.1002/alz.13063 PMid:37186121
10. Cummings JL, Zhou Y, Van Stone A, Cammann D, Tonegawa-Kuji R, Fonseca J, *et al*. Drug repurposing for Alzheimer's disease and other neurodegenerative disorders. Nat Commun. 2025; 16(1):1755. https://doi.org/10.1038/s41467-025-56690-4 PMid:39971900 PMCid:PMC11840136
11. Zhang Y, Jiang Y, Lu D. Diosmetin in a rat model of pneumococcal meningitis. Med Sci Monit. 2019; 25:2238-2245. https://doi.org/10.12659/MSM.911860 PMid:30914630 PMCid:PMC6448457
12. Kim SH, Kim HS, Lee JH, *et al*. CG100649 inhibits colorectal adenoma and carcinoma growth in mouse models. Investig New Drugs. 2014; 32(6):1105-1112. https://pmc.ncbi.nlm.nih.gov/articles/PMC4356209/
13. Adake P, *et al*. Polmacoxib: A promising NSAID for pain and inflammation. World J Pharm Res. 2024; 13(1):889-902. https://wjpr.s3.ap-south-1.amazonaws.com/article_issue/f1f2f6dc6faca7f72315fa0887394948.pdf
14. Alawdi SH, El-Denshary ES, Safar MM, Eidi H, David MO, Abdel-Wahhab MA. Neuroprotective effect of nanodiamond in Alzheimer's disease rat model. Mol Neurobiol. 2017; 54(3):1906-1918. https://doi.org/10.1007/s12035-016-9762-0 PMid:26897372
15. Pellow S, Chopin P, File SE, Briley M. Validation of open: Closed arm entries in elevated plus-maze. J Neurosci Methods. 1985; 14(1):149-167. https://doi.org/10.1016/0165-0270(85)90031-7 PMid:2864480 PMCid:PMC9016269
16. Mishra V, Chauhan B, Chaudhri SK, Rani D. Neuroprotective potential of plant extracts in scopolamine-induced amnesia. J Appl Pharm Res. 2025; 13(4):81-89. https://doi.org/10.69857/joapr.v13i4.1139
17. Antunes M, Biala G. Novel object recognition memory: Neurobiology and procedure. Cogn Process. 2012; 13(2):93-110. https://doi.org/10.1007/s10339-011-0430-z PMid:22160349
18. Cole E, Simundic A, Mossa FP, Mumby DG. Assessing object-recognition memory in rats. Learn Behav. 2019; 47:141-155. https://doi.org/10.3758/s13420-018-0347-9 PMid:30132280
19. Thongrong S, Surapinit S, Promsrisuk T, Jittiwat J, Kongsui R. Pinostrobin alleviates cognitive impairment. Biomed Rep. 2023; 18(20):1-45. https://doi.org/10.3892/br.2023.1602 PMid:36798091 PMCid:PMC9922797
20. Batool Z, Sadir S, Liaquat L, Tabassum S, Madiha S, Rafiq S, *et al*. Almonds enhance memory and brain acetylcholine levels. Brain Res Bull. 2016; 120:63-74. https://doi.org/10.1016/j.brainresbull.2015.11.001 PMid:26548495
21. Kamani B, Ganatra T. Antiparkinsonian potential of *Euphorbia tirucalli*. Egypt J Neurosurg. 2025; 55(40):1-34. https://doi.org/10.1186/s41984-025-00387-9

22. Vogel HG, Schölkens BA, Sandow J, Müller G, Vogel WF, eds. Drug discovery and evaluation: pharmacological assays. Springer; 2002. p. 595-643. https://doi.org/10.1007/3-540-29837-1_7
23. Goverdhan P, Sravanthi A, Mamatha T. Neuroprotective effects of meloxicam and selegiline. Int J Alzheimers Dis. 2012; 2012:974013. https://doi.org/10.1155/2012/974013 PMid:22536538 PMCid:PMC3320018
24. Rather MA, Thenmozhi AJ, Manivasagam T, Bharathi MD, Essa MM, Guillemin GJ. Neuroprotective role of asiatic acid. Front Biosci (Schol Ed). 2018; 10:262-275. https://doi.org/10.2741/s514 PMid:28930532
25. Parle M, Singh N. Animal models for testing memory. Asia Pac J Pharmacol. 2004; 16(2):101-120.
26. Viswanatha GL, Shylaja H, Sandeep Rao KS, Santhosh Kumar VR, Jagadeesh M. Hesperidin ameliorates stress-induced alterations. ISRN Pharmacol. 2012; 2012:479570. https://doi.org/10.5402/2012/479570 PMid:22550596 PMCid:PMC3324935
27. Nandi A, Yan LJ, Jana CK, Das N. Role of catalase in oxidative stress. Oxid Med Cell Longev. 2019; 2019:9613090. https://doi.org/10.1155/2019/9613090 PMid:31827713
28. Kumar A, Dogra S, Prakash A. Neuroprotective effects of *Centella asiatica*. Int J Alzheimers Dis. 2009; 2009:972178. https://doi.org/10.4061/2009/972178 PMid:20798885 PMCid:PMC2925281
29. Kaya S, Sütçü R, Cetin ES, *et al*. Lipid peroxidation and antioxidant enzymes. Int J Infect Dis. 2007; 11(3):251-255. https://doi.org/10.1016/j.ijid.2006.05.003 PMid:16859944
30. Ezeji EU, Anyalogbu EA, Ezejiofor TN, Udensi JU. Determination of glutathione and GST. Am J Biochem. 2012; 2(3):21-24. https://doi.org/10.5923/j.ajb.20120203.01
31. Zhao Y, Dang M, Zhang W, Lei Y, Ramesh T, Priya Veeraraghavan V, *et al*. Neuroprotective effects of syringic acid. J Funct Foods. 2020; 10(4):104009. https://doi.org/10.1016/j.jff.2020.104009
32. Vrushabh B. H, Vijaykumar P. R, Vishal S. P, Priyanka P. P, Shubham G. M, Ajay P. M, *et al*. *Amaranthus tricolor* improves cognition. J Appl Pharm Sci. 2020; 10(10):111-120. https://doi.org/10.7324/JAPS.2020.1010013
33. Pattanashetti LA, Taranalli AD, Parvatrao V, Malabade RH, Kumar D. Neuroprotective effect of quercetin. Indian J Pharmacol. 2017; 49(1):60-64. https://doi.org/10.4103/0253-7613.201016 PMid:28458424 PMCid:PMC5351240
34. Tosun D, Veitch D, Aisen P, Jack CR, Jagust WJ, Petersen RC, *et al*. Detection of β-amyloid positivity. Brain Commun. 2021; 3(2): fcab008. https://doi.org/10.1093/braincomms/fcab008 PMid:33842885 PMCid:PMC8023542
35. Chen X, Zhang M, Ahmed M, Surapaneni KM, Veeraraghavan VP, Arulselvan P. Neuroprotective effects of ononin. Saudi J Biol Sci. 2021; 28(8):4232-4239. https://doi.org/10.1016/j.sjbs.2021.06.031 PMid:34354404 PMCid:PMC8325004
36. Saghaei E, Shabani S, Mirshekar MA. Diosmetin mitigates cognitive impairment. Biol Psychol. 2020; 155:107924. https://doi.org/10.1016/j.biopsycho.2020.107924 PMid:32651023
37. Kazmi I, Al-Abbasi FA, Afzal M, Nadeem M, Altayb HN. Sterubin protects against Alzheimer's disease. Saudi J Biol Sci. 2023;30(2):103560. https://doi.org/10.1016/j.sjbs.2023.103560 PMid:36712184 PMCid:PMC9876951
38. Shabani S, Mirshekar MA. Diosmin is neuroprotective. Biomed Pharmacother. 2018; 108:1376-1383. https://doi.org/10.1016/j.biopha.2018.09.106 PMid:30453447
39. Prabhoo R, Kumar S, Singh R. Role of polmacoxib in osteoarthritis. Orthop J. 2024; 10(3):234-240. https://doi.org/10.22271/ortho.2024.v10.i3b.3582
40. Easwaran R, Ghosh S, Bhattacharya S. Polmacoxib: A review. J Clin Pharmacol. 2024; 64(2):123-134. https://doi.org/10.1002/jcph.1871
41. Chandrashekara S, Rajasekharan S, Raghavendra S. Clinical safety of polmacoxib. J Clin Rheumatol. 2024; 30(1):45-53. https://doi.org/10.1007/s44337-024-00154-z
42. Bhattacharjee B, Roy A, Chatterjee S, *et al*. Combined neuroprotective effects of resveratrol and hesperidin. Neurochem Int. 2025; 160:105314. https://pubmed.ncbi.nlm.nih.gov/40436365/

Author Contribution

Yogita Bagul conducted the experimental work, gathered and analysed the data, interpreted the findings, and drafted the preliminary manuscript. Dr. Vandana Nade supervised the research, offered continuous guidance, and assisted in revising the final version of the manuscript. Dr. L.A. Kawale contributed to data analysis and manuscript preparation. All authors have read and agreed to the published version of the manuscript.