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Research Article

Anti-Amnesic and Neuroprotective Potential of a Polyherbal Nutritional Supplement Against Lipopolysaccharide-Induced Neuroinflammation in Rats: Behavioral, Histopathological, and Molecular Insights

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Abstract

Background: The nutritional supplement (NS), composed of *Prunus amygdalus*, *Juglans regia*, and *Cucumis melo*, contains bioactive phytoconstituents such as tocopherol, linoleic acid, and phenolic compounds that contribute to its anti-inflammatory, antioxidant, and neuroprotective potential. The current investigation was conducted to evaluate the neuroprotective potential of the nutritional supplement in a lipopolysaccharide (LPS)-induced neuroinflammatory model and resulting memory deficit in Wistar rats by analyzing oxidative stress, histological features, and molecular outcomes. **Methods:** Adult Wistar rats were divided into five groups (n = 8/group): control (distilled water), LPS (0.25 mg/kg, i.p.), NS (500 mg/kg), NS (500 mg/kg, p.o.) + memantine (5 mg/kg, p.o.), and memantine (5 mg/kg, p.o.). Phytoconstituents of the NS were analyzed by gas chromatography–mass spectrometry (GC–MS). Total phenolic content and antioxidant activity were assessed using the Folin–Ciocalteu method and the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging assay, respectively. Cognitive function was assessed using the passive avoidance and Morris Water Maze tests. Rats were sacrificed on day 35, after 30 days of dosing. Brain tissue homogenates were evaluated for malondialdehyde (MDA), catalase (CAT), and superoxide dismutase (SOD); histopathological alterations were examined by hematoxylin–eosin staining; amyloid-like deposition was assessed by Congo red staining; cytokine and amyloid precursor protein (*APP*) gene expression were assessed by real-time polymerase chain reaction (PCR); enzyme-linked immunosorbent assay (ELISA) was used to measure protein concentrations of interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α). **Result:** The phytoconstituents identified were linoleic acid (65.04%) and tocopherol (9.29%). Total phenolic compounds were present in significant amounts, and the DPPH assay showed 67.9% radical-scavenging activity. The LPS group showed reduced cognitive function and spatial memory. Moreover, LPS increased MDA levels, decreased antioxidant levels, altered neuronal cell architecture on histology, increased amyloid-like deposition, and elevated cytokine gene and protein expression compared with the control. NS mitigates LPS-mediated neuroinflammation by improving cognitive function and memory. Furthermore, NS reduces MDA levels and elevates antioxidant levels, thereby restoring neuronal architecture compared with the LPS group. Real-time PCR analysis showed a significant decrease in the targeted gene, while ELISA showed a significant reduction in TNF-α and IL-6 protein levels in brain tissue of the nutritional supplement-treated group compared with the LPS group. **Conclusions:** These findings suggest that NS treatment protects brain tissue from LPS-induced neuroinflammation through antioxidant and anti-inflammatory mechanisms, supporting its potential as a neuroprotective agent to restore cognitive function.

Keywords: dietary supplement; neuroprotective agent; neuroinflammation; catalase; superoxide dismutase; lipopolysaccharides; cytokines; cognitive dysfunction

1. Introduction

Cognitive impairment is manifested by progressive neuronal and synaptic degeneration, accompanied by oxidative stress, inflammation, and synaptic dysfunction [1]. It has emerged as a notable global health problem among the elderly population, representing the leading cause of disability and mortality [2]. The prevalence of cognitive dysfunction or dementia is increasing rapidly, and the limited availability of safe and effective treatments to manage or improve its progression remains a challenge. Consequently, adopting a well-balanced and healthy diet has become a fundamental approach for preventing and managing numerous chronic conditions [3,4].

Primarily, persistent microglial activation promotes the upregulation and release of key proinflammatory cytokines, including interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α), which exacerbate oxidative stress and disrupt synaptic integrity [5,6]. Neuroinflammation and oxidative stress are considered central contributors to cogni-

tive impairment. Activated microglia initiate inflammatory cascades via Toll-like receptor 4-dependent signalling pathways, leading to the activation of nuclear factor kappa-B (NF-κB) and upregulation of cytokines [7,8]. The secretion of cytokines (IL-6 and TNF-α) exacerbates the production of reactive oxygen species (ROS), undermining antioxidant defences [9]. The aggregation of peptides to form plaques and amyloid protein accumulation activate glial cells and trigger the release of proinflammatory cytokines, promoting neuroinflammation and neuronal damage [10,11].

Lipopolysaccharide (LPS), a bacterial endotoxin, is widely used as a neuroinflammatory model for preclinical studies as it induces both central and systemic inflammatory responses. Circulating endotoxins originating in the gut can enter the bloodstream and trigger brain inflammation by crossing the blood-brain barrier, leading to neuronal toxicity [12]. Upon binding to Toll-like receptor 4 (TLR4), LPS activates the NF-κB pathway, leading to upregulation of proinflammatory cytokines. LPS provides a well-established platform for investigating inflammation-driven molecular alterations, including oxidative stress [1].

In contemporary research, nutritional and dietary supplements have received growing recognition as a neuroprotective approach. There is increasing research interest in nutritional strategies as adjunctive approaches to modulate neurodegenerative processes [13]. *Juglans regia*, *Prunus amygdalus*, and *Cucumis melo* have long been consumed as nutrient-rich foods in traditional dietary practices, particularly in South Asia and the Mediterranean region. These are rich sources of phytoconstituents, including linoleic acid (omega-6), α-linoleic acid (omega-3), vitamin E (α- *and* γ-tocopherol), phenolic compounds, and flavonoids [14,15]. Traditionally, these foods have been regarded as beneficial for maintaining general health, vitality, and cognitive function. They possess anti-inflammatory and antioxidant properties and have been associated with improved cognitive performance in preclinical studies. Omega-6 (linoleic acid) has been shown to suppress inflammatory mediators and microglial activation [1]. Simultaneously, tocopherols support redox homeostasis by enhancing levels of endogenous antioxidants, such as catalase and superoxide dismutase [16]. Additionally, certain nut-derived natural active compounds have been shown to interfere with cholinergic neurotransmission and amyloid-β activation [17]. Previous studies have reported that phytochemicals such as polyphenols, flavonoids, polyunsaturated fatty acids, and proteins possess antioxidant, anti-inflammatory, cardioprotective, and neuroprotective properties. Although the content of nutrients may vary among different types of dry nuts, their overall nutrient composition remains comparable [18]. Preclinical and experimental research has reported that natural compounds can attenuate neuroinflammation and act as antioxidants, thereby improving cognitive function and preventing neuronal damage [19]. Their traditional use, together with growing experimental evidence of their neuroprotective potential, provides a scientific rationale for combining these ingredients as a nutritional supplement for the management of neuroinflammation and cognitive impairment [20,21]. Previous findings support the growing interest in nutrition-based interventions as a safer alternative to conventional pharmacological therapies. They may also enhance therapeutic outcomes through complementary mechanisms to reduce the risk of age-related cognitive disorders [13]. Despite this evidence, important gaps remain in understanding the integrated effects of combined nut-derived bioactive compounds. Most studies focus on isolated phytochemicals rather than the synergistic effect of a combined nut-based supplement [22]. Moreover, few investigations have assessed inflammatory cytokines, oxidative stress markers, and cognitive dysfunction [23]. In particular, whether such nutritional supplementation and bioactive substances can concurrently modulate the inflammatory pathway in LPS-induced neuroinflammation warrants further investigation.

Therefore, the current study aimed to investigate the neuroprotective effects of a combination of *Prunus amygdalus*, *Juglans regia*, and *Cucumis melo*, when administered as the nutritional supplement, on LPS-induced cognitive impairment and neuroinflammation in a Wistar rat model. Furthermore, the study aimed to elucidate the molecular mechanisms underlying the neuroprotective effects of the nutritional supplement against inflammation and oxidative damage.

2. Materials and Methods

2.1 Chemicals and Reagents

Analytical-grade reagents and chemicals were used in the study. The endotoxin LPS (500×) was acquired from Invitrogen (Thermo Fisher Scientific, Carlsbad, CA, USA), and the source was *Escherichia coli* (026: B6, catalogue no. 00-4976). Memantine was obtained from High-Q International Pharmaceuticals (Karachi, Pakistan; Lot no. 1RM007). Malondialdehyde (MDA; 100683), thiobarbituric acid (TBA; 31670), and trichloroacetic acid (TCA; 120010500) were procured from Sigma-Aldrich (St. Louis, MO, USA). The micro-method antioxidant assay kit catalase (CAT; D799598-0100) and superoxide dismutase (SOD; D799594-0100) were purchased from Sangon Biotech (Shanghai, China), and sandwich enzyme-linked immunosorbent assay (ELISA) kits for IL-6 (SEA 079 RA) and TNF-α (SEA 133 RA) were purchased from USCN (Cloud-Clone Corp., Katy, TX, USA).

2.2 Collection of Samples and Nutritional Supplement Preparation

Prunus amygdalus (*PA*), *Juglans regia* (*JR*), and *Cucumis melo* (*CM*) were acquired from the local market and identified by the Department of Pharmacognosy at the Faculty of Pharmacy and Pharmaceutical Sciences, Ziauddin University. The herbarium numbers were assigned to

Prunus amygdalus (*PAS-037-25*), *Juglans regia* (*JRS-035-25*), and *Cucumis melo* (*CMS-034-25*). After washing and drying, the nuts were ground into a fine powder using a grinder mixer and stored at 4 °C until use. The nutritional supplement was formulated by combining 10 g each of *Prunus amygdalus*, *Juglans regia*, and *Cucumis melo* seed powders in a 1:1:1 (w/w) ratio, then thoroughly mixing to obtain a homogeneous formulation. The mixture was then stored in a sealed, moisture-free container to prevent degradation and maintain stability.

Dose Selection and Preparation

A pilot study was conducted before the model was established. The two doses of the nutritional supplement, 250 mg/kg and 500 mg/kg, were selected to evaluate a dose-dependent neuroprotective effect. The comparative analysis of behavioral and biochemical outcomes indicated that the 500 mg/kg dose produced the most pronounced beneficial effects without observable adverse responses. Consequently, the 500 mg/kg dose was selected for the experimental design [24]. The doses were freshly prepared as a paste in distilled water. The paste was prepared at a concentration of 1 g/5 mL. The dose was calculated based on the body weight of the individual animal [17]. LPS stock solution, 500× (2.5 mg/mL), was prepared in distilled water. The 1 mL stock solution was diluted with 9 mL of distilled water to obtain a working concentration of 0.25 mg/mL.

2.3 Experimental Animal

The study was conducted in Wistar rats of both sexes weighing 200 ± 30 g. The rats were bred locally at the animal house of Jinnah University for Women (JUW), Karachi. Before the experiment, animals were acclimatized to the laboratory environment for 7 days. The rats were kept at an appropriate temperature (25 °C ± 2 °C) and humidity (65% ± 5) with a 12-hr dark/light cycle [4]. The animals were housed in separate cages, with four animals per cage, and allowed free access to standard rat chow and water as required. All experimental procedures were conducted in accordance with the National Institutes of Health (NIH) guidelines for the care and use of laboratory animals (NIH publication No. 80-23).

Experimental Design

The study was conducted to assess the neuroprotective effects of the nutritional supplement by improving cognitive function. An illustration of an experimental workflow is shown in Fig. 1. The nutritional supplement (PA+JR+CM) and memantine (reference drug) were administered orally via orogastric intubation to groups III, IV, and V, respectively, for 30 days. Forty Wistar rats were randomly grouped into five groups (n = 8) [24]. (I) Control administered distilled water orally daily, (II) LPS alone was injected (0.25 mg/kg, i.p.) for 4 consecutive days, (III) NS-treated administered the nutritional supplement (500 mg/kg, p.o.) daily for thirty days as a pretreatment, (IV) NS (500 mg/kg, p.o.) + Memantine (5 mg/kg, p.o.) for thirty days, (V) Memantine served as a reference group, pre-administered memantine (5 mg/kg/day, p.o.) for 30 days. After pretreatment, LPS (0.25 mg/kg, i.p.) was administered on days 27–30 in Groups II, III, IV, and V to induce neuroinflammation and cognitive dysfunction. Behavioral assessments were performed from Day 30 to 34. Following the completion of the behavioural assessment, all experimental animals were anaesthetised on Day 35 by intraperitoneal administration of ketamine (100 mg/kg) and xylazine (10 mg/kg). The depth of anaesthesia was confirmed by the absence of corneal and pedal withdrawal reflexes and lack of response to a noxious stimulus. Once deep anaesthesia was confirmed, the animals were euthanised by exsanguination through cardiac blood withdrawal. Death was confirmed by absence of spontaneous respiration and heartbeat. The brains were subsequently removed and processed for biochemical and histopathological analyses [25].

2.4 Assessment of Behavior and Cognitive Function

The passive avoidance and Morris water maze tests were conducted to evaluate the effect of the nutritional supplement and to compare it with LPS and memantine on cognitive functions [26].

2.4.1 Passive Avoidance Test (PAT)

The test was performed to evaluate learning and memory in rats. The apparatus included a “shuttle box”, a specially designed apparatus for a passive avoidance experiment. The box comprised two equal-sized compartments, a light-coloured and a dark-coloured chamber [27]. A guillotine-style door (5 cm × 5 cm) separated the dark chamber from the light area. The floor of both chambers was lined with stainless steel rods, spaced 6 mm apart. Each rat underwent the learning test during three consecutive days. During the first two days of testing, experimental rats spent 5 min in the apparatus to acclimatize. The learning assessment was performed on the third day. In the learning phase, the rats received a mild shock (1 mA, 50 Hz) for 1 second whenever they entered the dark compartment with all four paws. Training continued until each rat learned to stay in the light chamber for 120 seconds without moving into the dark chamber. After completing this phase, the animals were returned to their cages [28].

Step-Through Latency (STL). A step-through latency was performed 24 hours after training on day 4. As in a training or learning test, the rat was re-entered into the light chamber, and after 5 seconds, the guillotine gate was opened, allowing it to enter the dark chamber. In this phase, the step-through latency (STL) was observed for 5 minutes. The test was discontinued after 5 minutes if an animal could not enter the dark compartment [7].

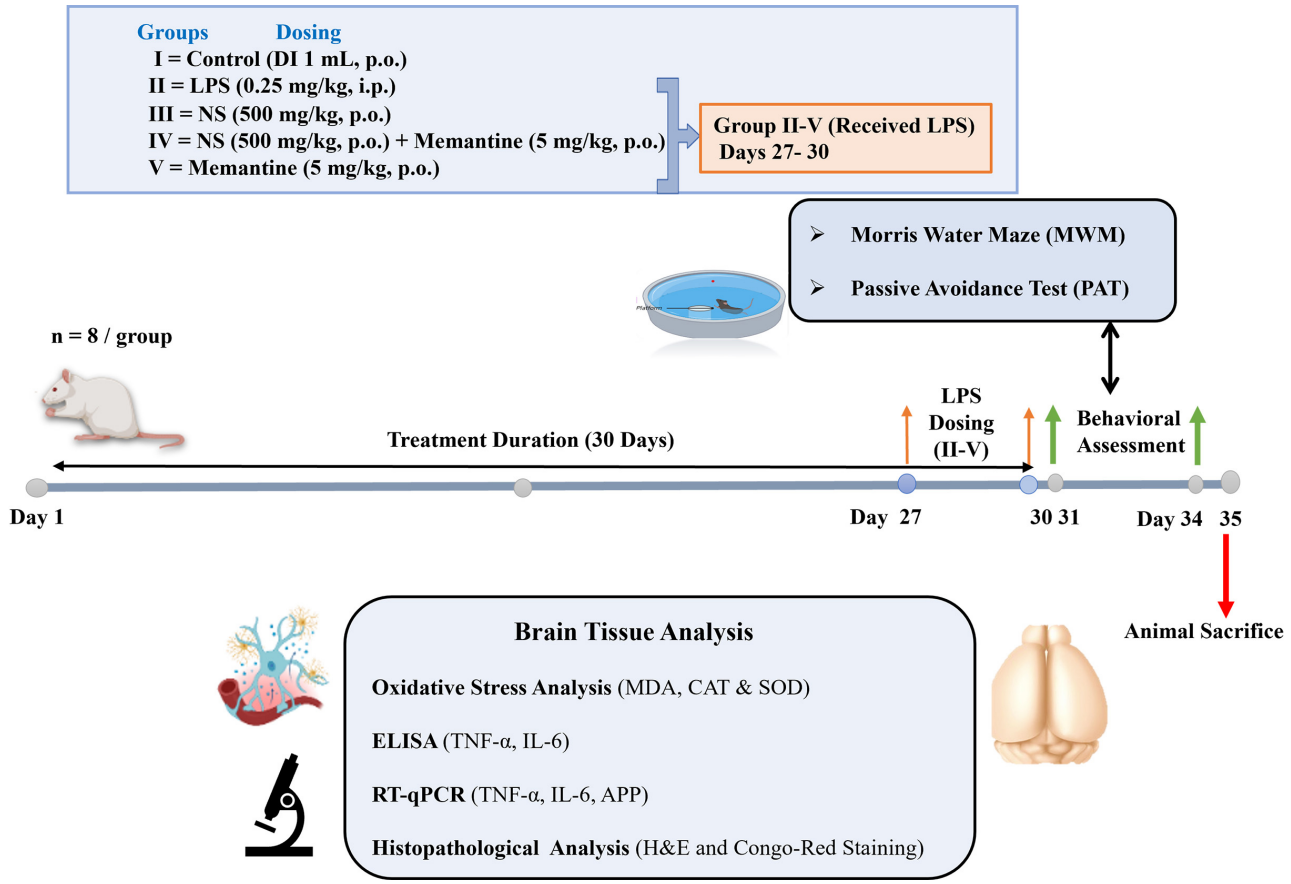


Fig. 1. Schematic workflow for evaluating the anti-inflammatory and antioxidant effect of the nutritional supplement in LPS-induced cognitive impairment in rats. The workflow was created using Microsoft PowerPoint. LPS, lipopolysaccharide.

2.4.2 Morris Water Maze (MWM) Behavioral Assessment Test

To accomplish the Morris water maze assessment, a polypropylene-based pool with a depth of 35 cm and a height of 2 m was used, filled with water and kept at a constant temperature (25 ± 1 °C) [4]. A small white platform (10 cm wide, 30 cm tall) was placed about 50 cm from the edge of one quadrant and was hidden just below the water surface. The training was done over four days, with a total of 16 trials. Each day, the rats were trained to find the hidden platform, and they were kept there for an additional 10 seconds. If the animal could not locate the platform in time, it was gently placed on it and also allowed to stay there for 10 seconds.

Probe Trial. The platform was removed for the probe trial on the fifth day of the experiment. Each rat was placed in the pool from the opposite side and allowed to swim for 90 seconds. The duration that the rat spent exploring the previous placement of the platform was noted as retention time, an indication of memory retention [29].

2.5 Isolation and Preparation of the Brain Sample

At the end of the experiment, the animals were decapitated, and brains were rapidly dissected. The whole brain was then removed and immediately chilled in an ice-cold saline solution. Furthermore, after the brain removal, the hippocampus was placed into separate aliquots, and the tissue samples were stored at −80 °C until the assay was performed [30].

2.6 Estimation of Total Phenolic Content

For the estimation of total phenolic content, the methanolic extract was prepared (10 g in 50 mL of methanol). The total phenolic content was estimated through slight variations of the Folin-Ciocalteu spectrophotometric method [31]. Gallic acid was used as a reference standard. Extract (1 mL) was mixed with 10% Folin's reagent (2.5 mL) and Na_2CO_3 (2.5 mL) in a tube. The prepared sample was incubated at room temperature (25 °C ± 1 °C) in the dark for 2 hours. The absorbance was determined at 740 nm against a blank (methanol) in a spectrophotometer (UV-1800) [32].

2.7 Assessment of 1,1-diphenyl-2-picrylhydrazyl (DPPH) Radical Scavenging Activity

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging ability was measured using a modified method described by Nguyen et al. (2022) [33]. The methanolic extract(1 mL) at concentrations of 300 mg/mL and 500 mg/mL was separately added to the DPPH solution (3.5 mL) and incubated for 30 minutes in the dark. The absorbance was measured at 517 nm using a spectrophotometer (Shimadzu UV-1800) with methanol as the blank. An ascorbic acid calibration curve (0.03–0.27 mM) was prepared, and all measurements were performed three times [33]. The antioxidant potential was estimated using the standard percentage inhibition formula.

$$\% \text{ inhibition} = (A_C - A_T) \times 100/A_C$$

Where, A_C = Absorbance of Control, A_T = Absorbance of test Sample.

2.8 Gas Chromatography Mass Spectrometry (GC-MS)

The characterisation was performed to identify bioactive phytoconstituents in the nutritional supplement. The extract was prepared using a Soxhlet apparatus (50 g sample /100 mL hexane). GC-MS was performed using an Agilent Technology 7000 GC-MS triple-quadrupole system [34]. Chromatographic separation was performed using an OPTIMA-5 column, maximum column temperature 360 °C, with helium as the carrier gas (1.129 mL/min flow rate, 5:1 split ratio) and total run time 70.716 min. A 2.5 µL aliquot was injected using an automated liquid sampler. The detected compounds were identified by comparing their mass spectra with the reference spectra in the National Institute of Standards and Technology (NIST) Mass Spectral Library. The relative abundance of each identified compound was estimated from its peak area as a percentage of the total integrated peak [35].

2.9 Estimation of Lipid Peroxidation and Antioxidant Activity

Lipid peroxidation in brain tissue was determined using the TBARS (Thiobarbituric Acid Reactive Substances) assay by measuring MDA levels, and the results were expressed as nmol/mg. 100 mg of brain tissue was homogenised in 1 mL of phosphate-buffered saline (pH 7.4), and the supernatant was collected after centrifugation at 10,000 ×g for 15 minutes. Protein denaturation was achieved by centrifugation and precipitation with 20% (v/v) TCA. TBA 0.33% was mixed with the supernatant, and the sample was boiled for 1 hour at 95 °C to allow the MDA-TBA reaction. After cooling, the samples were extracted with butanol to increase the intensity of the pink colour. The samples were finally centrifuged, and absorbance was measured at 532 nm using a microplate photometer (Thermo Scientific Multiskan, USA). 1000 µM stock solution of MDA was prepared to make dilutions over the 0–40 nmol/mL range to quantify MDA levels in samples using a standard curve linear equation [36]. CAT and SOD levels were estimated in brain tissue to measure antioxidant enzyme status in experimental animal groups using commercial assay kits and following the manufacturer's protocol by Sangon Biotech. CAT activity was determined using a micro method based on H_2O_2 decomposition at 240 nm, measured with a UV-Visible spectrophotometer (Shimadzu UV-1800, Shimadzu Corporation). CAT activity was analysed by measuring the decrease in absorbance at 240 nm over time and calculating the rate of change in absorbance. The SOD enzymatic reaction was detected using the xanthine-xanthine oxidase reaction to generate superoxide anions, which form blue formazan by reducing nitroblue tetrazolium. SOD inhibits formazan formation by neutralising O_2^-, and a decrease in absorbance value at 560 nm was measured.

2.10 Estimation of Neuroinflammatory Biomarkers

2.10.1 Pro-Inflammatory Cytokine Gene Expression Analysis via RT-qPCR

The brain samples were homogenised in TRIzol reagent (RT 111, USA) to extract RNA from the hippocampal region. The purity and concentration of the isolated RNA were assessed by measuring the 260/230 and 260/280 ratios using a NanoDrop 2000 (USA). Reverse transcription cDNA was performed using Superscript II Reverse Transcriptase (Thermo Scientific, Invitrogen, USA). Quantitative reverse transcription real-time polymerase chain reaction (RT-qPCR) was performed on the Eco-TM Real-time PCR system (Illumina, USA) using SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA). Each RT-qPCR reaction was carried out in a final volume of 20 µL containing 1 µL cDNA and 0.2 µM each of the Forward and Reverse primers. The amplification conditions consisted of an initial denaturation at 95 °C, for 5 min, followed by 40 cycles of denaturation at 94 °C for 15 seconds, annealing at the primer-specific temperatures listed in Table 1, and extension at 72 °C for 20 seconds. The specificity of the amplification products was verified by melting curve analysis. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the housekeeping gene for normalisation, and relative gene expression was calculated using the ΔΔCq method to measure fold change. The primers for the targeted genes IL-6, TNF-α, and amyloid precursor protein were generated using the NCBI Primer BLAST tool, as shown in Table 1.

2.10.2 Estimation of Cytokine Levels

After isolation of the hippocampus from each brain sample stored in a separate Falcon tube, each sample was homogenised in 10 volumes of cold buffer containing 20 mM Tris-HCl (pH 7.4), 0.5 mM benzamidine, 1.0 mM

Table 1. RT-qPCR primers for gene expression analysis.

Primer	Sequence 5′-3′	Primer length (bp)	Amplicon length (bp)	Annealing temperature °C
IL-6	F: CTCACACTCCTCTCACAGTCT	21	115	54 °C
	R: CGAAAGTCAACTCCATCTGCC	21		54 °C
TNF-α	F: CCAGACCCTCACACTCAGTAAG	22	135	55 °C
	R: CTTAGGTTTCCCAGCAAGCA	20		55 °C
Amyloid precursor protein	F: TCTTCTTCGCTGCAGACCAC	20	167	56 °C
	R: AGCTCCCAGGATCTTGCATAC	21		56 °C
GAPDH	F: CCATTCCTAATTTCCATTCGTCTCC	25	143	59 °C
	R: CCCACCATCCAGTTCCTATAAATAC	25		59 °C

IL-6, interleukin-6; TNF-α, tumor necrosis factor alpha; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

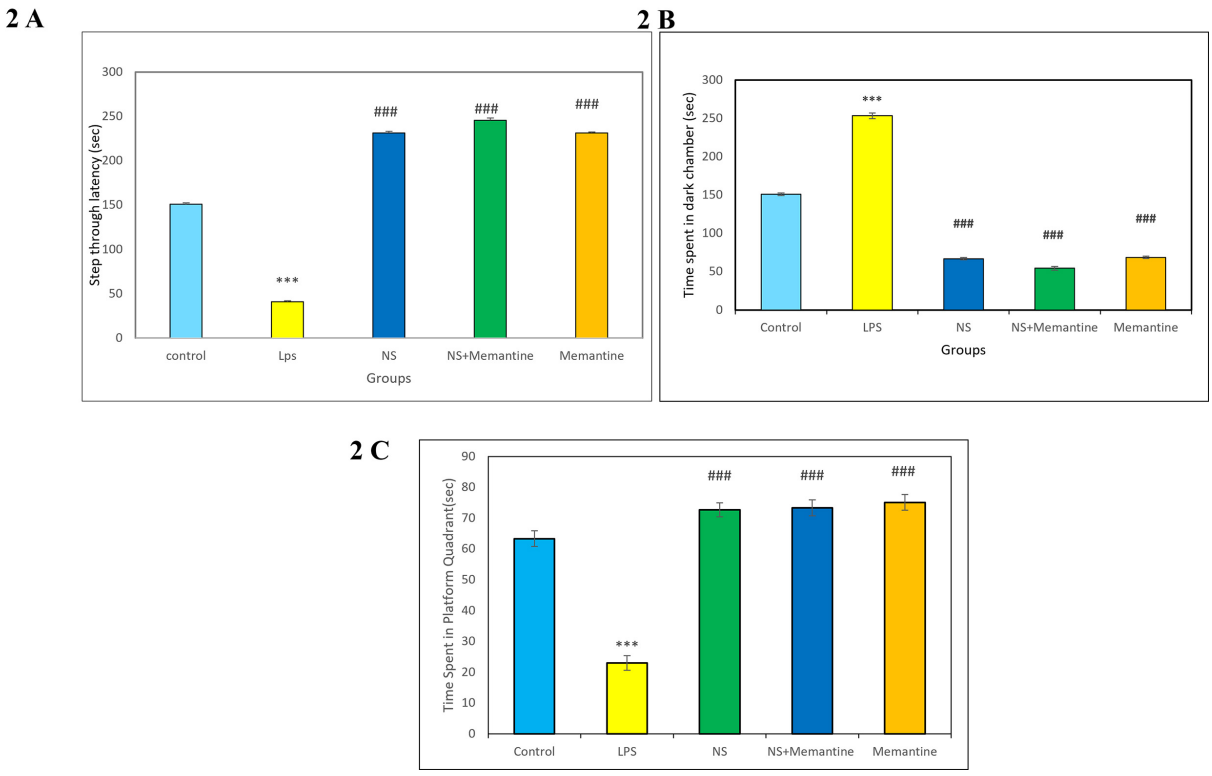


Fig. 2. NS memory-restoration effect in LPS-induced cognitive dysfunction in rats. Behavioral tests were performed using the passive avoidance test and the Morris water maze to assess memory impairment across all five groups (n = 8). (A) Step-through latency (STL) in seconds, (B) time spent in the dark chamber, and (C) Probe trial in the MWM test. Significance is indicated as ***$p < 0.001$ compared with control; $^{###}p < 0.001$ compared with LPS. MWM, Morris Water Maze.

EDTA, and 1.0 mM DTT. The proteins were extracted from the tissue homogenate by ultrasonic disruption with a sonicator (WUC-A02H, Daihan Scientific Co., Republic of Korea), and the samples were immediately centrifuged at 20,000 ×g for 30 minutes at 4 °C using a refrigerated benchtop centrifuge (K2015R, Centurion Scientific, UK). The supernatant was carefully collected and stored at –40 °C until analysis. The concentrations of TNF-α (lot: L230208134) and IL-6 (lot: L230412958) in tissue homogenates were quantified using a commercially available sandwich ELISA kit from UCSN (Cloud-Clone Corp., USA) according to the manufacturer's instructions.

2.11 Histopathological Examination

The sagittal plane brain sections were fixed in formalin and dehydrated using ethanol, cleaned in xylene, and mounted in paraffin blocks. After that, 5 µm-thick serial sections were cut and stained. Hematoxylin and eosin (H&E) and Congo Red staining were performed. The images were observed using a light microscope to analyse the hippocampal regions [4]. Histopathological alterations were assessed by semi-quantitative analysis based on pyramidal layer disorganization, neuronal shrinkage, nuclear pyknosis, vacuolation/tissue damage, and overall neuronal degeneration, using a severity-based scoring approach

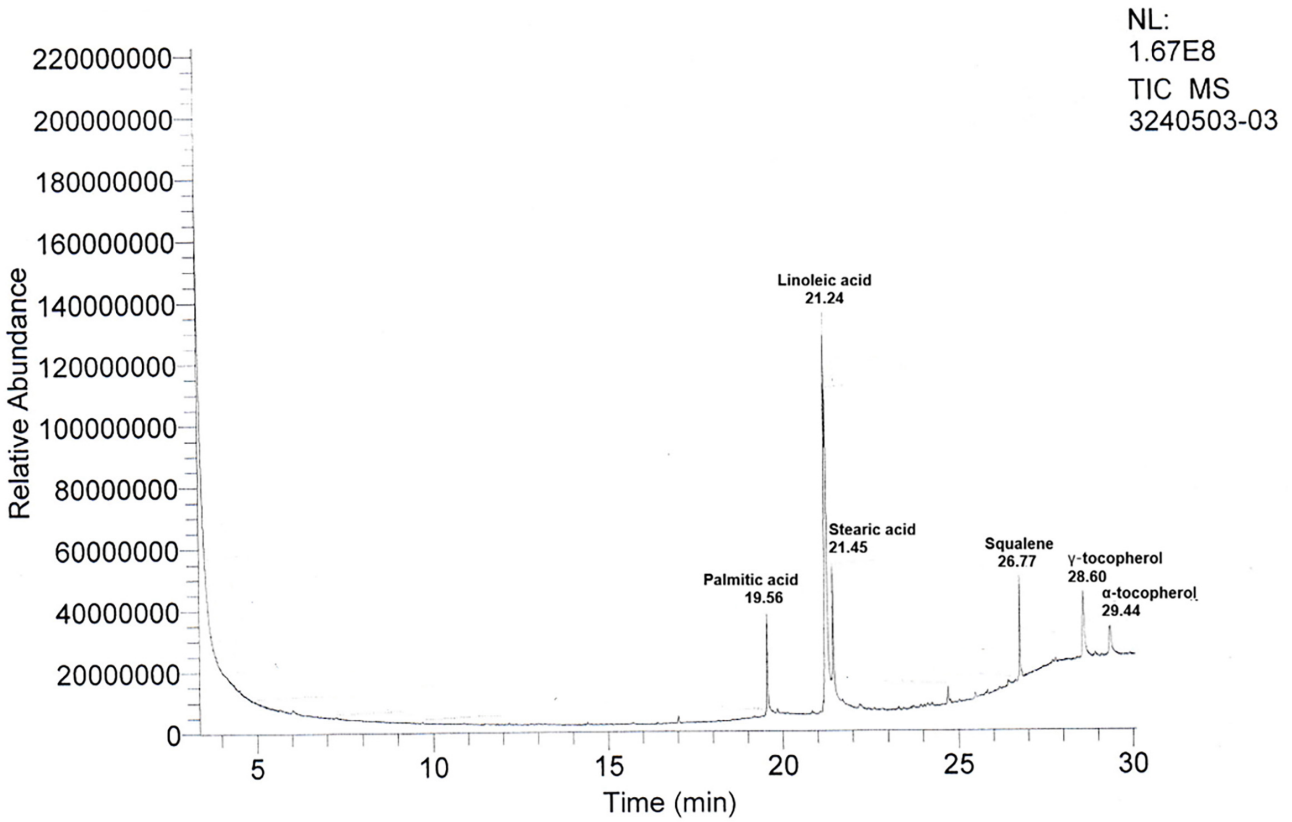


Fig. 3. Chromatogram; Represents the presence of phytoconstituents in the nutritional supplement extract.

Table 2. Total phenolic content (TPC) (GAE) mg/g of dry weight of methanolic and aqueous extracts of the nutritional supplement.

Sample	Concentration (mg/mL)	TPC (GAE mg/gm)
NS-m	300	ND
NS-m	500	7.70 ± 2.5
NS-aq	300	5.5 ± 1.2
NS-aq	500	9.45 ± 1.5

Values expressed as Mean ± SD. ND, not detected; GAE, gallic acid equivalents; NS-m, nutritional supplement methanolic extract; NS-aq, nutritional supplement aqueous extract.

adapted from Khalil et al. (2022) [37]. Each histopathological feature was graded on a 0–4 scale, where 0 = normal, 1–2 = mild, 3 = moderate, and 4 = severe [37].

2.12 Statistical Analysis

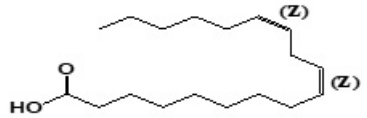
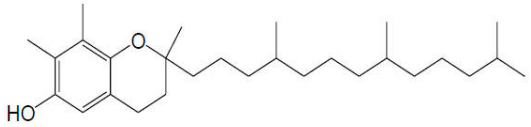
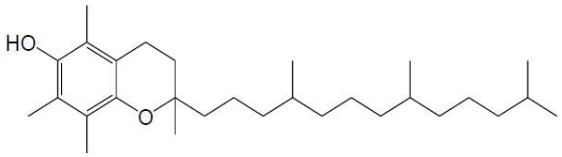
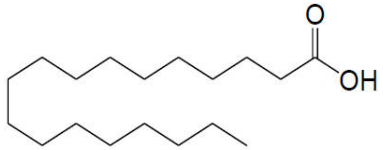
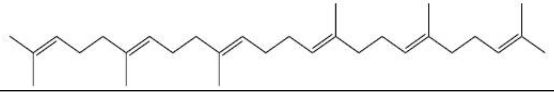
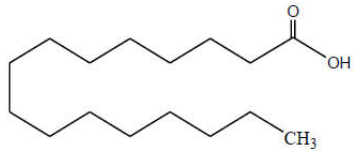
Data are expressed as mean ± standard deviation (SD) for each group. Statistical analysis across multiple groups was performed using SPSS version 22.0 (IBM Corp., Armonk, NY, USA). Data normality was assessed by the Shapiro–Wilk test. Homogeneity of variances was evaluated using Levene's test. As the assumptions of normality and homogeneity of variance were satisfied, differences among groups were analysed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparisons. The statistical significance level was set at $p < 0.05$.

3. Results

3.1 Nutritional Supplement (PA, JR, and CM) Restores Cognitive Impairment

Restoration of cognitive impairment was assessed using passive avoidance tests and the Morris water maze, as illustrated in Fig. 2. In the PAT, step-through latency was significantly decreased, and time spent in the dark chamber was increased in the LPS group compared with the control ($p < 0.001$), indicating memory decline. The NS-treated group and NS co-administration with memantine showed significantly increased STL. Both treated groups significantly reduced time spent in the dark area compared with the LPS group ($p < 0.001$), demonstrating improved memory and learning performance following treatment (Fig. 2A,B). In the MWM probe trial test, LPS administration significantly reduced time spent in the target quadrant compared with the control group ($p < 0.001$), indicating impaired spatial memory. Treatment with the Nutritional supplement, co-administered with memantine (Fig. 2C), significantly increased target quadrant time compared with the LPS group ($p < 0.001$), demonstrating improved spatial memory.

Table 3. Key bioactive constituents identified in the nutritional supplement by GC-MS analysis.

Compounds	Chemical formula	Chemical name	Class	RT (min)	GC Peak Area%	% match
Linoleic acid	$C_{18}H_{32}O_2$ 	(9Z,12Z)-Octadeca-9,12-dienoic acid	Polyunsaturated fatty acid (PUFA)	21.24	65.04%	93.2
γ-tocopherol	$C_{28}H_{48}O_2$ 	2,7,8-trimethyl-2-[(4R,8R)-4,8,12-trimethyltridecyl]-3,4dihydrochromen-6-ol	Phenolic compound	28.6	9.29%	91.7
α-tocopherol	$C_{29}H_{50}O_2$ 	(2R)-2,5,7,8-Tetramethyl-2-[(4R,8R)-4,8,12-trimethyltridecyl]-3,4-dihydro-2H-1-benzopyran-6-ol	Phenolic compound	29.44	4.13%	89.1
Stearic acid	$C_{18}H_{36}O_2$ 	Octadecanoic acid	Saturated Fatty acid	21.45	8%	77.5
Squalene	$C_{30}H_{50}$ 	2,6,10,15,19,23-Hexamethyltetracosa-2,6,10,14,18,22-hexaene	Triterpene hydrocarbon	26.77	6.56%	83.6
Palmitic acid	$C_{16}H_{32}O_2$ 	Hexadecanoic acid	Saturated Fatty acid	19.56	6.98%	92.4

RT, retention time; % area indicates the percentage of the constituents. Chemical structure was drawn using ChemDraw software. The compounds were identified using the National Institute of Standards and Technology (NIST) mass spectral Library.

Table 4. DPPH assay of nutritional supplement.

Methanolic extract	Conc. mg/mL	RSA%
NS 1	300	54.8 ± 0.38
NS 2	500	67.9 ± 0.30

%RSA; Percentage of free radical-scavenging activity; Values are expressed as Mean ± S.D.

3.2 Estimation of Total Phenolic Content in the Nutritional Supplement

The total phenolic content was determined in the aqueous and methanolic extracts of the nutritional supplement. AT 500 mg/mL, TPC values were 7.70 and 9.45 mg GAE/g dry weight in the methanolic (NS-m) and aqueous (NS-aq) extracts, respectively. At 300 mg/mL, the TPC value was 5.50 mg GAE/g dry weight in the NS-aq, whereas it was not detected in the methanolic extract (Table 2).

3.3 Composition and Characterisation by GC-MS

The GC-MS profile of the nutritional supplement showed the presence of unsaturated fatty acids, including monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) (Fig. 3). Table 3 shows that the extract is predominantly composed of linoleic acid, stearic acid, squalene, and palmitic acid, along with notable levels of the antioxidants α- and γ-tocopherols. This composition suggests potential health-promoting effects and good oxidative stability, supporting its neuroprotective potential when administered as a nutritional supplement. The compounds were identified by comparing their mass spectra with reference spectra in the NIST Mass Spectral Library based on spectral match factors. The relative abundance (% peak area), retention time (RT) and match factor of each identified compound are listed in Table 3.

3.4 NS Mediated Scavenging Activity Determined by DPPH Assay

The free radical-scavenging activity of the nutritional supplement was measured as a percentage of DPPH inhibition. The nutritional supplement exhibits significant DPPH radical-scavenging activity (RSA) at 500 mg/mL, as shown in Table 4. The methanolic extract exhibits a concentration-dependent increase in antioxidant activity, reflecting antioxidant potential correlated with phenolic content.

3.5 NS Mitigates Oxidative Stress

Lipid Peroxidation and oxidative stress were quantified in the hippocampus region of brain tissue by measuring MDA levels, expressed as nmol/mg of protein. As shown in Table 5, the MDA level was higher in the LPS group (11.68 ± 1.84 nmol/mg protein) than in the control (1.91 ± 0.58 nmol/mg protein), indicating pronounced oxidative damage following LPS-induced oxidative stress. Pretreatment with the nutritional supplement reduced MDA levels (7.10 ± 2.10 nmol/mg protein). Co-administration of memantine + NS significantly attenuated lipid peroxidation (4.59 ± 0.89 nmol/mg protein). Memantine alone also significantly reduced oxidative stress (5.26 ± 1.60 nmol/mg protein) compared to LPS. However, the findings indicate that the nutritional supplement mitigates lipid peroxidation, supporting its oxidative and neuroprotective potential against oxidative stress.

3.6 NS Reinforces Antioxidant Defense

The antioxidant enzymes CAT and SOD were measured in the brain tissue homogenate of each group. In the LPS group, CAT and SOD levels were significantly decreased compared to the control group ($p < 0.0001$). The treated groups (NS, NS+ memantine, and memantine) significantly improved catalase and superoxide dismutase levels compared to LPS ($p < 0.001$). The results are also comparable with the reference drug (memantine) and showed a significant ($p < 0.05$) increase in catalase and superoxide dismutase levels in the NS-treated group.

3.7 Nutritional Supplement Suppresses Pro-Inflammatory Cytokine Gene Expression

The expression of gene transcripts using real-time PCR in the rat hippocampus is shown in Fig. 4A,B. After intraperitoneal LPS administration, we observed increased expression of TNF-α and IL-6 (3.0- and 2.7-fold, respectively) compared with the control group. Nutritional supplement treatment reduced the overexpression of TNF-α and IL-6 (1.90- and 1.5-fold, respectively), and memantine also reduced their expression (2.3- and 1.85-fold, respectively), compared with the LPS group, indicating suppression of inflammatory cytokines. Co-administration of the nutritional supplement with memantine significantly attenuated LPS-induced inflammation, with a fold change of TNF-α and IL-6 (1.6 and 1.3, respectively) when compared with the LPS group.

3.8 NS-Mediated Downregulation of Amyloid Precursor Protein (APP) Gene Expression

As shown in Fig. 4C, LPS triggered a marked increase in amyloid precursor protein levels in the rat hippocampus, indicating significant upregulation. mRNA expression of the amyloid precursor protein increased by 3.4-fold. NS treatment and memantine alone also significantly downregulated targeted gene expression (1.2- and 1.62-fold, respectively) relative to the LPS group. In contrast, co-treatment of NS with memantine (1.1-fold change) attenuated the downregulation of APP gene expression.

3.9 Estimation of Protein Concentration

As illustrated in Fig. 5A,B, pro-inflammatory cytokine levels (TNF-α and IL-6) were estimated.

Quantitative analysis of hippocampal proteins was performed to evaluate the neuroprotective effects of the nu-

Table 5. Estimation of MDA (nmol/mg) and antioxidant enzymes.

Groups	Treatment	MDA (nmol/mg protein)	Catalase (U/mg Protein)	Superoxide dismutase (U/mg Protein)
I	Control	1.91 ± 0.58	5.60 ± 0.06	19.06 ± 0.36
II	LPS	11.68 ± 1.84***	3.22 ± 0.07***	14.09 ± 0.40***
III	NS	7.10 ± 2.10$^{\#\#\#}$	4.28 ± 0.23$^{\#\#\#}$+	16.26 ± 0.36$^{\#\#\#}$+
IV	NS + Memantine	4.59 ± 0.89$^{\#\#\#}$	4.19 ± 0.04$^{\#\#\#}$	15.18 ± 0.54$^{\#\#\#}$
V	Memantine	5.26 ± 1.60$^{\#\#\#}$	4.00 ± 0.08$^{\#\#\#}$	15.5 ± 0.29$^{\#\#}$

Data are represented as mean ± SD, and one-way analysis of variance (ANOVA) (n = 8) is used for analysis, with post hoc comparisons by Tukey's test. Significance is shown as $^{***}p < 0.001$ in comparison with control; $^{\#\#\#}p < 0.001$, $^{\#\#}p < 0.01$ in comparison with LPS, and $^{+}p < 0.05$ when compared with memantine.

Table 6. Semi-quantitative injury scoring of brain tissue.

Histopathological feature	A = Control	B = LPS	C = NS	D = NS + Memantine	E = Memantine
Pyramidal layer disorganisation	0	4	1	1	1
Neuronal shrinkage	0	4	1	1	2
Nuclear pyknosis	0	3	2	1	1
Vacuolation/tissue damage	0	4	1	2	1
Overall neuronal degeneration	0	4	2	1	1

Scoring: 0 = Normal; 1–2 = Mild; 3 = Moderate; 4 = Severe.

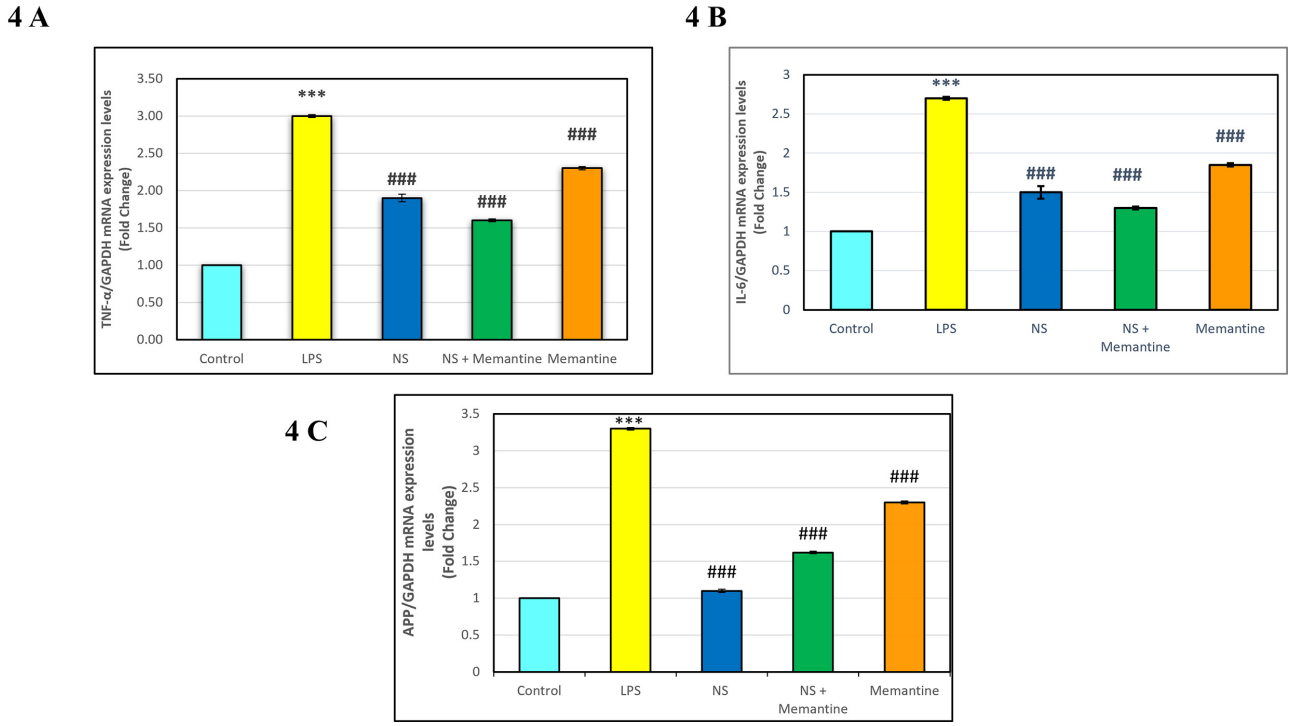


Fig. 4. Nutritional supplement (NS) mitigated LPS-induced neuroinflammation in rats. The effect of NS was assessed by measuring mRNA expression levels (fold change) of target genes (A) TNF-α, (B) IL-6, and (C) Amyloid precursor protein by RT-qPCR. One-way ANOVA was applied for statistical analysis, followed by post hoc Tukey's test. $^{***}p < 0.001$ when compared with control, $^{\#\#\#}p < 0.001$ when compared with LPS.

tritional supplement. Elevated levels of proinflammatory cytokines in the LPS group compared with the control indicate neuroinflammation. In contrast, NS treatment, co-administration of NS + memantine, and memantine treatment significantly ($p < 0.001$) decreased TNF-α and IL-6 levels compared with the LPS group.

3.10 NS Alleviated LPS-Induced Histopathological Alteration in Rat Brain Tissue

3.10.1 Hematoxylin-Eosin Staining

The histopathological evaluation of hippocampal brain tissue revealed distinct morphological changes among the treatment groups. The control group (A) maintained

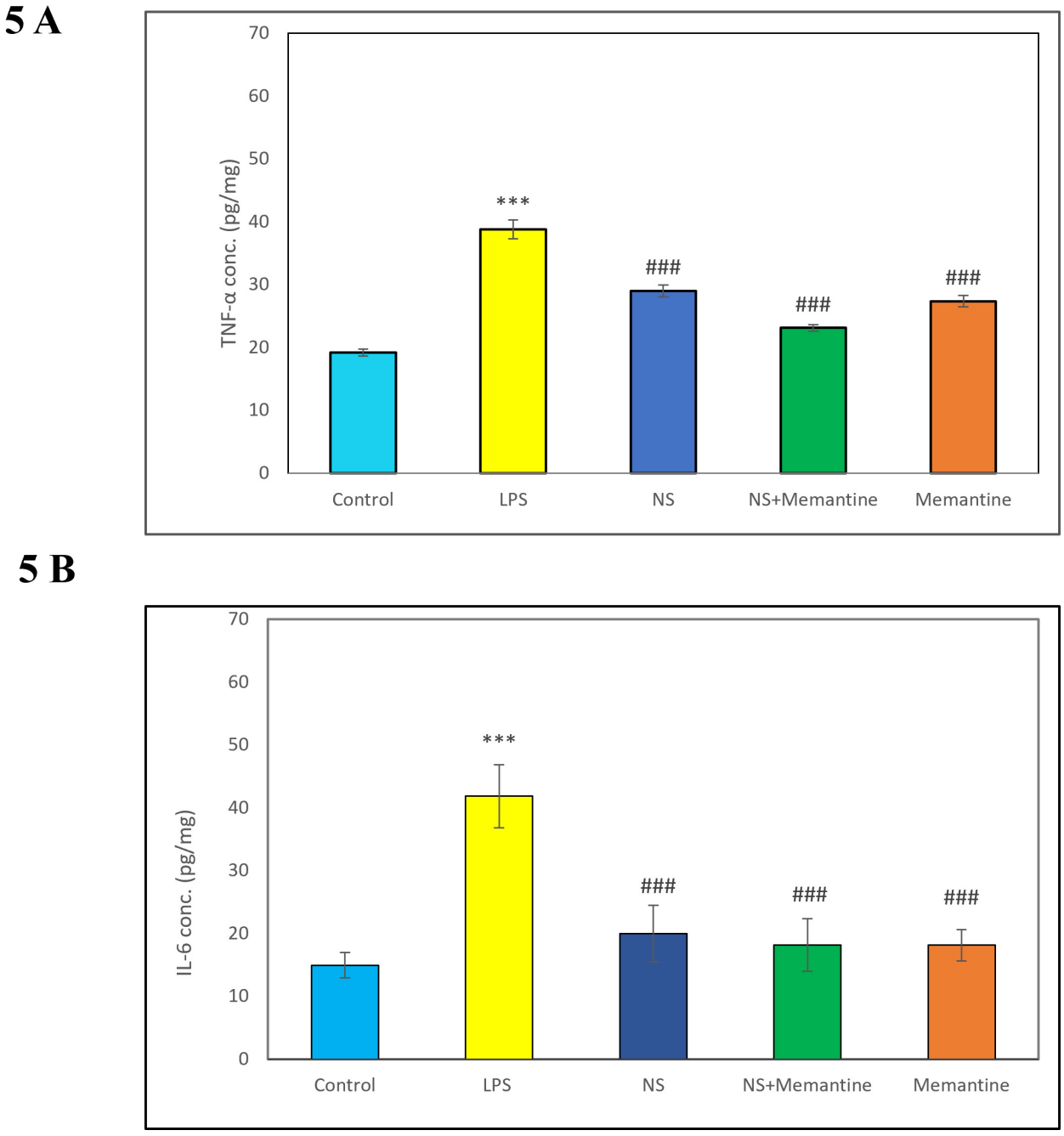


Fig. 5. Effect of NS (nutritional supplement) on the protein levels of pro-inflammatory cytokines. (A) TNF-α and (B) IL- 6 levels. Statistical significance between groups is indicated by: ***$p < 0.001$; compared with the control; $^{###}p < 0.001$; when treatment groups were compared with LPS.

normal neuronal morphology, while the LPS group (B) exhibited marked neuronal degeneration and neuroinflammatory changes, including disruption of the CA1 pyramidal layer, neuronal shrinkage, nuclear pyknosis, hyperchromatism (black arrow), cytoplasmic vacuolation (red arrow), necrotic features, and inflammatory cell infiltration. Conversely, NS-treated (C) memantine (E) showed partial restoration of neuronal architecture and a significant reduction in histopathological alterations (Fig. 6). Co-administration of NS+ memantine (D) significantly attenuated LPS-induced histopathological alterations, as evidenced by restoration of hippocampal architecture. These observations were further supported by semi-quantitative injury scores (Table 6), which showed the LPS group exhibited a high score, whereas the treatment groups showed comparatively lower scores, indicating attenuation of neural injury and neuroinflammation.

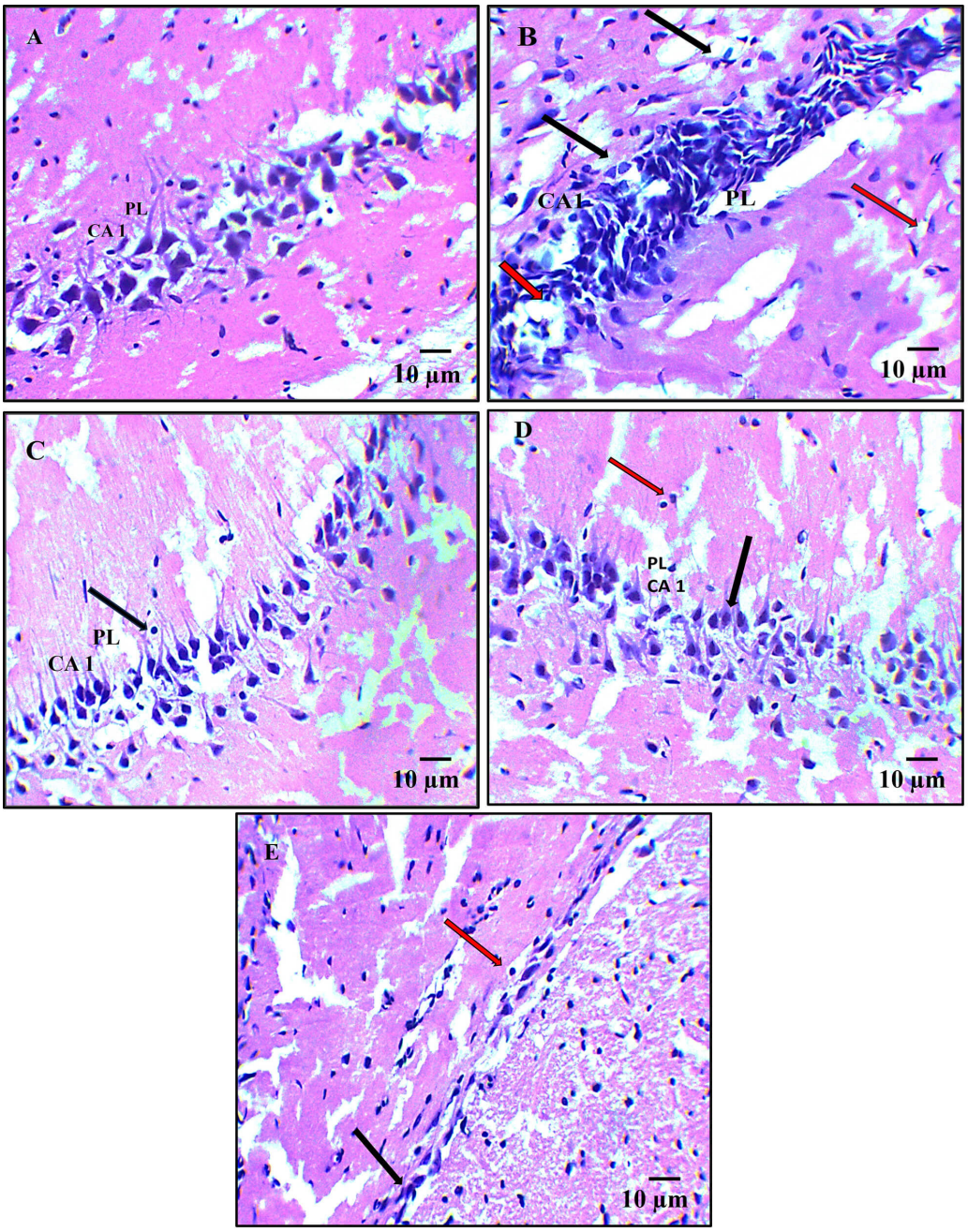


Fig. 6. NS alleviated LPS-induced histopathological alteration in rat brain tissue. The hippocampal region of the rat brain was stained with hematoxylin and eosin and observed under 40× magnification with a light microscope. (A) Exhibits the control group with normal morphology of the pyramidal layer in the CA1 region. (B) shows the LPS group with the pyramidal layer that underwent inflammatory changes as indicated by the disorganised arrangement, hyperchromatism (black arrow), eosinophilic cytoplasm, nuclear pyknosis, and shrinkage of neurons and vacuolated cell morphology (red arrow). (C) represents the NS-treated group with normal neuronal morphology, indicative of a neuroprotective effect; (D) shows a combined treatment of NS + memantine, with neuronal structural preservation; (E) the memantine-treated group showed preserved neuronal architecture and reduced nuclear pyknosis compared with the LPS group. Scale bar = 10 µm.

3.10.2 Congo Red Staining

Fig. 7 shows that amyloid protein accumulation in the brain is a major factor in the progression of memory impairment. Congo red staining was used to determine Congo red-positive amyloid-like deposits. LPS administration intraperitoneally in rats resulted in a significant increase in amyloid-like protein accumulation (blue arrow). NS-treated and co-administered memantine groups showed markedly reduced amyloid-like protein deposits compared to the LPS group.

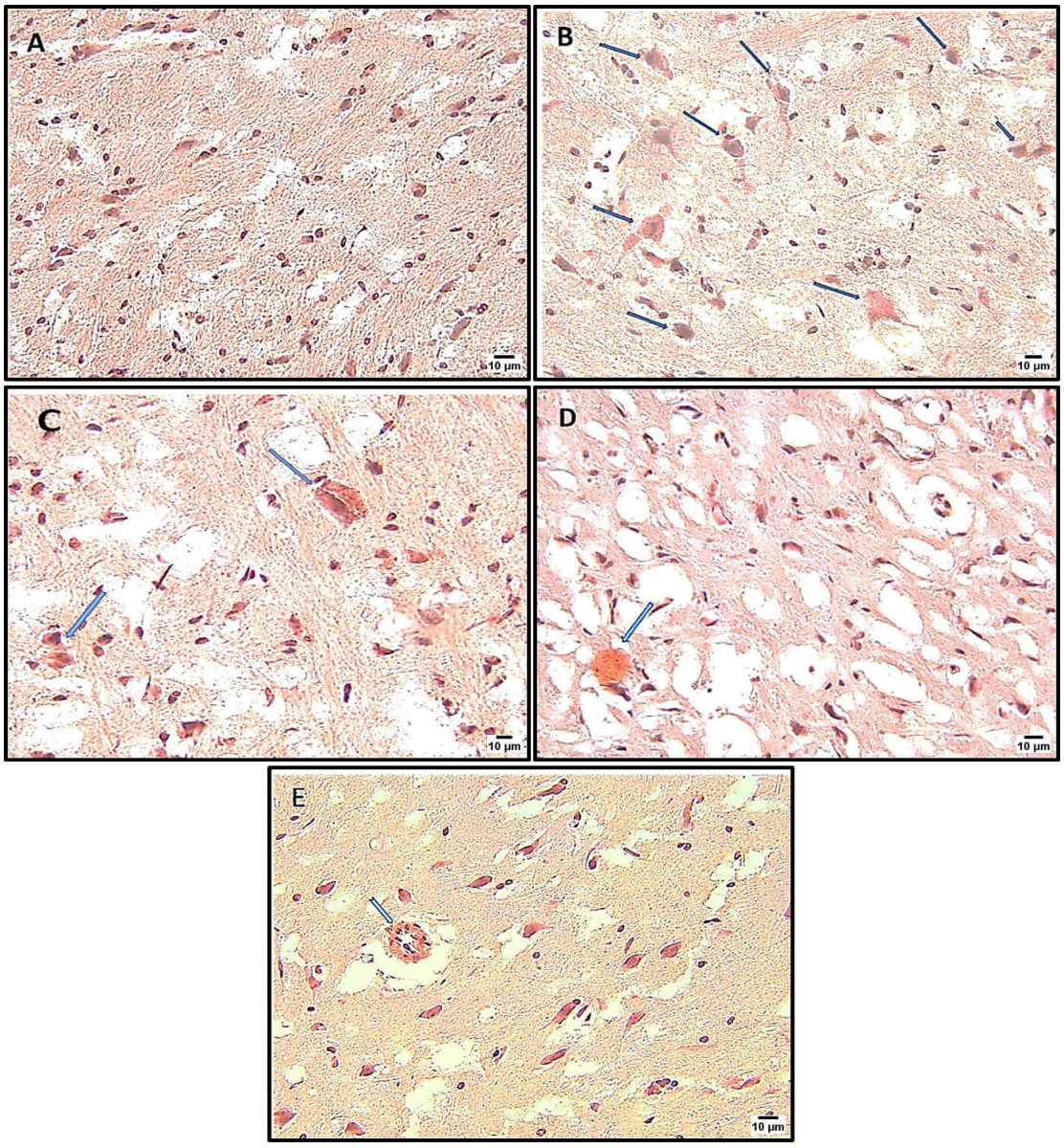


Fig. 7. NS alleviated LPS-induced neuroinflammation and histopathological alteration in rat brain tissue after Congo Red staining. The rat brain was stained with Congo red and observed under 40× magnification with a light microscope. Congo Red staining was performed to provide evidence for the presence of amyloid-like protein in the brain tissue (blue arrow). (A) Control; (B) LPS-induced neuroinflammation; (C) NS-treated; (D) NS + Memantine; (E) Memantine-treated group. Scale bar = 10 µm.

4. Discussion

The study aimed to demonstrate the neuroprotective activity of the nutritional supplement (*Prunus amygdalus*, *Juglans regia*, and *Cucumis melo*) enriched with phytoconstituents, including linoleic acid (polyunsaturated fatty acid) and α- and γ-tocopherol (phenolic compounds). Our findings demonstrate that the nutritional supplement improved behavioral performance while modulating fundamental pathological mechanisms underlying cognitive dysfunction. The multitarget neuroprotective and anti-inflammatory effects suggest that the nutritional supplement may represent a promising nutritional intervention for cognitive decline.

The memory assessment outcomes provide functional evidence of the detrimental effect of LPS on cognitive function tests. Animals receiving LPS showed significant impairments in memory retention and spatial learning as reflected by poor acquisition and longer escape latency in the MWM test and significantly increased time in the dark chamber in the passive avoidance test, as shown in Fig. 2. A deficit in a particular parameter indicates hippocampal injury, a hallmark of neuroinflammation. A limitation of the present study is that due to the lack of an automated video-recording system, animal swimming trajectories were not recorded during the Morris Water Maze activity. Accordingly, qualitative assessments of movement

trajectories and animal search behaviour could not be analysed. Future studies using computerised tracking systems will provide more comprehensive behavioural characterisation. The hippocampus is vulnerable to oxidative stress and inflammatory cytokines due to its increased metabolic demand and sensitivity to redox imbalances. Reactive oxygen species and pro-inflammatory cytokines, when excessively generated, impair neurotransmission and disrupt synaptic plasticity, leading to memory impairment and a deficit in the learning process. The behavioral characteristics noted in the NS and NS + memantine-treated groups closely paralleled the reduction in oxidative stress and inflammatory cytokines, indicating that the attenuation of molecular neurotoxicity directly contributed to the restoration of cognitive function. A similar study conducted by Batool et al. (2019) [38] reported that walnuts and almonds significantly improved cognitive function in a neurotoxicity model. The improvement in cognitive behaviour was substantially corroborated by biochemical findings, which showed a significant increase in endogenous antioxidant levels and suppression of oxidative stress. Oxidative stress and neuroinflammation are integrated sequential process that exacerbates neuronal injury in response to neurodegeneration. LPS administration activates toll-like receptor-mediated inflammatory pathways, promoting NF-κB activation and transcription of proinflammatory cytokines. A sustained neuroinflammatory response augments ROS production, increases lipid peroxidation, and induces mitochondrial dysfunction and cellular energy dysregulation, thereby promoting neuronal dysfunction and cognitive decline [30]. In the current study, LPS significantly elevated MDA levels while reducing SOD and CAT activities, indicating oxidative imbalance and membrane disruption within brain tissue. Elevated lipid peroxidation not only damages neuronal membranes but also impairs mitochondrial bioenergetics and synaptic function, both of which are essential for memory formation and neuronal survival [5,39]. However, the NS treatment and co-administration of NS with memantine attenuated these alterations by reducing MDA levels and restoring antioxidant enzyme activity, demonstrating recovery of cellular redox homeostasis. The antioxidant activity of the nutritional supplement may therefore interrupt the self-propagating cycle between neuroinflammation and oxidative stress, reducing progressive neuronal injury and preserving cognitive performance. A similar study conducted by Rusu et al. (2020) [40] reported that walnut extract improved antioxidant levels, reduced oxidative stress, and protected neuronal structure in aged rat models, highlighting the anti-inflammatory and neuroprotective potential of natural antioxidant-based interventions against cognitive dysfunction.

Dysregulation of pro-inflammatory cytokines contributes to persistent microglial activation, neuronal toxicity, and disruption of signalling pathways that support memory consolidation and synaptic plasticity [5]. The current investigation shows upregulation of TNF-α and IL-6 gene expression and protein concentration, indicating activation of neuroinflammatory cascades associated with cognitive dysfunction after LPS administration. Conversely, NS-treated groups showed significant downregulation of both cytokine expression and protein levels, indicating effective modulation of the inflammatory pathway. Alterations in TNF-α and IL-6 are important because neuroinflammation not only induces direct neuronal damage but also exacerbates oxidative stress and promotes amyloidogenic processing. Pro-inflammatory cytokines impair the amyloid protein clearance mechanism and facilitate amyloid accumulation, thereby accelerating neuronal disease progression [41]. Thus, the downregulation of cytokines observed following administration of the nutritional supplement may have contributed not only to reducing neuroinflammation but also to preserving neuronal function. The findings of the present study suggest that although both the nutritional supplement and memantine administration alone produced significant improvements in the behavioral, biochemical, and molecular parameters compared with the LPS-treated group, the combination treatment did not show statistically remarkable benefit over either monotherapy under the present experimental conditions. Further studies investigating optimised dose combinations, extended treatment durations, and mechanistic pathways could determine whether co-administration of the nutritional supplement and memantine confers additive or synergistic neuroprotective effects.

Neuroinflammation and oxidative stress are fundamentally involved in amyloid-associated disease, another hallmark of neurodegeneration. Inflammatory activation and augmented ROS production impair the degeneration and clearance of pathological tau protein and amyloid accumulation, leading to synaptic dysfunction, cytotoxic protein accumulation, and neuronal death [11,42].

Consistent with the reported mechanism, our findings showed that LPS administration increased amyloid-associated staining in the brain tissue. However, the nutritional supplement dosing significantly reduced amyloid-like protein deposition, as shown in Fig. 7, suggesting modulation of amyloidogenic pathways. The reduction in Congo red-positive amyloid-like protein accumulation observed in the current study may be attributed to the synergistic anti-inflammatory and antioxidant properties of α, γ-tocopherol identified in the poly nut formulation. Pei et al. (2022) [43] conducted a similar study, consistent with the present findings and with the prominent presence of phytoconstituents in nuts. The present study reflects the accumulation of amyloid-like proteins rather than mature extracellular amyloid plaques on Congo red staining. Although LPS administration promotes neuroinflammation and amyloidogenic processing, it does not induce the formation of mature fibrillar plaques in acute dosing. In addition, Congo red staining alone cannot distinguish between soluble and in-

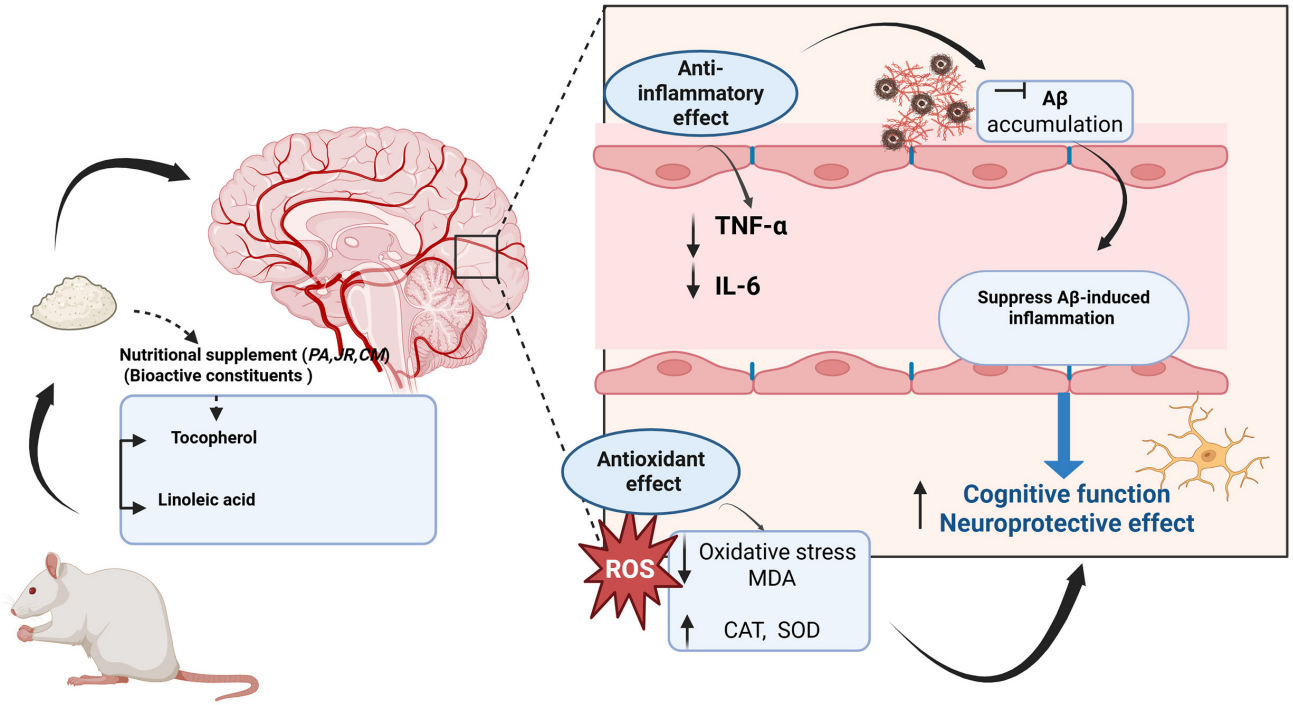


Fig. 8. Suggested neuroprotective effect of the nutritional supplement in LPS-induced cognitive dysfunction, created in https://BioRender.com.

soluble Aβ species. Therefore, future studies incorporating specific Aβ immunohistochemistry or ELISA are suggested for further characterization of observed amyloid-associated changes.

By attenuating oxidative stress and anti-inflammatory signaling, the nutritional supplement may interfere with the downregulation of molecular events involved in neuronal degeneration and amyloid genesis, thereby limiting disease progression. These findings corroborate previous data suggesting that the walnut supplement mitigates lipid peroxidation and oxidative stress in AD models [44]. Another study reported that walnuts, a component of the nutritional supplement, support neural health by reducing oxidative stress and inflammation [40]. The proposed mechanism of the anti-inflammatory and antioxidant potential of the nutritional supplement is presented in Fig. 8. These effects were associated with improved cognitive function.

The histomorphological alterations corroborated the neurobehavioral, biochemical, and molecular consequences of the study. The structural alterations are known to impair synaptic integrity and to contribute to hippocampal-dependent cognitive dysfunction. In contrast, treatment with the nutritional supplement preserved neuronal morphology and improved hippocampal organization, which closely corresponded with enhanced cognitive performance in behavioral analysis. The observed neuroprotection may be attributed to the downregulation of IL-6 and TNF-α gene expression and the attenuation of oxidative stress, thereby preserving neuronal and functional memory within neural networks. Cumulatively, these findings demonstrate that the nut-based therapy exerts neuroprotective potential by simultaneously modulating integrated pathological mechanisms underlying cognitive decline. The amelioration in learning and memory was closely related to the restoration of the antioxidant defenses, the downregulation of pro-inflammatory cytokines, the attenuation of amyloid-associated pathology, and the preservation of hippocampal neuronal structure [11]. These integrated findings emphasise the therapeutic potential of phytoconstituent-enriched nut-based nutritional supplements, which contain linoleic acid and tocopherol, as multitarget interventions capable of mitigating neuroinflammation-driven cognitive impairment and slowing the progression of cognitive dysfunction and neurodegeneration. Despite the observed neuroprotective effect, the present study has some limitations. Although Wistar rats of both sexes were included, sex-specific differences were not evaluated. Since biological sex may influence neuroinflammatory response, the observed effects of the nutritional supplement could broadly be interpreted with this consideration. Future studies are recommended to incorporate balanced male and female cohorts and perform gender-specific analysis to further validate the present findings.

Limitations of the Study

A key limitation of this study is that the LPS model induces neuroinflammation, rather than the chronic progres-

sion of neurodegenerative diseases. Therefore, our findings should be interpreted in the context of neuroinflammation-induced cognitive decline. Further studies using transgenic models of neurodegeneration are required to validate the nutritional supplement's neuroprotective potential.

5. Conclusions

These findings reveal that the nutritional supplement attenuated the LPS-induced oxidative stress, neuroinflammation, and learning and memory deficits. Due to its natural phytoconstituents, anti-inflammatory and antioxidant effects, and downregulation of proinflammatory cytokines, it could contribute to neuroprotection and the restoration of cognitive impairment. Co-administration of nutritional supplements with memantine showed a supportive treatment effect on memory restoration. Further investigations are recommended to identify the effect of this nut combination on neurogenesis and to explore other molecular pathways associated with its phytoconstituents. The nutritional supplement could represent a promising dietary intervention warranting further clinical evaluation in cognitive impairment.

Availability of Data and Materials

Data are available from the corresponding author on request.

Author Contributions

AE conceived and conducted the experimental work, collected data, and participated in study design. HK supervised the research project, contributed to the study design, data analysis, interpretation of results, and overall guidance of the research. SS co-supervised the study and provided expertise in the design and execution of the molecular investigations. AA assisted in the experimental procedures, data collection, and validation of the research findings. SA contributed to the interpretation of results, manuscript writing, critical revision, and editing of the final manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Ethical approval for animal care and handling was carried out in accordance with the NIH guidelines for the care and use of laboratory animals (8th ed., National Research Council, 2011). The study was approved by the Institutional Ethical Review Board of Jinnah University for Women, Karachi, Pakistan (Reference No. JUW/IERB/PHARMARA-006/2023). The study was carried out in accordance with institutional ethical requirements and established animal welfare principles for the care and use of laboratory animals, as described by Hubrecht and Kirkwood (2010). All animal experimental procedures were conducted in accordance with the principles of the 3Rs (Replacement, Reduction, and Refinement), and the study was reported following the ARRIVE guidelines for animal research.

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Conflicts of Interest

The authors declare no conflicts of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/IJP54494.

References

[1] Lee JW, Lee YK, Yuk DY, Choi DY, Ban SB, Oh KW, et al. Neuro-inflammation induced by lipopolysaccharide causes cognitive impairment through enhancement of beta-amyloid generation. Journal of Neuroinflammation. 2008; 5: 37. https://doi.org/10.1186/1742-2094-5-37

[2] GBD 2019 Dementia Forecasting Collaborators (2022). Estimation of the global prevalence of dementia in 2019 and forecasted prevalence in 2050: an analysis for the Global Burden of Disease Study 2019. The Lancet. Public health. 2022; 7: e105–e125. https://doi.org/10.1016/S2468-2667(21)00249-8

[3] Wu XM, Ji MH, Yin XY, Gu HW, Zhu TT, Wang RZ, et al. Reduced inhibition underlies early life LPS exposure induced-cognitive impairment: Prevention by environmental enrichment. International Immunopharmacology. 2022; 108: 108724. https://doi.org/10.1016/j.intimp.2022.108724

[4] Bahaidrah KA, Alzahrani NA, Aldhahri RS, Mansouri RA, Alghamdi BS. Effects of different lipopolysaccharide doses on short-and long-term spatial memory and hippocampus morphology in an experimental Alzheimer's disease model. Clinical and Translational Neuroscience. 2022; 6: 20. https://doi.org/10.3390/ctn6030020

[5] Bello-Medina PC, González-Franco DA, Vargas-Rodríguez I, Díaz-Cintra S. Oxidative stress, the immune response, synaptic plasticity, and cognition in transgenic models of Alzheimer disease. Neurologia. 2022; 37: 682–690. https://doi.org/10.1016/j.nrleng.2019.06.008

[6] Demirel G, Sanajou S, Yirün A, Çakır DA, Özyurt AB, Berkkan A, et al. Walnut oil: a promising nutraceutical in reducing oxidative stress and improving cholinergic activity in an *in vitro* Alzheimer's disease model. Toxicology Research. 2024; 13: tfae097. https://doi.org/10.1093/toxres/tfae097

[7] Kim JM, Lee U, Kang JY, Park SK, Shin EJ, Kim HJ, et al. Anti-Amnesic Effect of Walnut via the Regulation of BBB Function and Neuro-Inflammation in $A\beta_{1-42}$-Induced Mice. Antioxidants (Basel, Switzerland). 2020; 9: 976. https://doi.org/10.3390/antiox9100976

[8] Arangia A, Ragno A, Cordaro M, D'Amico R, Siracusa R, Fusco R, et al. Antioxidant Activity of a Sicilian Almond Skin Extract Using In Vitro and In Vivo Models. International Journal of Molecular Sciences. 2023; 24: 12115. https://doi.org/10.3390/ijms241512115

[9] Decandia D, Gelfo F, Landolfo E, Balsamo F, Petrosini L, Cutuli D. Dietary Protection against Cognitive Impairment, Neuroinflammation and Oxidative Stress in Alzheimer's Disease Animal Models of Lipopolysaccharide-Induced Inflammation. International Journal of Molecular Sciences. 2023; 24: 5921. https://doi.org/10.3390/ijms24065921

[10] Hampel H, Hardy J, Blennow K, Chen C, Perry G, Kim SH, et al. The Amyloid-β Pathway in Alzheimer's Disease. Molecular Psychiatry. 2021; 26: 5481–5503. https://doi.org/10.1038/s41380-021-01249-0

[11] Vaishnavi G, Justin Thenmozhi A. Nuts and their potential role in Alzheimer's disease. In Nutraceuticals for Alzheimer's Disease: A Promising Therapeutic Approach (pp. 125–149). Springer: Singapore. 2023.

[12] Mani V, Arfeen M, Ali HM, Abdel-Moneim AM, Alhowail A. Neuroprotective Effect of Clobenpropit in Lipopolysaccharides-induced Mice via Enhancing Cholinergic Transmission. International Journal of Pharmacology. 2022; 18: 321–330. https://doi.org/10.3923/ijp.2022.321.330

[13] Arslan J, Jamshed H, Qureshi H. Early Detection and Prevention of Alzheimer's Disease: Role of Oxidative Markers and Natural Antioxidants. Frontiers in Aging Neuroscience. 2020; 12: 231. https://doi.org/10.3389/fnagi.2020.00231

[14] Ros E, Sala-Vila A. Nuts and brain health: Cognition, depression, and neurodegenerative diseases. In Health benefits of nuts and dried fruits (pp. 261–288). CRC Press: Boca Raton. 2020.

[15] Alami K, Nazari Z, Bayat R, Bayat A, Qasemi S, Karimi F, et al. Cognitive Effects of Almond Consumption: A Review of Animal Studies. Nutrition and Dietary Supplements. 2024; 16: 105–128. https://doi.org/10.2147/NDS.S469179

[16] Yap HM, Lye KL. An insight of vitamin E as neuroprotective agents. Progress In Microbes & Molecular Biology. 2020; 3. https://doi.org/10.36877/pmmb.a0000071

[17] Gorji N, Moeini R, Memariani Z. Almond, hazelnut and walnut, three nuts for neuroprotection in Alzheimer's disease: A neuropharmacological review of their bioactive constituents. Pharmacological Research. 2018; 129: 115–127. https://doi.org/10.1016/j.phrs.2017.12.003

[18] Nishi SK, Babio N, Gómez-Martínez C, Martínez-González MÁ, Ros E, Corella D, et al. Mediterranean, DASH, and MIND Dietary Patterns and Cognitive Function: The 2-Year Longitudinal Changes in an Older Spanish Cohort. Frontiers in Aging Neuroscience. 2021; 13: 782067. https://doi.org/10.3389/fnagi.2021.782067

[19] Fumia A, Cicero N, Gitto M, Nicosia N, Alesci A. Role of nutraceuticals on neurodegenerative diseases: neuroprotective and immunomodulant activity. Natural Product Research. 2022; 36: 5916–5933. https://doi.org/10.1080/14786419.2021.2020265

[20] Bhardwaj A, Singh A, Patnaik RS, Bhardwaj S. Juglans Regia L: A Review of its Traditional Uses Phytochemistry and Therapeutic Applications. Journal of Pharmaceutical Negative Results. 2023; 14: 11–16.

[21] Fahamiya N, Aslam M, Siddiqui A, Shiffa M. Review on cucumis melo: ethnobotany and Unani medicine. World Journal of Pharmacy and Pharmaceutical Sciences. 2016; 5: 621–636.

[22] Rajaram S, Damasceno NRT, Braga RAM, Martinez R, Kris-Etherton P, Sala-Vila A. Effect of Nuts on Markers of Inflammation and Oxidative Stress: A Narrative Review. Nutrients. 2023; 15: 1099. https://doi.org/10.3390/nu15051099

[23] Tan B, Wang Y, Zhang X, Sun X. Recent Studies on Protective Effects of Walnuts against Neuroinflammation. Nutrients. 2022; 14: 4360. https://doi.org/10.3390/nu14204360

[24] Chen X, Drew J, Berney W, Lei W. Neuroprotective Natural Products for Alzheimer's Disease. Cells. 2021; 10: 1309. https://doi.org/10.3390/cells10061309

[25] Percie du Sert N, Hurst V, Ahluwalia A, Alam S, Avey MT, Baker M, et al. The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. PLoS Biology. 2020; 18: e3000410. https://doi.org/10.1371/journal.pbio.3000410

[26] Karimi SA, Salehi I, Shykhi T, Zare S, Komaki A. Effects of exposure to extremely low-frequency electromagnetic fields on spatial and passive avoidance learning and memory, anxiety-like behavior and oxidative stress in male rats. Behavioural Brain Research. 2019; 359: 630–638. https://doi.org/10.1016/j.bbr.2018.10.002

[27] Moon JH, Kim JM, Lee U, Kang JY, Kim MJ, Lee HL, et al. Walnut Prevents Cognitive Impairment by Regulating the Synaptic and Mitochondrial Dysfunction via JNK Signaling and Apoptosis Pathway in High-Fat Diet-Induced C57BL/6 Mice. Molecules (Basel, Switzerland). 2022; 27: 5316. https://doi.org/10.3390/molecules27165316

[28] Keymoradzadeh A, Hedayati CM, Abedinzade M, Khakpour TB. Enriched environment restores passive avoidance memory impairment in a rat model of neuroinflammation. Physiology and Pharmacology. 2022; 26: 127–137. http://dx.doi.org/10.52547/phypha.26.3.4

[29] Jin J, Maren S. Prefrontal-Hippocampal Interactions in Memory and Emotion. Frontiers in Systems Neuroscience. 2015; 9: 170. https://doi.org/10.3389/fnsys.2015.00170

[30] Azzam SM, Abdel Rahman AAS, Ahmed-Farid OA, Abu El-Wafa WM, Salem GEM. Lipopolysaccharide induced neuroprotective effects of bacterial protease against Alzheimer's disease in male Wistar albino rats. International Journal of Biological Macromolecules. 2023; 230: 123260. https://doi.org/10.1016/j.ijbiomac.2023.123260

[31] Galvão MA, Arruda AO, Bezerra IC, Ferreira MR, Soares LA. Evaluation of the Folin-Ciocalteu method and quantification of total tannins in stem barks and pods from Libidibia ferrea (Mart. ex Tul) LP Queiroz. Brazilian Archives of Biology and Technology. 2018; 61: e18170586. https://doi.org/10.1590/1678-4324-2018170586

[32] Barbosa Abrantes KK, Colombo Pimentel T, da Silva C, Santos Junior ODO, Barão CE, Cardozo-Filho L. Brazil Nut Semi-Defatted Flour Oil: Impact of Extraction Using Pressurized Solvents on Lipid Profile, Bioactive Compounds Composition, and Oxidative Stability. Plants (Basel, Switzerland). 2024; 13: 2678. https://doi.org/10.3390/plants13192678

[33] Nguyen TH, Vu DC, Alvarez S, Nguyen KD, Nguyen TL, Tuan NN, et al. Comparative examination of phytonutrients and antioxidant activity of commonly consumed nuts and seeds grown in Vietnam. Horticulturae. 2022; 8: 521. https://doi.org/10.3390/horticulturae8060521

[34] Masoodi L, Gani A, Sircar D, Jan K. Quality analysis of walnut oil extracted by cold press and solvent methods for in vitro phytochemicals, antioxidant activity and GCMS analysis. Measurement: Food. 2025; 18: 100223. https://doi.org/10.1016/j.meafoo.2025.100223

[35] Qamar F, Sana A, Naveed S, Faizi S. Phytochemical characterization, antioxidant activity and antihypertensive evaluation of *Ocimum basilicum* L. in l-NAME induced hypertensive rats and its correlation analysis. Heliyon. 2023; 9: e14644. https://doi.org/10.1016/j.heliyon.2023.e14644

[36] Sidorova Y, Domanskyi A. Detecting Oxidative Stress Biomarkers in Neurodegenerative Disease Models and Patients. Methods and Protocols. 2020; 3: 66. https://doi.org/10.3390/mps3040066

[37] Khalil HMA, Mahmoud DB, El-Shiekh RA, Bakr AF, Boseila AA, Mehanna S, et al. Antidepressant and Cardioprotective

Effects of Self-Nanoemulsifying Self-Nanosuspension Loaded with Hypericum perforatum on Post-Myocardial Infarction Depression in Rats. AAPS PharmSciTech. 2022; 23: 243. https://doi.org/10.1208/s12249-022-02387-6

[38] Batool Z, Agha F, Tabassum S, Batool TS, Siddiqui RA, Haider S. Prevention of cadmium-induced neurotoxicity in rats by essential nutrients present in nuts. Acta Neurobiologiae Experimentalis. 2019; 79: 169–183.

[39] Barreira JCM, Ferreira ICFR, Oliveira MBPP, Pereira JA. Antioxidant activity and bioactive compounds of ten Portuguese regional and commercial almond cultivars. Food and Chemical Toxicology : an International Journal Published for the British Industrial Biological Research Association. 2008; 46: 2230–2235. https://doi.org/10.1016/j.fct.2008.02.024

[40] Rusu ME, Georgiu C, Pop A, Mocan A, Kiss B, Vostinaru O, et al. Antioxidant Effects of Walnut (*Juglans regia* L.) Kernel and Walnut Septum Extract in a D-Galactose-Induced Aging Model and in Naturally Aged Rats. Antioxidants (Basel, Switzerland). 2020; 9: 424. https://doi.org/10.3390/antiox9050424

[41] Simpson DSA, Oliver PL. ROS Generation in Microglia: Understanding Oxidative Stress and Inflammation in Neurodegenerative Disease. Antioxidants (Basel, Switzerland). 2020; 9: 743. https://doi.org/10.3390/antiox9080743

[42] Esselun C, Dieter F, Sus N, Frank J, Eckert GP. Walnut Oil Reduces Aβ Levels and Increases Neurite Length in a Cellular Model of Early Alzheimer Disease. Nutrients. 2022; 14: 1694. https://doi.org/10.3390/nu14091694

[43] Pei Q, Liu Y, Peng S. Fatty Acid Profiling in Kernels Coupled with Chemometric Analyses as a Feasible Strategy for the Discrimination of Different Walnuts. Foods (Basel, Switzerland). 2022; 11: 500. https://doi.org/10.3390/foods11040500

[44] Pandareesh MD, Chauhan V, Chauhan A. Walnut Supplementation in the Diet Reduces Oxidative Damage and Improves Antioxidant Status in Transgenic Mouse Model of Alzheimer's Disease. Journal of Alzheimer's Disease : JAD. 2018; 64: 1295–1305. https://doi.org/10.3233/JAD-180361