

Omega-3 Fatty Acids Attenuates Scopolamine-Induced Cognitive Dysfunction, Oxidative Stress and Hippocampal Structural Alterations in Wistar Rats

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Abstract

Scopolamine is a non-selective muscarinic antagonist widely used as a pharmacological model of cholinergic dysfunction and cognitive impairment. Omega-3 fatty acids may modulate neuronal, oxidative, and inflammatory processes; however, their effects following scopolamine exposure remain incompletely characterised. This study evaluated the effects of post-scopolamine omega-3 fatty acid supplementation on metabolic, behavioural, serum biochemical, and hippocampal CA1 structural parameters in adult male Wistar rats. Sixty, adult male Wistar rats (120–150 g) were randomly assigned to six groups ($n = 10$): control, omega-3 (250 and 500 mg/kg), scopolamine, and scopolamine + omega-3 (250 and 500 mg/kg). Scopolamine (0.7 mg/kg, intraperitoneally) was administered for 7 days, followed by oral omega-3 supplementation for 21 days. Metabolic, behavioural, serum biochemical, and hippocampal histomorphometric parameters were subsequently assessed. Scopolamine significantly altered body weight, feed intake, locomotor and exploratory activity, spontaneous alternation, and serum levels of MDA, TAC, inflammatory cytokines, and acetylcholine ($p < 0.05$). Post-scopolamine omega-3 supplementation attenuated several of these alterations. Histological and quantitative analyses revealed scopolamine-associated alterations in the hippocampal CA1 region, including reduced cellular and neuronal profiles, increased vacuolation, and changes in optical density and layer thickness. These alterations were attenuated following omega-3 supplementation, with the 500 mg/kg dose generally showing greater numerical improvement across several parameters. Omega-3 supplementation attenuated several behavioural, biochemical, and hippocampal structural alterations associated with scopolamine-induced cholinergic dysfunction, supporting further investigation of its potential ameliorative effects.

KEYWORDS: Omega-3 fatty acids, Scopolamine, Cholinergic dysfunction, Oxidative Stress, Hippocampus

1. Introduction

Neurodegenerative disorders are characterised by progressive deterioration of neuronal structure and function, resulting in impairments of cognition, memory, and behaviour, with Alzheimer's disease accounting for the majority of dementia cases worldwide and its global burden continuing to rise alongside increasing life expectancy [1, 2, 3]. Current pharmacotherapies remain largely symptomatic, targeting

neurotransmitter deficits without meaningfully altering disease progression, underscoring the need for agents capable of addressing the underlying cholinergic, oxidative, and inflammatory drivers of neurodegeneration [4].

Scopolamine, a non-selective muscarinic acetylcholine receptor antagonist, is extensively used to model cognitive impairment by competitively blocking central muscarinic receptors, thereby disrupting cholinergic neurotransmission essential for learning

and memory [5]. Beyond its anticholinergic action, scopolamine increases reactive oxygen species production, elevates lipid peroxidation, depletes endogenous antioxidant defences, and upregulates pro-inflammatory cytokines such as TNF- α , IL-1 β , and IL-6 within the hippocampus, reproducing key pathological hallmarks of Alzheimer's disease [6, 7, 8]. Its reproducibility and reversibility make it a particularly suitable model for evaluating candidate neuroprotective and anti-inflammatory agents.

Omega-3 polyunsaturated fatty acids, particularly docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), are important components of neuronal membranes, where they contribute to membrane properties, receptor function, synaptic plasticity, and neurotransmission [9, 10]. Omega-3 fatty acids may also influence inflammatory regulation through modulation of inflammatory signalling and the generation of specialised pro-resolving mediators derived from EPA and DHA [11, 12, 13, 14, 15]. These biological properties provide a rationale for investigating omega-3 fatty acids in experimental models of cognitive and neural dysfunction. However, clinical evidence indicates that the cognitive effects of omega-3 supplementation are not consistently demonstrated across populations and study conditions [16]. This variability supports cautious interpretation of preclinical findings and discourages broad therapeutic claims. Omega-3 fatty acids may therefore influence neuronal and inflammatory processes, but the magnitude and context of their cognitive or ameliorative effects require further investigation.

The hippocampus, owing to its dense cholinergic innervation and high metabolic demand, is particularly relevant to studies of cholinergic dysfunction and memory [17, 18]. Cholinergic dysfunction within the hippocampal trisynaptic circuit can disrupt long-term potentiation, a cellular substrate for memory encoding and consolidation [19], contributing to deficits in spatial working memory and related behavioural functions [5, 17, 20]. Scopolamine is widely used as a pharmacological model of cholinergic-related cognitive impairment; however, it is a non-selective muscarinic antagonist with both central and peripheral actions, and its behavioural effects can vary with species, sex, dose, and task characteristics [21]. Peripheral and non-cognitive effects may also complicate interpretation of tasks involving locomotion, exploration, or other behavioural domains [21]. Thus, the scopolamine model is useful for investigating cholinergic dysfunction but should not be regarded as a complete representation of human neurodegenerative disease. Within this context, it remains important to determine whether omega-3 supplementation initiated after scopolamine exposure is associated with concurrent changes in behaviour, cholinergic status, systemic oxidative and inflammatory biomarkers, and hippocampal structural integrity.

Although omega-3 fatty acids have established membrane, antioxidant, and inflammation-resolving functions, it remains unclear whether post-insult omega-3 supplementation can

simultaneously improve cholinergic-related biochemical status, behavioural performance, and hippocampal microstructural integrity following pharmacologically induced cholinergic dysfunction. This study therefore evaluated the effects of omega-3 fatty acid supplementation initiated after scopolamine exposure on metabolic, behavioural, serum biochemical, and hippocampal CA1 structural outcomes in adult male Wistar rats.

2. Materials and Methods

2.1. Chemicals and Drugs

Scopolamine (LABORATE Pharmaceuticals India Ltd., India), omega-3 fatty acids (NOW Foods Molecularly Distilled Omega-3 Fish Oil; 1,000 mg fish oil per softgel, containing 180 mg EPA and 120 mg DHA per softgel; NOW Foods, Bloomingdale, IL, USA), normal saline, and assay kits for MDA, IL-1 β , IL-6, IL-10, TNF- α , acetylcholine, and TAC (BioVision Inc., Milpitas, CA, USA) were used in the study. Omega-3 fatty acids were administered orally at doses of 250 and 500 mg/kg, calculated based on the total fish-oil content of the preparation. The required dose was determined according to the individual body weight of each animal and administered directly by oral gavage.

2.2 Animals

Healthy adult male Wistar rats weighing 120–150 g were obtained from Empire Breeders, Osogbo, Osun State, Nigeria, and housed in wooden cages (20 × 10 × 12 inches) at room temperature (25°C ± 2.5°C) under a 12-hour light/dark cycle (lights on at 7:00 am and off at 7:00 pm), with unrestricted access to feed and water. Animals were allowed to acclimatise for two weeks before commencement of the experimental procedures. Animals were selected based on health status and male sex, and no animals were excluded from the analysis. All procedures conformed to European Council Directive 2010/63/EU.

2.3 Diet

Animals received standard rodent chow (Top Feeds®, Nigeria; 29% protein, 11% fat, 58% carbohydrate) with *ad libitum* access to feed and water.

2.4 Experimental Design

Sixty adults male Wistar rats (120–150 g) were randomly assigned to six experimental groups (n = 10 per group) using block randomisation. Allocation was performed in blocks of six, with each block containing one allocation to each experimental group. The order of allocations within each block was computer-generated, and the allocation sequence was concealed from the researcher responsible for animal allocation until assignment was made. Group A (Control) received oral normal saline (2 mL/kg). Groups B and C received omega-3 fatty acids orally at 250 mg/kg and 500 mg/kg, respectively. Groups D, E, and F received scopolamine intraperitoneally at 0.7 mg/kg for the first 7 days. Groups E and F subsequently received omega-3 fatty acids orally at 250 mg/kg and 500 mg/kg, respectively, for 21

days, commencing after completion of the 7-day scopolamine exposure. Thus, Groups E and F constituted a post-insult intervention model in which omega-3 supplementation was initiated following scopolamine exposure to evaluate its potential ameliorative effects on the resulting behavioural, biochemical, and hippocampal structural alterations. Scopolamine and omega-3 fatty acid administrations were performed between 7:00 and 8:00 am each day. Scopolamine dosing was based on previously reported protocols [22, 23], while the omega-3 doses were selected based on previous reports [24-27]

Groups	Normal Saline	Scopolamine	Omega-3 Fatty Acid
Control	YES	NO	NO
OMG 250	NO	NO	YES
OMG 500	NO	NO	YES
SCO Control	NO	YES	NO
SCO+ OMG250	NO	YES	YES
SCO + OMG500	NO	YES	YES

Table 1: Experimental groups. SCO: Scopolamine; OMG: Omega-3 fatty acids; i.p.: Intraperitoneal; P.O.: Oral by gavage.

2.5 Determination of Body Weight and Feed Intake

Body weight and feed intake were measured weekly and daily respectively using an electronic weighing scale. Relative change in body weight or feed intake was calculated as:

$$\frac{\text{Final body weight (or feed intake)} - \text{Initial body weight (or feed intake)}}{\text{Initial body weight (or feed intake)}} (g)$$

2.6 Behavioural Testing

Animals underwent behavioural testing in the following sequence: open field, Y-maze, and elevated plus maze, following completion of the administration period. Behavioural testing was conducted between 07:00 and 13:00 h, beginning 24 h after the final treatment administration. A 10-min interval was maintained between successive behavioural tests. Animals were not subjected to a separate habituation period before testing. The behavioural tests were conducted under quiet environmental conditions without deliberate additional lighting or other major environmental disturbances. The apparatus was cleaned with ethanol between animals, ensuring adequate coverage of the surfaces contacted by each animal, and allowed to dry before the subsequent trial to minimise residual olfactory cues. All behavioural scoring was performed using coded animal/cage labels assigned by a researcher not involved in scoring, and the

experimenter conducting and scoring the behavioural tests was blinded to group identity until data collection was complete.

2.6.1 Open Field Test

Ten minutes of open-field behaviour, comprising horizontal locomotion (line-crossing), rearing, and self-grooming, were monitored in a square arena (36 x 36 x 26 cm) with a floor segmented into 16 equal squares.

2.6.2 Y-maze (Spatial Working Memory)

Spontaneous alternation was assessed in a Y-shaped maze with three arms (120° apart, 41 cm long, 15 cm high). Percentage alternation was calculated as previously described [28, 29].

$$\text{Percentage alternation} = \frac{\text{Number of actual alternations}}{\text{Maximum alternation}} \times 100$$

2.6.3 Elevated Plus Maze (Anxiety-Related Behaviour)

The elevated plus maze comprised two open arms (25 x 5 x 5 cm) and two enclosed arms (25 x 5 x 5 cm, wall height 16 cm) joined by a central platform. Time spent in open and closed arms was recorded over 10 minutes [30-32].

2.7 Biochemical Assays

At the end of the experimental period, animals were deeply anaesthetised and blood was collected via intracardiac puncture; death was subsequently confirmed by cervical dislocation [33]. Serum was separated by centrifugation at 3500 rpm for 10 minutes. Brains were subsequently extracted and immersion-fixed as described in Section 2.8. Serum malondialdehyde (MDA) was assessed as an index of lipid peroxidation using the thiobarbituric acid reactive substances (TBARS) assay [34], while serum total antioxidant capacity (TAC) was determined using the ABTS-based method [28]. Serum concentrations of interleukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-10 (IL-10), tumour necrosis factor-alpha (TNF-α), and acetylcholine were determined using commercial assay kits in accordance with the manufacturers' instructions.

2.8 Tissue Processing and Histological Staining

Brains were fixed in 10% neutral buffered formalin, dehydrated through graded alcohols, cleared in xylene, infiltrated and embedded in paraffin wax, and sectioned at 5 μm [35]. Sections of the hippocampal Cornu Ammonis 1 (CA1) region were stained with Haematoxylin and Eosin (H&E) for general cytoarchitecture [36] and Cresyl Fast Violet (CFV) to demonstrate Nissl substance [37].

2.9 Histomorphometric and Computational Image Analysis

Photomicrographs were captured under standardised illumination and magnification and analysed using ImageJ. Slides were assigned coded labels by a researcher not involved in image analysis, and histomorphometric analysis was therefore performed blind to group identity. Three CA1 sections were analysed from each animal, with four non-overlapping microscopic fields selected from predefined anatomical landmarks within the CA1 region in each section. Quantitative

parameters assessed included cellular profile density (cells/mm²), mean cellular area, stained area fraction, vacuolation index, optical density, texture entropy, and RGB colour histogram distribution. Measurements obtained from the four microscopic fields within each section were aggregated, and measurements across the three sections from each animal were subsequently combined to generate a single animal-level value for each parameter. The individual animal was treated as the experimental unit ($n = 10$ per group), while multiple sections and microscopic fields obtained from the same animal were treated as subsamples rather than independent biological replicates. Image measurements were calibrated in Image J using the recorded pixel-to-micrometre conversion factor.

2.10 Statistical Analysis

Data were analysed using appropriate statistical procedures according to the structure of each outcome. For body weight and feed intake measured repeatedly across the experimental period, two-way repeated-measures analysis of variance (ANOVA) was performed to assess the effects of treatment, time (week), and the treatment $\times$ time interaction, followed by appropriate post-hoc multiple comparisons where significant effects were detected. Single-endpoint parameters were analysed using one-way ANOVA followed by Tukey's Honestly Significant Difference (HSD) test. Results are presented as mean $\pm$ standard error of the mean (SEM), and $p < 0.05$ was considered statistically significant. The experimental design comprised six groups, with 10 animals per group ($n = 10/\text{group}$). The individual animal served as the experimental unit, while repeated measurements obtained from the same animal were treated as repeated observations rather than independent biological replicates.

3. Result

3.1 Effect of Omega-3 Fatty Acid on Body Weight and Feed Intake

Body weight showed distinct temporal patterns across the experimental groups (Figure 1). Control, OMG250, OMG500, SCO+OMG250, and SCO+OMG500 groups showed progressive increases in body weight, whereas the SCO group declined from 74.0 to 68.6 g between Weeks 1 and 4. The greatest numerical increase occurred in the OMG500 group (76.0–86.8 g). Two-way repeated-measures ANOVA assessed the effects of treatment, week, and treatment $\times$ week interaction, with $p < 0.05$ considered statistically significant; significant effects were followed by appropriate post-hoc comparisons. Feed intake was similarly evaluated across the experimental period (Figure 2). Relative and weekly changes in feed intake were compared among the six experimental groups. Two-way repeated-measures ANOVA assessed the effects of treatment, week, and treatment $\times$ week interaction, with $p < 0.05$ considered statistically significant; significant effects were followed by appropriate post-hoc comparisons.

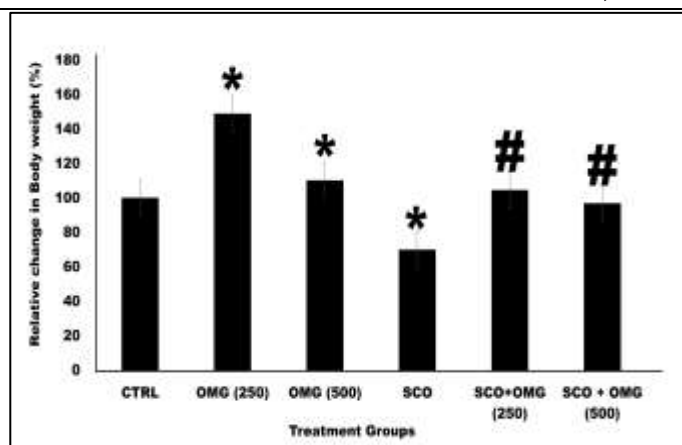


Figure 1a: Effect of omega-3 fatty acid supplementation on relative change in body weight. Data presented as mean $\pm$ SEM ($n = 10$ per group). * $p < 0.05$ vs Control; # $p < 0.05$ vs SCO. CTRL: Control, SCO: Scopolamine, OMG: Omega -3 fatty acids

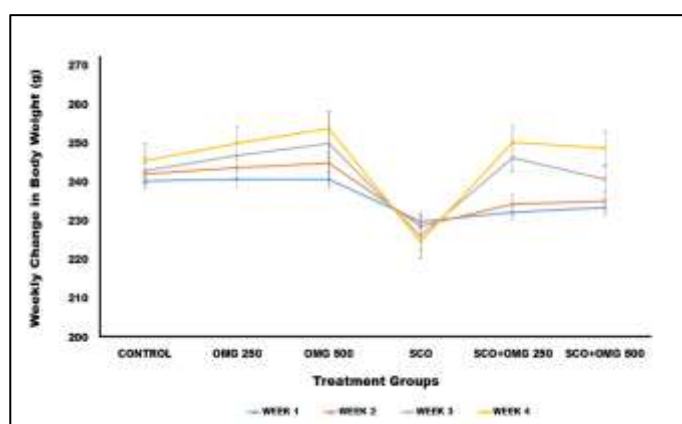


Figure 1b: Effect of omega-3 fatty acid supplementation on weekly body-weight change. Data presented as mean $\pm$ SEM ($n = 10$ per group). Body weight was assessed weekly over the experimental period. * $p < 0.05$ vs Control; # $p < 0.05$ vs SCO at the corresponding week. CTRL: Control, SCO: Scopolamine, OMG: Omega -3 fatty acids

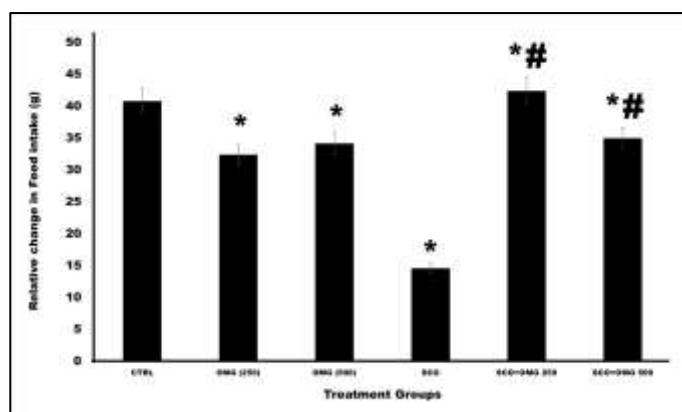


Figure 2a: Effect of omega-3 fatty acid supplementation on relative change in feed. Data are presented as mean $\pm$ SEM ($n = 10$ per group). * $p < 0.05$ vs Control; # $p < 0.05$ vs SCO. Statistical significance was set at $p < 0.05$. CTRL: Control, SCO: Scopolamine, OMG: Omega -3 fatty acids.

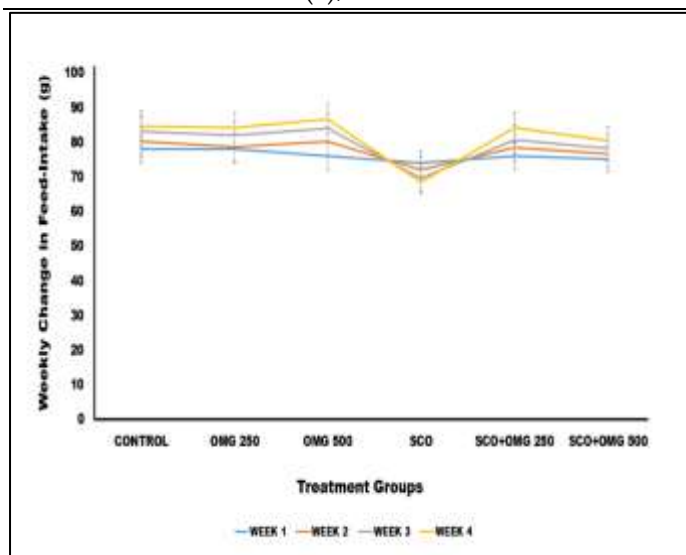


Figure 2b: Effect of omega-3 fatty acid supplementation on weekly feed intake. Data presented as mean $\pm$ SEM ($n = 10$ per group). Feed intake was assessed over the experimental period. * $p < 0.05$ vs Control; # $p < 0.05$ vs SCO. CTRL: Control, SCO: Scopolamine, OMG: Omega -3 fatty acids.

3.2 Open Field (Locomotor and Exploratory Behaviour)

Horizontal locomotion (line-crossing) decreased significantly ($p < 0.005$) in OMG250, OMG500, SCO, SCO+OMG250, and SCO+OMG500 relative to control; compared with SCO, locomotor activity increased with SCO+OMG250 and SCO+OMG500 (Figure 3). Rearing activity followed a similar pattern, decreasing significantly ($p < 0.05$) across treatment groups relative to control but improving in SCO+OMG250 and SCO+OMG500 relative to SCO (Figure 4). Self-grooming decreased significantly across treatment groups relative to control, with significant recovery in SCO+OMG250 and SCO+OMG500 relative to SCO (Figure 5).

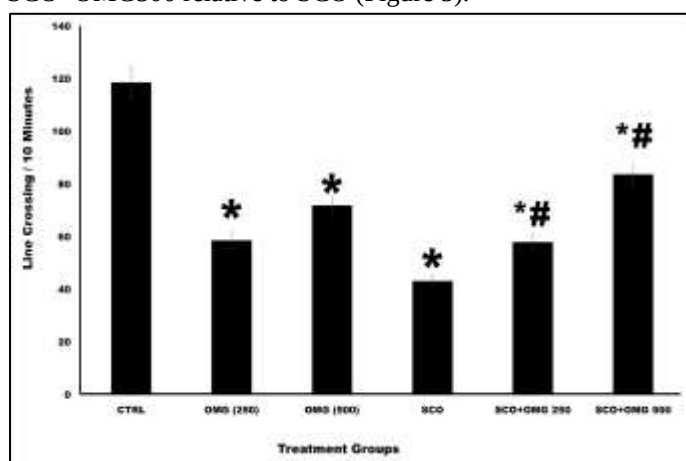


Figure 3: Effect of omega-3 fatty acid on horizontal locomotor activity (line crossing). Each bar represents Mean $\pm$ S.E.M, * $p < 0.05$ vs control, # $p < 0.05$ vs SCO. $n = 10$ per group. CTRL: Control, SCO: Scopolamine, OMG: Omega -3 fatty acids.

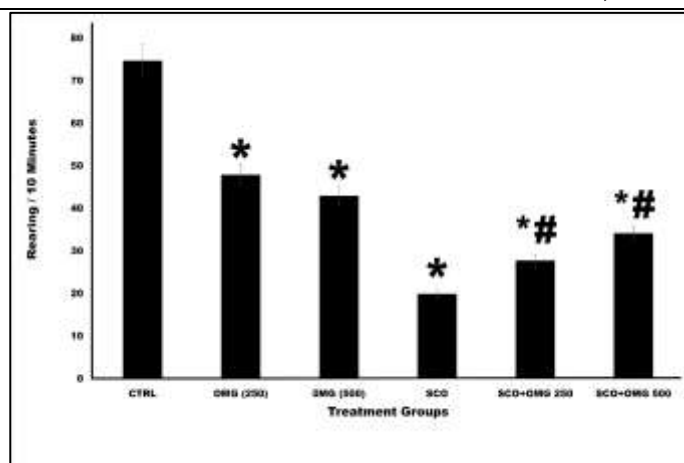


Figure 4: Effect of omega-3 fatty acid on vertical locomotor activity (rearing). Each bar represents Mean $\pm$ S.E.M, * $p < 0.05$ vs control, # $p < 0.05$ vs SCO. $n = 10$ per group. CTRL: Control, SCO: Scopolamine, OMG: Omega -3 fatty acids.

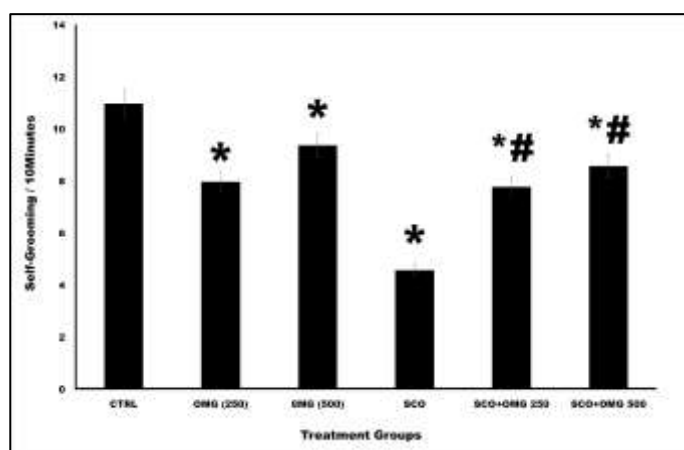


Figure 5: Effect of omega-3 fatty acid on self-grooming behaviour. Each bar represents Mean $\pm$ S.E.M, * $p < 0.05$ vs control, # $p < 0.05$ vs SCO. $n = 10$ per group. CTRL: Control, SCO: Scopolamine, OMG: Omega -3 fatty acids.

3.3 Effect of Omega-3 fatty acids on Anxiety-Related Behaviours

Open-arm time increased significantly with OMG250 and OMG500 relative to control, and decreased significantly with SCO, SCO+OMG250, and SCO+OMG500; compared with SCO, open-arm time increased with SCO+OMG250 and SCO+OMG500 (Figure 6). Closed-arm time increased significantly with SCO, SCO+OMG250, and SCO+OMG500 relative to control, and decreased significantly with OMG250 and OMG500; compared with SCO, closed-arm time decreased with SCO+OMG250 and SCO+OMG500 (Figure 7).

3.4 Effect of Omega-3 fatty acids on Spatial Working Memory (Y-Maze)

Spontaneous alternation decreased with OMG250, OMG500, and SCO, and increased with SCO+OMG250 and SCO+OMG500 relative to control; compared with SCO,

alternation increased significantly with SCO+OMG250 and SCO+OMG500 (Figure 8).

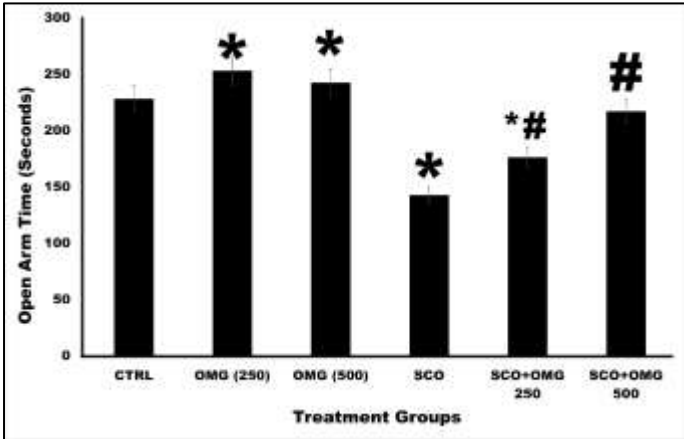


Figure 6: Effect of omega-3 fatty acid on open-arm time in the elevated plus maze. Each bar represents Mean $\pm$ S.E.M, * $p < 0.05$ vs control, # $p < 0.05$ vs SCO. n=10 per group. CTRL: Control, SCO: Scopolamine, OMG: Omega -3 fatty acids.

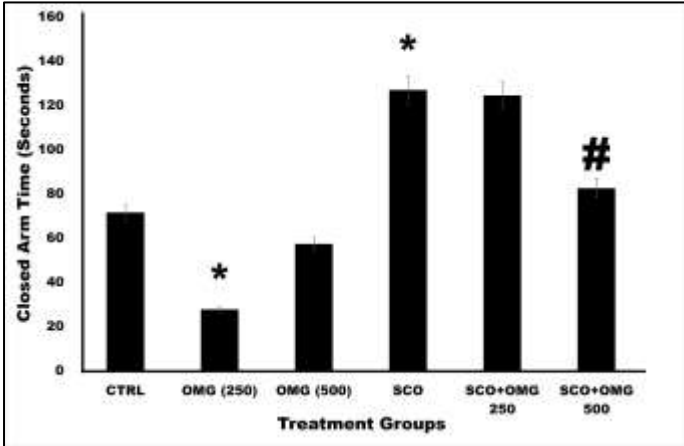


Figure 7: Effect of omega-3 fatty acid on closed-arm time in the elevated plus maze. Each bar represents Mean $\pm$ S.E.M, * $p < 0.05$ vs control, # $p < 0.05$ vs SCO. n=10 per group. CTRL: Control, SCO: Scopolamine, OMG: Omega -3 fatty acids.

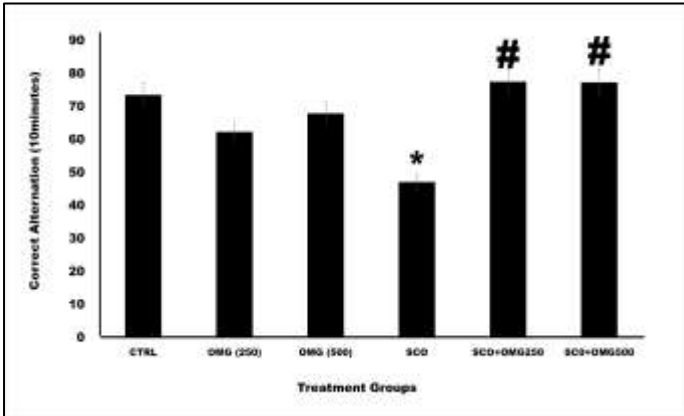


Figure 8: Effect of omega-3 fatty acid on spontaneous alternation index (Y-maze). Each bar represents Mean $\pm$ S.E.M, * $p < 0.05$ vs control, # $p < 0.05$ vs SCO. n=10 per group. CTRL: Control, SCO: Scopolamine, OMG: Omega -3 fatty acids.

3.5 Effects of Omega-3 Fatty acids on Oxidative stress markers and Acetylcholine levels

Table 2 shows the effect of omega -3 fatty acids on oxidative stress markers. Total antioxidant capacity (TAC) increased significantly with SCO+OMG250 and SCO+OMG500 compared to control. Compared with the SCO group, TAC levels increased significantly with SCO+OMG250 and SCO+OMG500. Malondialdehyde (MDA) levels decreased significantly with SCO+OMG250 and SCO+OMG500 compared to control and also compared to the SCO group. Acetylcholine levels decreased significantly with SCO and increased significantly with OMG250, OMG500, SCO+OMG250, and SCO+OMG500 compared to control. Compared with SCO, acetylcholine levels increased significantly with SCO+OMG250, and SCO+OMG500.

Table 2: Effect of omega-3 fatty acid on oxidative stress markers

Groups	TAC (mM)	MDA (µM)	Acetylcholine (mM)
CTRL	11.98 $\pm$ 1.35	0.69 $\pm$ 0.01	0.84 $\pm$ 0.03
OMG250	10.79 $\pm$ 0.03	0.68 $\pm$ 0.08	1.11 $\pm$ 0.09*
OMG500	11.75 $\pm$ 0.04	0.65 $\pm$ 0.02	1.24 $\pm$ 0.05*
SCO	8.69 $\pm$ 0.03*	0.90 $\pm$ 0.06*	0.45 $\pm$ 0.01*
SCO+OMG250	13.42 $\pm$ 0.06*#	0.73 $\pm$ 0.04*#	0.89 $\pm$ 0.03*#
SCO+OMG500	13.76 $\pm$ 0.05*#	0.70 $\pm$ 0.13*#	1.10 $\pm$ 0.02#

Each bar represents Mean $\pm$ S.E.M, * $p < 0.05$ vs control, # $p < 0.05$ vs SCO. n=10 per group. TAC: Total Antioxidant Capacity; MDA: Malondialdehyde. CTRL: Control, SCO: Scopolamine, OMG: Omega -3 fatty acids.

3.6 Effects of Omega-3 Fatty acids on Inflammatory cytokines

Table 3 shows the effect of omega-3 fatty acids on inflammatory cytokines including Tumour necrosis factor - α , interleukin 6-, 10 and 1 β . Interleukin -6 levels increased significantly with SCO and decreased with OMG250, OMG500, SCO+OMG250, and SCO+OMG500 compared to control. Compared to SCO, IL-6 decreased significantly with SCO+OMG250, and SCO+OMG500. Tumour necrosis factor - α (TNF- α) levels decreased with OMG250 and OMG500, and increased significantly with SCO, SCO+OMG250, and SCO+OMG500 compared to control. Compared to SCO, TNF- α levels decreased significantly with SCO+OMG250, and SCO+OMG500. Interleukin-1 β increased with OMG250, OMG500, and SCO compared to control, and decreased significantly with SCO+OMG250 and SCO+OMG500 compared to SCO. Interleukin-10 levels decreased with OMG250, OMG500, SCO, and SCO+OMG250, and increased with SCO+OMG500

compared to control. Compared with SCO, IL-10 increased significantly with SCO+OMG250 and SCO+OMG500.

Table 3: Effect of omega-3 fatty acid on inflammatory markers

Groups	IL-6 (pg/ml)	TNF-α (pg/ml)	IL-1β (pg/ml)	IL-10 (pg/ml)
CTRL	2.68±0.09	32.06±0.61	4.57±0.50	5.12±0.61
OMG250	2.23±0.03*	19.84±0.75*	4.90±0.19	2.81±0.39*
OMG500	2.37±0.14	31.89±0.69	4.98±0.54	0.71±0.05*
SCO	3.10±0.22	45.37±1.83*	5.04±0.02	2.06±0.32*
SCO+OMG250	2.10±0.14*#	33.02±0.15*#	3.31±0.16*#	3.45±0.44
SCO+OMG500	1.88±0.03*#	33.26±0.39*#	3.87±0.12#	6.10±0.43#

Each bar represents Mean ± S.E.M, *p<0.05 vs control, #p<0.05 vs SCO. n=10 per group. CTRL: Control, SCO: Scopolamine, OMG: Omega -3 fatty acids.

3.7 Effects of Omega-3 Fatty acids on Hippocampal Cornus ammonis 1 histoarchitecture

Plate 1(A-F) and 2(A-F) show representative photomicrographs of haematoxylin and eosin and cresyl fast violetstained sections of the cornus ammonis 1 region of the rat hippocampus respectively. The control group (1A, 2A) showed preserved hippocampal CA1 cytoarchitecture. The omega-3-only groups (B and C) also retained broadly preserved cytoarchitecture, whereas the scopolamine group (D) showed altered pyramidal-cell organisation and increased vacuolation. In the groups co-administered scopolamine and omega-3 fatty acids [SCO+OMG250(1E, 2E) and SCO+OMG500 (1F, 2F)] there was preservation of cornus ammonis histoarchitecture compared to the scopolamine alone group, with the 500 mg/kg group (1F, 2F) demonstrating significant preservation.

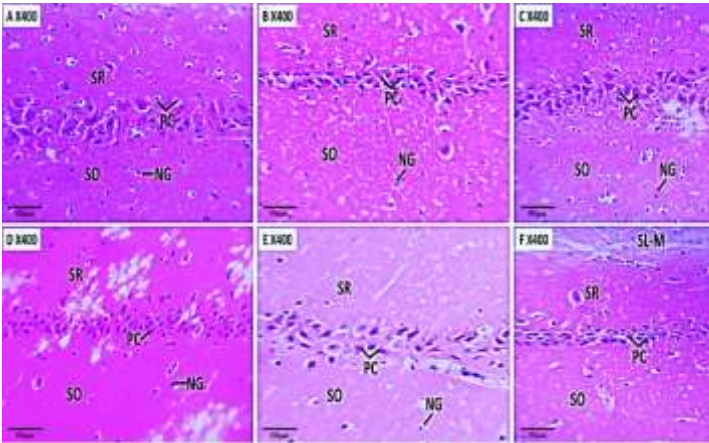


Plate 1: Representative photomicrographs of the hippocampal Cornu Ammonis 1 (CA1) region stained with H&E at ×400 magnification. A–C: relatively compact and organised pyramidal cell layers; D: reduced apparent pyramidal-cell density with cellular disorganisation and fragmentation; E–F: comparatively improved cellular organisation and preservation relative to D, more evident in F.

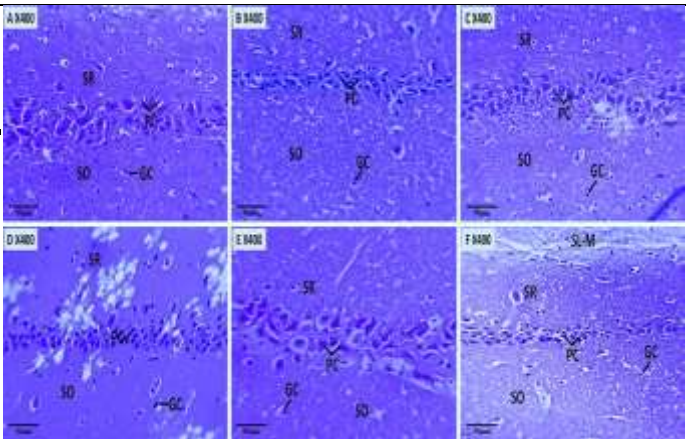


Plate 2: Representative photomicrographs of the hippocampal Cornu Ammonis 1 (CA1) region stained with Cresyl Fast Violet at ×400 magnification. A–C: relatively densely arranged pyramidal neurons with visible Nissl substance; D: altered cellular organisation and Nissl staining pattern; E–F: comparatively improved cellular organisation and preservation relative to D, more evident in F.

3.8 Quantitative Histomorphometric Analysis (H&E-Stained Sections)

Quantitative histomorphometric analysis (Table 4) demonstrated differences in hippocampal CA1 morphology among the experimental groups. The control group exhibited a cellular profile density of 267.0 ± 13.25 cells/mm², mean cellular area of 6321.01 ± 220.24 µm², stained area of 31.49 ± 8.32%, vacuolation of 4.88 ± 0.30%, optical density of 0.291 ± 0.01, and layer thickness of 35.56 ± 1.00 µm. The omega-3-only groups (B and C) showed broadly preserved histomorphometric features, with Group C (OMG500) recording the highest cellular profile density (290.0 ± 13.15 cells/mm²). The scopolamine-only group (D) recorded a lower cellular profile density (145.0 ± 14.12 cells/mm²) and mean cellular area (4916.90 ± 215.32 µm²) compared with the control group, together with a lower stained area (8.71 ± 1.21%), optical density (0.184 ± 0.01) and layer thickness (24.11 ± 1.10 µm), and higher vacuolation (12.74 ± 0.91%). Following post-scopolamine omega-3 administration, Group E (SCO+OMG250) showed a cellular profile density of 196.0 ± 12.12 cells/mm², while Group F (SCO+OMG500) recorded 233.0 ± 12.32 cells/mm². Group F also showed a stained area of 24.81 ± 3.55%, vacuolation of 6.82 ± 0.74%, optical density of 0.262 ± 0.01, and layer thickness of 31.78 ± 1.10 µm. Overall, the combination groups showed numerical changes in several histomorphometric parameters relative to the scopolamine-only group, with the 500 mg/kg group generally showing greater numerical changes than the 250 mg/kg group.

3.9 Comparative RGB Colour Histogram Analysis (H&E)

Comparative RGB colour histogram analysis (Figure 9) showed differences in the distribution of red, green, and blue colour intensities among the experimental groups. The control and omega-3-only groups displayed broadly similar colour-distribution patterns, whereas the scopolamine-only group

showed a visibly different distribution. The SCO+OMG250 and SCO+OMG500 groups displayed distributions that were visually closer to the control pattern than the scopolamine-only group. Because the histogram-derived distributions were used as an exploratory image-analysis measure and were not subjected to independent inferential statistical testing, these findings are presented as supportive image-analysis observations rather than statistically validated treatment effects.

Table 4: Quantitative histomorphometric analysis of hippocampal CA1 (H&E).

Group	Cellular Profiles (cells/m m²)	Mean Cellular Area (µm²)	Stained Area (%)	V(%)	Optical Density	Layer Thickness (µm)
Control	267.0±13.25	6321.01 ± 220.24	31.49±8.32	4.88±0.30	0.291±0.01	35.56±1.00
OMG250	248.0±12.22	5986.73 ± 210.22	26.84±2.15	5.63±0.62	0.278±0.01	34.12±0.95
OMG500	290.0±13.15	6151.04 ± 205.22	29.28±2.22	4.95 ± 0.51	0.287±0.01	35.01±1.02
SCO	145.0±14.12*	4916.90 ± 215.32	8.71±1.21*	12.71±0.91*	0.184±0.01*	24.11±1.10*
SCO + OMG250	196.0±12.12*#	5429.55 ± 212.54	17.13±2.22*#	8.64±0.82*#	0.228±0.01*#	28.43±0.92*#
SCO + OMG500	233.0±12.32*#	5875.09 ± 202.67	24.81±3.55*#	6.82±0.74*#	0.262±0.01*#	31.78±1.10*#

Each bar represents Mean ± S.E.M, *p<0.05 vs control, #p<0.05 vs SCO. n=10 per group. CTRL: Control, SCO: Scopolamine, OMG: Omega -3 fatty acids, V: Vacuolation

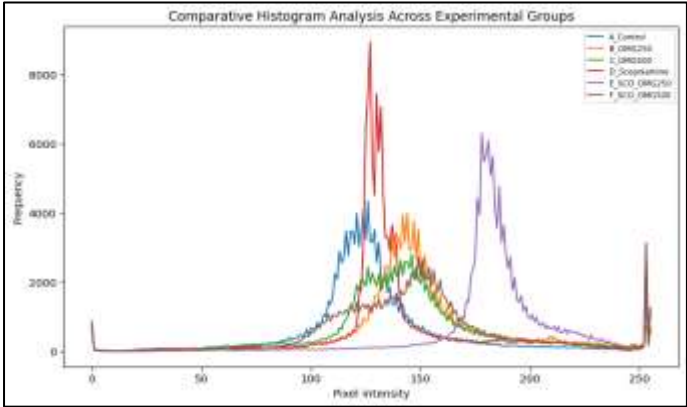


Figure 9: Comparative RGB colour histogram analysis of H&E-stained hippocampal sections across the experimental groups.

3.10 Quantitative Histomorphometric Analysis (Cresyl Fast Violet)

Quantitative histomorphometric analysis of Cresyl Fast Violet-stained hippocampal CA1 sections (Table 5) demonstrated differences among the experimental groups in neuronal count, vacuolation, optical density, layer thickness, texture entropy, and Nissl positivity. The control group (A) recorded a neuronal

count of 64.0 ± 1.11, vacuolation of 3.33 ± 0.01%, optical density of 0.35 ± 0.01, layer thickness of 34.44 ± 0.11 µm, texture entropy of 4.77 ± 0.01, and Nissl positivity of 97.86 ± 0.20%. The OMG250 group (B) recorded a neuronal count of 61.0 ± 1.08, while OMG500 (C) recorded the highest neuronal count (68.0 ± 1.15). Nissl positivity was 98.20 ± 0.18% in B and 98.04 ± 0.19% in C. The scopolamine-only group (D) recorded a neuronal count of 48.0 ± 1.02, vacuolation of 5.86 ± 0.04%, optical density of 0.32 ± 0.01, layer thickness of 33.89 ± 0.10 µm, texture entropy of 4.49 ± 0.01, and Nissl positivity of 96.53 ± 0.19%. Relative to the control group, the scopolamine group therefore showed a numerically lower neuronal count, optical density, layer thickness, texture entropy, and Nissl positivity, together with higher vacuolation. Following post-scopolamine omega-3 administration, Group E (SCO+OMG250) recorded a neuronal count of 56.0 ± 1.04, vacuolation of 4.18 ± 0.03%, optical density of 0.33 ± 0.01, layer thickness of 34.08 ± 0.09 µm, texture entropy of 4.61 ± 0.01, and Nissl positivity of 97.15 ± 0.20%. Group F (SCO+OMG500) recorded a neuronal count of 60.0 ± 1.06, vacuolation of 3.58 ± 0.02%, optical density of 0.34 ± 0.01, layer thickness of 34.22 ± 0.10 µm, texture entropy of 4.73 ± 0.01, and Nissl positivity of 97.74 ± 0.19%. Relative to the scopolamine-only group, both omega-3-treated groups showed numerically higher neuronal counts, optical density, layer thickness, texture entropy, and Nissl positivity, together with lower vacuolation. These numerical changes were generally more pronounced in the SCO+OMG500 group.

Table 5: Quantitative histomorphometric analysis of hippocampal CA1 (Cresyl Fast Violet).

Group	Neuro n Count	V (%)	Optical Density	Layer Thickness (µm)	Texture Entropy	Nissl Positive (%)
Control	64.0±1.11	3.33±0.01	0.35±0.01	34.44±0.11	4.77±0.01	97.86±0.20
OMG250	61.0±1.08	2.85±0.01	0.35±0.01	33.52±0.10	4.72±0.01	98.20±0.18
OMG500	68.0±1.15	3.21±0.02	0.36±0.01	34.01±0.10	4.81±0.01	98.04±0.19
SCO	48.0±1.02*	5.86±0.04*	0.32±0.01	33.89±0.10	4.49±0.01	96.53±0.19
SCO+OMG250	56.0±1.04*#	4.18±0.03*#	0.33±0.01	34.08±0.09	4.61±0.01	97.15±0.20
SCO+OMG500	60.0±1.06*#	3.58±0.02*#	0.34±0.01	34.22±0.10	4.73±0.01	97.74±0.19

Each bar represents Mean ± S.E.M, *p<0.05 vs control, #p<0.05 vs SCO. n=10 per group. CTRL: Control, SCO: Scopolamine, OMG: Omega -3 fatty acids, V: Vacuolation

3.11 Comparative Colour Histogram Analysis of Cresyl Fast Violet Sections

Comparative colour histogram analysis (Figure 10) demonstrated differences in staining distributions among the experimental groups. The control and omega-3-only groups displayed broadly similar staining patterns, whereas the scopolamine-only group showed a visibly different distribution. The SCO+OMG250 and SCO+OMG500 groups displayed distributions that were visually closer to the control pattern. As with the H&E colour histogram analysis, these findings were exploratory and were not subjected to independent inferential statistical testing; therefore, they are presented as supportive image-analysis observations rather than statistically validated treatment effects.

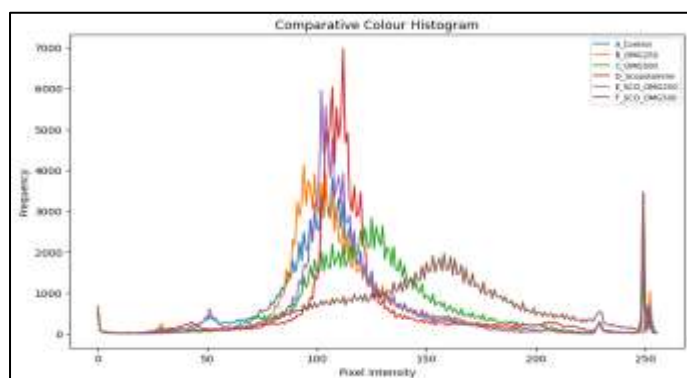


Figure 10: Comparative colour histogram analysis of Cresyl Fast Violet-stained hippocampal sections across the experimental groups.

Discussion

This study evaluated the ability of omega-3 fatty acid supplementation commenced following a prior 7-day scopolamine administration to ameliorate behavioural, biochemical, and hippocampal structural alterations in adult male Wistar rats. The post-insult design is an important feature of the study, as omega-3 supplementation was commenced after completion of the scopolamine exposure period. Overall, the findings indicate that subsequent omega-3 administration was associated with attenuation of several scopolamine-associated alterations across behavioural, biochemical, and hippocampal histomorphometric outcomes.

Scopolamine administration was associated with reductions in body weight and feed intake, suggesting an alteration in general physiological status during the experimental period. Cholinergic signalling is involved in feeding-related behaviour and physiological regulation [21]. The subsequent improvement in body weight and feed intake following omega-3 supplementation may be related to its effects on membrane composition, cellular signalling, oxidative balance, and inflammatory regulation [15]. These findings suggest that omega-3 supplementation may have supported recovery of physiological function following scopolamine exposure.

The behavioural findings demonstrated alterations in locomotor activity, exploratory behaviour, grooming, anxiety-related behaviour, and spatial working memory following scopolamine exposure. These findings were consistent with the established effects of scopolamine on cholinergic neurotransmission and its use in experimental models of cognitive dysfunction [38]. Reduced locomotor and exploratory activity in the Open Field Test, together with alterations in grooming behaviour, may reflect disruption of central cholinergic signalling and associated neural processes [39]. Oxidative stress and inflammatory responses may further contribute to alterations in neuronal signalling and behavioural performance [40, 41]. Post-scopolamine omega-3 supplementation attenuated several of these behavioural alterations, consistent with the antioxidant, membrane-stabilising, and neurofunctional properties attributed to omega-3 fatty acids [15]. Notably, the omega-3-only groups (B and C) did not behave identically to control on several open-field measures, showing lower locomotor, rearing, and grooming scores despite receiving no scopolamine. This observation warrants explicit consideration rather than being set aside. High-dose fish-oil supplementation in otherwise healthy rodents has occasionally been associated with mild sedative-like or anxiolytic-like shifts in spontaneous activity, potentially reflecting membrane-level or neurotransmitter-modulating effects of EPA and DHA that are not specific to a pathological state. An alternative, non-mutually-exclusive possibility is that the daily gavage procedure itself, independent of scopolamine, contributed a mild stress-related suppression of exploratory behaviour common to all gavaged groups. Because the present study did not include a vehicle-gavage-only comparison distinct from the saline control, these explanations cannot be distinguished with the current data, and this pattern should be interpreted as a caveat on the assumption that omega-3 alone is behaviourally neutral, rather than as evidence against its ameliorative effect in the scopolamine-exposed groups.

In the Elevated Plus Maze, scopolamine exposure altered anxiety-related behaviour, reflected by changes in open- and closed-arm exploration. Such behavioural changes are consistent with the effects of cholinergic disruption on neural circuits involved in emotional and exploratory behaviour [21]. Omega-3 supplementation attenuated several of the observed alterations, suggesting modulation of behavioural responses following scopolamine exposure. Similarly, improvement in spontaneous alternation in the Y-maze following post-scopolamine omega-3 treatment indicates improved spatial working-memory performance. Taken together, the behavioural findings suggest that omega-3 supplementation following scopolamine exposure was associated with improvement across several domains of neurobehavioural function. Because locomotor activity was also substantially altered by scopolamine and by omega-3 treatment, this raises an interpretive caveat for

the Y-maze and elevated plus maze findings. Spontaneous alternation in the Y-maze depends on the animal generating a sufficient number of arm entries, and reduced locomotor drive could in principle lower the total number of entries and alternations independently of any change in spatial working memory; likewise, elevated-plus-maze arm-time measures are influenced by general exploratory drive as well as anxiety-related behaviour. The present study cannot fully dissociate these possibilities, as arm-entry counts were used to calculate the alternation percentage rather than analysed as an independent locomotor covariate. Consequently, the Y-maze and elevated-plus-maze findings should be interpreted as reflecting a combination of cognitive, anxiety-related, and locomotor influences rather than spatial working memory or anxiety in isolation.

The oxidative stress findings are broadly consistent with evidence that omega-3 fatty acids influence lipid peroxidation and antioxidant processes [15, 42]. Scopolamine exposure was associated with increased serum MDA, indicating enhanced lipid peroxidation, whereas omega-3 supplementation reduced MDA in the scopolamine-exposed groups. This pattern suggests attenuation of systemic lipid peroxidation following omega-3 administration. The TAC findings showed a less uniform pattern across the experimental groups, indicating that the effects of omega-3 supplementation on systemic antioxidant capacity were not consistent across all treatment conditions. The inflammatory findings further demonstrated changes in systemic cytokine status following scopolamine exposure and omega-3 administration. Changes in serum IL-1 β , IL-6, TNF- α , and IL-10 following omega-3 supplementation are consistent with the recognised ability of omega-3 fatty acids to influence inflammatory signalling and resolution processes [14, 15, 42]. EPA- and DHA-derived mediators may contribute to regulation of inflammatory responses, although these specific mediators were not assessed in the present study. The pattern observed for IL-10, an anti-inflammatory cytokine, is of particular interest: rather than a uniform decline across all groups, IL-10 was numerically higher in the SCO+OMG500 group than in control, alongside reductions in several pro-inflammatory markers in the same group. This combination is consistent with an active resolution-oriented shift in cytokine balance, rather than a simple, non-specific suppression of the inflammatory response, although this interpretation remains speculative given that only systemic, serum-level cytokine concentrations were measured and the causal cellular sources of IL-10 were not examined. Importantly, the cytokines measured in this study were obtained from serum; therefore, these findings represent systemic inflammatory changes. The observed reductions in serum pro-inflammatory cytokines should consequently be considered alongside, rather than interpreted as direct measurements of, the independent hippocampal histological findings. Serum

acetylcholine concentrations were also altered across the experimental groups. The increase observed following omega-3 supplementation may be associated with modulation of cholinergic-related biochemical status, consistent with the recognised influence of omega-3 fatty acids on neuronal membrane properties and neurotransmission [15, 38]. Because acetylcholine was measured in serum, the finding reflects systemic acetylcholine concentrations and supports an association between omega-3 administration and altered cholinergic-related biochemical status.

The histological findings provided tissue-level evidence of hippocampal CA1 alterations following scopolamine exposure. H&E staining demonstrated changes in cellular organisation, cellular profile density, vacuolation, stained area, optical density, and pyramidal layer thickness in the scopolamine group. These alterations were attenuated following post-scopolamine omega-3 administration, with the 500 mg/kg group generally showing greater numerical preservation across several histomorphometric parameters. The Cresyl Fast Violet findings showed a similar pattern, with scopolamine-associated reductions in neuronal profiles and Nissl-related measures accompanied by increased vacuolation, while subsequent omega-3 administration was associated with improved neuronal preservation and Nissl-related characteristics. These findings provide morphological evidence supporting an association between post-scopolamine omega-3 administration and attenuation of hippocampal CA1 structural alterations. Specifically, the reductions in cellular and neuronal profile density and the increased vacuolation observed in the scopolamine-only group are consistent with a comparable pattern of scopolamine-associated hippocampal structural alteration reported using similar histological and histomorphometric methods in a related model of plant-extract neuroprotection [43]; that report is cited here only in relation to the structural pattern produced by scopolamine, not as evidence for the omega-3 findings themselves. The preservation observed following omega-3 administration may be related to the ability of EPA and DHA to influence neuronal membrane integrity, oxidative balance, inflammatory signalling, and cellular function [14, 15, 42]. The greater numerical preservation generally observed with 500 mg/kg compared with 250 mg/kg suggests a dose-related pattern within the doses examined. However, the findings are better described as a dose-related pattern rather than a definitive dose-response relationship. Comparative RGB colour-histogram analysis provided additional observations of differences in staining distributions among the experimental groups. The control and omega-3-treated sections showed broadly similar staining distributions, whereas scopolamine produced observable changes. The combination groups displayed distributions that were visually closer to the control pattern. These observations complement the

quantitative histomorphometric findings by providing additional image-based characterisation of the tissue staining patterns.

The relationship between the biochemical and histological findings is also noteworthy. The serum biochemical results indicate systemic changes in oxidative, inflammatory, and cholinergic-related status, whereas the histological and histomorphometric findings demonstrate structural changes within the hippocampal CA1 region. The parallel improvement observed across these domains following post-scopolamine omega-3 administration suggests that systemic biochemical changes occurred alongside improved hippocampal structural characteristics. This integrated pattern supports the potential involvement of oxidative, inflammatory, and membrane-related processes in the observed effects of omega-3 supplementation. The post-insult nature of the intervention further strengthens the relevance of the findings. Omega-3 administration was initiated after the 7-day scopolamine exposure, and improvement was subsequently observed across several behavioural, biochemical, and hippocampal structural outcomes. Thus, the findings support the potential of omega-3 supplementation as an intervention following scopolamine-associated alterations rather than solely as a preventive treatment. The generally greater numerical changes observed with 500 mg/kg compared with 250 mg/kg further suggest that the higher dose may provide greater attenuation of several scopolamine-associated alterations under the present experimental conditions.

Collectively, the findings indicate that post-scopolamine omega-3 supplementation was associated with improvement across several behavioural, systemic biochemical, and hippocampal structural outcomes. The findings from the serum biomarkers and hippocampal histology complement one another, with the former demonstrating systemic biochemical changes and the latter providing direct evidence of altered hippocampal CA1 morphology. Overall, the study supports an ameliorative effect of post-scopolamine omega-3 supplementation on selected neurobehavioural, systemic biochemical, and hippocampal structural alterations in adult male Wistar rats.

Conclusion

This study demonstrates that scopolamine exposure was associated with alterations in behavioural, systemic biochemical, and hippocampal CA1 structural parameters in adult male Wistar rats. Omega-3 fatty acid supplementation initiated after the 7-day scopolamine exposure was associated with attenuation of several of these alterations, including selected behavioural outcomes, serum oxidative and inflammatory biomarkers, serum acetylcholine, and hippocampal histomorphometric parameters. The biochemical findings represent systemic responses, while the H&E and Cresyl Fast Violet findings provide tissue-level evidence of changes within the hippocampal CA1 region. The 500 mg/kg omega-3 regimen generally produced greater numerical changes than the 250 mg/kg regimen across several

outcomes, indicating a dose-related pattern within the experimental conditions.

Overall, the findings support an ameliorative effect of post-scopolamine omega-3 fatty acid supplementation on selected behavioural, systemic biochemical, and hippocampal structural alterations in adult male Wistar rats. The study therefore provides evidence supporting further investigation of omega-3 fatty acids as a post-insult intervention in scopolamine-associated neurobehavioural dysfunction.

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Availability of data and materials

Data generated during and analysed during the course of this study are available from the corresponding author on request.

Competing interests

All authors of this paper declare that there is no conflict of interest related to the content of this manuscript.

Declarations Ethics approval

Ethical approval was obtained from the Research Ethical Committee of the Faculty of Basic Medical Sciences, Ladoke Akintola University of Technology, Ogbomoso (ERCFBMSLAUTECH:140/1/2026).

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