

Neuroprotection by Vinpocetine and Omega-3 Fatty Acids in Haloperidol-Induced Cognitive Dysfunction: Neurobehavioral, Biochemical, and Histological Evidence

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ABSTRACT

Haloperidol, an antipsychotic drug, is a first-line agent but has cognitive impairment and neurotoxicity and is not a long-term agent. The aim of this research was to evaluate the synergistic effects of ω -3 FA and vinpocetine on spatial working memory, oxidative stress, and neuroinflammation in a haloperidol-induced rat model alone and in their combination. Twenty-five (25) adult male Wistar rats (200-250 g) were acclimatized for two weeks, after which they were randomly divided into five groups (n=5). Treatments were administered in 21 days: Group 1 (control) was given distilled water; Group 2 was given haloperidol (2 mg/kg i.p.); Group 3 was given haloperidol + vinpocetine (10 mg/kg p.o.); Group 4 was given haloperidol + ω -3 FA (300 mg/kg p.o.). A T-maze and classic labyrinth test were used to test spatial memory. Brains were collected to undergo biochemical (SOD, CAT, GPx, MDA, IL-6, TNF-2, and IL-1 β) and histopathological (H&E and GFAP) analysis. Haloperidol had a significant negative effect on spatial memory, enhanced oxidative stress levels (high MDA, low antioxidants), and stimulated neuroinflammation (increased pro-inflammatory cytokines and GFAP immunoreactivity). Vinpocetine or ω -3 FA monotherapies were also effective in reducing these deficits. It was, however, found that the combination therapy was more effective, as it almost restored memory performance, oxidative balance, and histological architecture in the hippocampus and the prefrontal cortex. The combination of omega-3 fatty acids and vinpocetine has a strong, synergistic neuroprotective effect on cognitive impairment by haloperidol, probably via a combination of antioxidant and anti-inflammatory effects.

Keywords: Haloperidol, Vinpocetine, Omega-3 fatty acids, Catalepsy, Working memory, Neuroprotection.

ARTICLE HISTORY

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1. INTRODUCTION

Cognitive issues are common with neurological disorders, particularly memory problems (Shallice, 1981; Harsh et al., 2025). These problems can also result from drugs such as haloperidol, a popular antipsychotic used to treat schizophrenia and other disorders (Dold et al., 2015). However, long-term use of haloperidol can result in extrapyramidal side effects and even neurotoxicity, which manifest as motor impairments and cognitive issues such as memory loss and cataplexy (Nasrallah and Chen, 2017; Saeedi et al., 2006). Narcolepsy research revolves around the study of cataplexy, which is defined as a sudden episode of muscle weakness. Researchers regularly employ haloperidol to simulate cataleptic states in animals for research purposes (Swick, 2015; Waku et al., 2021). Such models enable researchers to understand the disorder's essential neurological mechanisms. Working memory, which is necessary for executive tasks, is vulnerable to chemical imbalances in the brain (Ahluquist, 2023). The haloperidol-induced catalepsy paradigm in rats is a dependable tool for detecting memory deficits and establishing the efficacy of therapeutic interventions.

Omega-3 fatty acids, particularly EPA and DHA, are essential polyunsaturated fats known to protect the brain, reduce inflammation, and improve cognitive function (Atasie et al., 2022). Vinpocetine, derived from vincamine, has been shown to improve brain metabolism and neuroplasticity (Dubey et al., 2020; Bee et al., 2024). Combining these two therapies may provide a formidable shield, preventing or even correcting the memory problems that haloperidol might produce.

Given the rising prevalence of neurological illnesses and the limitations of current therapeutic methods, there is a need to investigate alternative treatments. The deleterious impact of haloperidol on cognitive function undermines its status as a primary antipsychotic medicine, resulting in poor medication adherence and a lower quality of life.

Omega-3 and vinpocetine might be useful in mitigating these

undesirable issues. The purpose of this study is to investigate the effect of omega-3 fatty acids and vinpocetine on spatial working memory in haloperidol-induced cataplexy in male Wistar rats.

2. MATERIALS AND METHODS**2.1 Chemicals and Reagents**

Haloperidol was purchased at Sigma-Aldrich, USA; the omega-3 fatty acid supplement (commercial-grade capsule, standardized for EPA and DHA) and vinpocetine (procured from a licensed pharmaceutical vendor). All reagents were of analytical grade and prepared freshly before use.

2.2 Samples

25 adult male Wistar rats (200-250 g) were obtained from the Animal House, Department of Physiology, [AE-FUNAI]. Animals were housed in plastic cages (5 rats per cage), maintained under standard laboratory conditions (12-hour light/dark cycle, temperature 22-25 °C, relative humidity 50-60 %). Animals had free access to food (rat chow) and water *ad libitum*. Animals acclimatized for two weeks under stable laboratory conditions before the commencement of treatment.

2.3 Ethical clearance

All protocols adhered strictly to the ethical guidelines for animal experimentation, and the Faculty of Basic Medical Sciences Research Ethics Committee at AE-FUNAI granted approval, referencing AE-FUNAI-2024/00450.

2.4 Research Design

A randomized controlled experimental design was adopted. The animals were divided into six groups (n = 5 per group). Cataplexy-like symptoms were induced with haloperidol (2 mg/kg i.p.), administered once daily for 21 days. This dosage was selected based on previous study (Nair et al., 2019).

Omega-3 fatty acids (300 mg/kg) and vinpocetine (10 mg/kg) were administered once daily for 21 days via oral gavage. Drugs were given 30 minutes before the haloperidol injection to ensure systemic absorption. The experimental grouping is as follows:

Group 1 (Control): 1 mL of distilled water

Group 2: administered with 2 mg/kg haloperidol only for 21 days.

Group 3: given 2 mg/kg of haloperidol plus 10 mg/kg of vinpocetine for 21 days.

Group 4: Administered with 2 mg/kg haloperidol plus 300 mg/kg omega-3 fatty acid for 21 days.

Group 5: administered with 2 mg/kg haloperidol plus 100 mg/kg of vinpocetine plus 300 mg/kg of omega-3 fatty acid for 21 days.

2.5 Spontaneous alternation assessment using a T-maze

This study took place on day 36 of the experiment. It is a model in behavioral research used for the evaluation of spatial working memory in rodents. It is made up of two goal arms and a base. It consists of the acquisition and testing phase (Etti, 2024). The rats were exposed to two trials (two sample runs per trial) per day for two days with a 10-minute interval (acquisition phase). This was to establish a baseline behavior and ensure they freely explore the T-maze. Furthermore, food was left at both arms of the maze. In the training trial, rats were introduced to the T-maze with one arm blocked. During the subsequent test trial, access to both arms was granted. An animal was considered to have exhibited functional working memory if it spontaneously entered the novel arm that was closed during habituation.

The absence of spontaneous alternation was noted if the animal re-entered the previously visited arm. The apparatus was sanitized with 80 % ethanol between trials to remove scent trails. Trials were conducted with a 10-second inter-trial interval, and each animal underwent one unique trial daily over five consecutive days. Data collected included the duration and frequency of entries into each arm.

2.6 Classic labyrinth test

On the 38th day of the study, spatial navigation was evaluated using the Classic Labyrinth Test (CLT) as previously described (Inwang *et al.*, 2023). This testing took place 2 days after the T-maze and was conducted in a square labyrinth featuring defined start and goal areas.

Behavioral testing occurred in a room illuminated by a dim white light. Prior to formal testing, animals underwent a 10-minute habituation session in the maze. The protocol consisted of five trials per animal; rewards were provided at the terminal point during the first four training trials to encourage learning. The fifth and final test trial was conducted without a reward. The primary measure was the latency, recorded as the time taken to travel from the start box to the goal.

2.7 Animal sacrifice

On experimental day 39, rats were anesthetized using isoflurane (1.5-3%) and perfused with 0.01 mmol/l phosphate-

buffered saline, adhering to the protocol of Burkhardt *et al.* (2012). The brain was then extracted, and the hippocampus and prefrontal cortex were rapidly dissected. A portion of the tissue was homogenized and centrifuged (4000 rpm, 10 min, 4°C), and the supernatant was aliquoted and stored at -80°C for analyzing antioxidants, oxidative stress, and pro-inflammatory markers. The corresponding tissues from the opposite hemisphere were placed in 4% paraformaldehyde for histopathological processing.

2.8 Biochemical assays

2.8.1 Assay of antioxidant activities

Catalase activity was quantified using the established method of Aebi (1984). The reaction mixture, prepared in a cuvette, consisted of 1.9 ml of 50 mM phosphate buffer (pH 7.0) and 0.1 ml of tissue homogenate. The reaction was started by the addition of freshly prepared 30 % H₂O₂. The change in absorbance at 240 nm was recorded spectrophotometrically to calculate the rate of H₂O₂ oxidation. To measure the activity of glutathione peroxidase (GPx) as well as the oxidation of protein, brain tissue samples were homogenized in a sucrose buffer (15 mM Tris/HCl, pH 7.4, 250 mM sucrose, 1 mM EDTA, and 1 mM DTT) in an ice bath, and the resulting supernatant was used for biochemical assays (Nogales *et al.*, 2017).

For the evaluation of superoxide dismutase (SOD) activity, the brain was quickly removed and either directly frozen by immersion in 2-methylbutane cooled in dry ice or first homogenized on ice for 2-3 min (tissue standards) and then transferred into small tubes and frozen in dry ice. Frozen brains and brain pastes were then stored at -80°C until sectioning with the cryostat. Cryostat sections (50 µm thick) were cut at -20°C and mounted on precleaned glass slides (single frosted; Sigma, Milan, Italy) (Viggiano *et al.*, 2003). According to Ohkawa *et al.* (1979), the thiobarbituric acid reactive substances (TBARS) method was used to evaluate the amount of malondialdehyde (MDA), an indicator of lipid peroxidation.

The tissue homogenate was combined with an aqueous solution of thiobarbituric acid, acetate buffer (pH 3.5), and sodium dodecyl sulfate. After heating the combination for 60 minutes at 95°C, the red pigment was extracted using an n-butanol-pyridine mixture and quantified spectrophotometrically at 532 nm.

2.8.2 Determination of pro-Inflammatory cytokines

Homogenization of brain tissues was performed on ice using PBS with protease inhibitors at a weight-to-volume ratio of 1:9 to 1:20. The clarified supernatants were stored at -80°C for subsequent analysis. Total protein was measured using the BCA method to normalize the cytokine data. The concentrations of IL-6 and TNF-α were then measured using commercial sandwich ELISA kits as per the provided guidelines, with readings taken at 450 nm and results expressed in pg/mg of protein.

2.8.3 Immunohistochemical staining technique

Following fixation, sectioning, and mounting, tissue sections

were processed for GFAP immunostaining. To minimize background, nonspecific sites were blocked with fetal calf serum (FCS). This was followed by incubation with an anti-GFAP primary antibody and a corresponding HRP-tagged secondary antibody. The resulting immunocomplex was detected via microscopic examination.

2.8.4 Quantification of GFAP immunoreactivity

Quantitative analysis of GFAP-positive immunoreactivity in the cerebellum was carried out using ImageJ, version 1.54.

2.8.5 Histopathological studies

After completing the behavioral and biochemical evaluations, brains from designated groups were fixed via perfusion with 4% PFA in a 0.1 M phosphate buffer. The brains were extracted and post-fixed overnight in the same fixative at 4°C. Following paraffin embedding and sectioning, tissues were stained with hematoxylin and eosin (H&E). An optical microscope was used to assess morphological lesions at 400x magnification. The hippocampus was specifically evaluated based on its well-documented significance in learning and memory (Inwang, 2025).

3. RESULTS

3.1 Evaluation of spatial working memory

The findings of spatial working memory are displayed in Fig. 1a-b. Percentage Alternation was assessed in Fig. 1a. The result revealed that percentage alternation significantly ($p<0.05$) decreased in the haloperidol, vin + halo, and ω -3 FA + halo groups, respectively, when compared to the control group. Compared to the haloperidol group, percentage alternation increased significantly ($p<0.05$) in the vin + halo, ω -3 FA + halo, and vin + ω -3 FA + halo groups, respectively. In Fig. 1b, there was a significant ($p<0.05$) increase in arrival time latency in haloperidol compared to the control group. The haloperidol group spent more time in the labyrinth before arriving at the terminal point compared to the vin + halo, ω -3 FA + halo, and vin + ω -3 FA + halo groups ($p<0.05$), respectively.

3.2 Pro-inflammatory mediators' activity

Interleukin-6 (IL-6) activity was evaluated in Fig. 1c. The result showed a significant ($p<0.05$) increase in IL-6 activity in the haloperidol and Vin + halo groups when compared with the control group. Compared to the haloperidol group, IL-6 activity decreased significantly ($p<0.05$) in the Vin + halo, ω -3 FA + halo, and Vin + ω -3 FA + halo groups, respectively.

Interleukin-1 β activity was evaluated in Fig. 1d. The result showed a significant ($p<0.05$) increase in IL-1 β activity in the haloperidol, Vin + halo, and ω -3 FA + halo groups when compared with the control group. Compared to the haloperidol group, IL-1 β activity decreased significantly ($p<0.05$) in the Vin + halo, ω -3 FA + halo, and Vin + ω -3 FA + halo groups, respectively.

Similarly, there was a significant ($p<0.05$) increase in Tumor Necrosis Factor Alpha (TNF- α) activity in the haloperidol, Vin + halo, and ω -3 FA + halo groups when compared with the control group. Compared to the haloperidol group, TNF- α activity decreased significantly ($p<0.05$) in the Vin + halo, ω -3 FA + halo, and Vin + ω -3 FA + halo groups, respectively.

3 FA + halo, and Vin + ω -3 FA + halo groups, respectively (Fig. 1e).

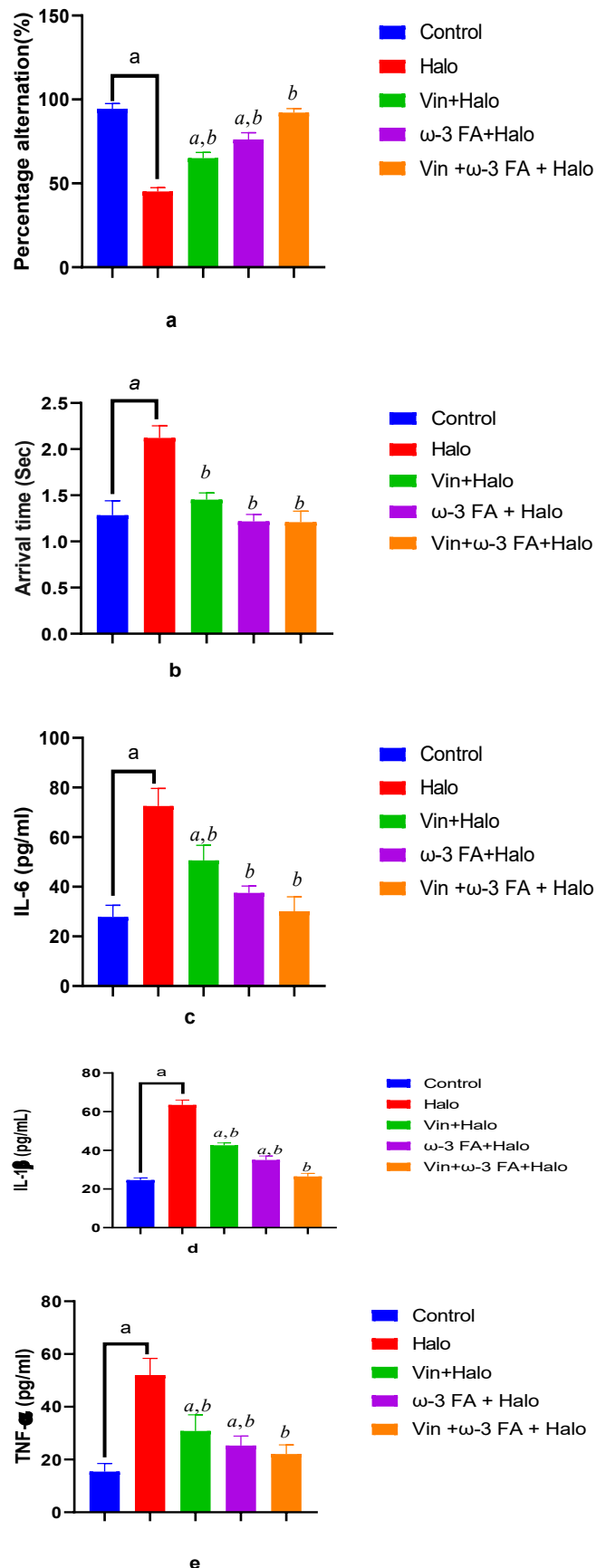


Figure 1: Effect of vinpocetine and omega-3 fatty acid monotherapy and synergy (ω -3 FA) on a. percentage alternation, b. arrival time latency, and c. IL-6 activity. d. IL-1 activity; e. TNF- α activity. Results are expressed as mean $\pm$ SD (n=5). The results were analyzed by one-way ANOVA followed by Turkey post hoc tests. (n=5; aP<0.05 versus vehicle-treated group; bP<0.05 versus HAL (2 mg/kg, i.p.) treated group) **Key:** Ctrl = control, Halo = haloperidol, Vin = vinpocetine, and ω -3 FA = omega-3 fatty acids.

3.3 Evaluation of antioxidant activity

Superoxide dismutase (SOD) activity was evaluated in Fig. 1a. The result showed a significant (p<0.05) decrease in SOD activity in the haloperidol, Vin + halo, and ω -3 FA + halo groups when compared with the control group. Compared to the haloperidol group, SOD activity increased significantly (p<0.05) in the Vin + halo, ω -3 FA + halo, and Vin + ω -3 FA + halo groups, respectively.

There was a significant (p<0.05) decrease in glutathione peroxidase (GPx) activity in the haloperidol, Vin + halo, and ω -3 FA + halo groups when compared with the control group. Compared to the haloperidol group, GPx activity increased significantly (p<0.05) in the Vin + halo, ω -3 FA + halo, and Vin + ω -3 FA + halo groups, respectively (Fig. 1b).

Similarly, there was a significant (p<0.05) decrease in catalase (CAT) activity in the haloperidol, Vin + halo, and ω -3 FA + halo groups when compared with the control group. Compared to the haloperidol group, (CAT) activity increased significantly (p<0.05) in the Vin + halo, ω -3 FA + halo, and Vin + ω -3 FA + halo groups, respectively (Fig. 1c).

Similarly, there was a significant (p<0.05) increase in malondialdehyde (MDA) activity in the haloperidol group when compared with the control group. Compared to the haloperidol group, MDA activity increased significantly (p<0.05) in the Vin + halo, ω -3 FA + halo, and Vin + ω -3 FA + halo groups, respectively (Fig. 1d).

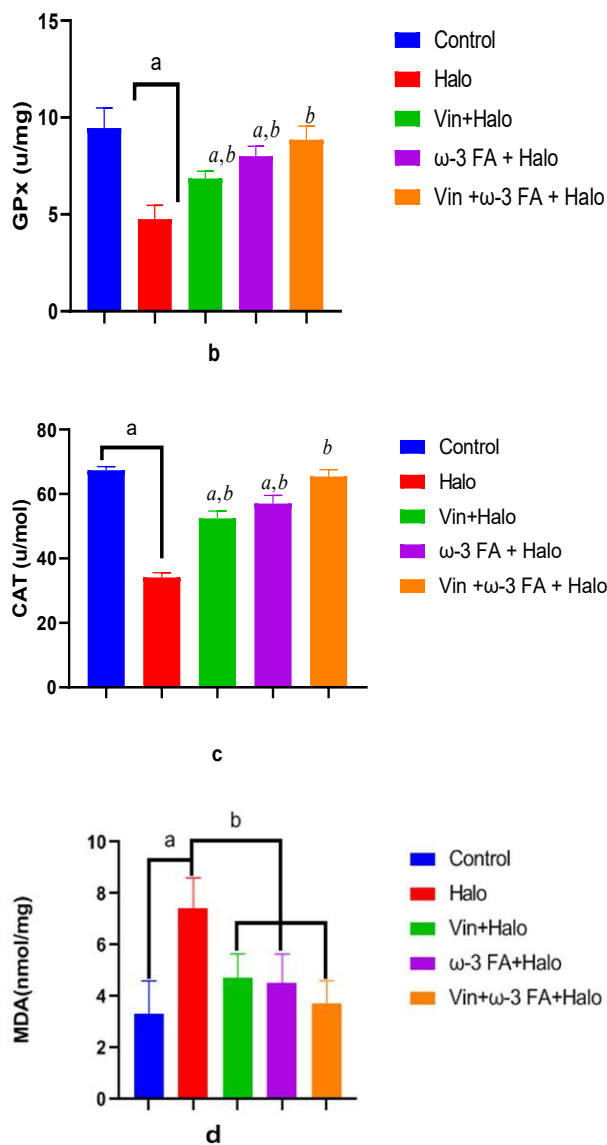
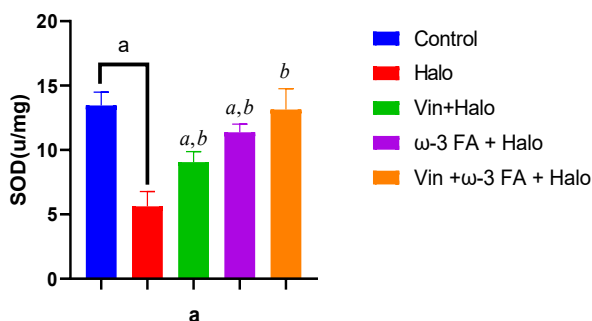


Figure 2: Effect of Vinpocetine and Omega-3 fatty acid monotherapy and synergy (ω -3 FA) on a. SOD activity, b. GPx activity, and c. CAT activity. d. MDA activity. Results are expressed as mean $\pm$ SD (n=5). The results were analyzed by one-way ANOVA followed by Turkey post hoc tests. (n=5; aP<0.05 versus vehicle-treated group; bP<0.05 versus HAL (2 mg/kg, i.p.) treated group) **Key:** Ctrl = control, Halo = haloperidol, Vin = vinpocetine, and ω -3 FA = omega-3 fatty acids.

3.4 Histology of the hippocampus

Plate 1 represents the histology of the hippocampus. The photomicrograph of the hippocampus of control rats given feed and water indicated a well-defined polymorphic layer (PL), pyramidal cell layer (PCL) with distinct and rounded pyramidal neurons (**arrows**), and molecular layer (ML). The photomicrograph of the hippocampus of group 2 rats given 2 mg/kg of haloperidol indicated severe pyramidal cell pyknosis in the entire population of the neurons. There was also nuclear shrinkage and condensation in the pyramidal neurons. The photomicrograph of the hippocampus of group 3 rats given 2 mg/kg haloperidol plus 10 mg/kg of vinpocetine indicated improvement in the pyramidal cell histo-structure.

The photomicrograph of the hippocampus of group 4 rats given 2 mg/kg haloperidol plus 300 mg/kg omega-3 fatty acid indicated improved pyramidal cell structure; the round shape of the pyramidal neurons with centrally placed nuclei was also noted. The photomicrograph of the hippocampus of group 5 rats given 2 mg/kg haloperidol plus 300 mg/kg omega-3 fatty acid plus 10 mg/kg vinpocetine indicated improved and distinct pyramidal cell histo-structure.

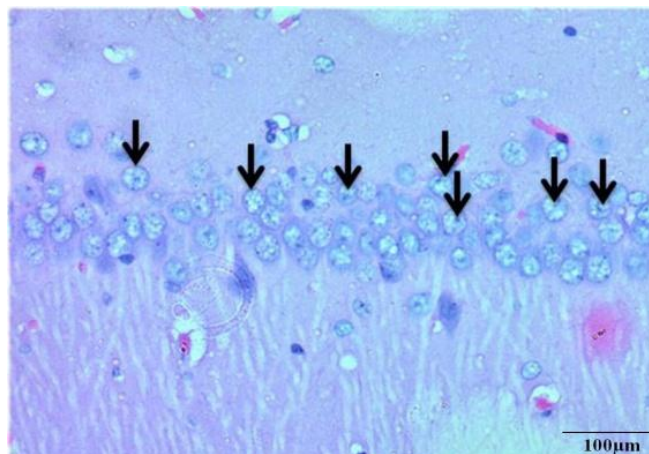
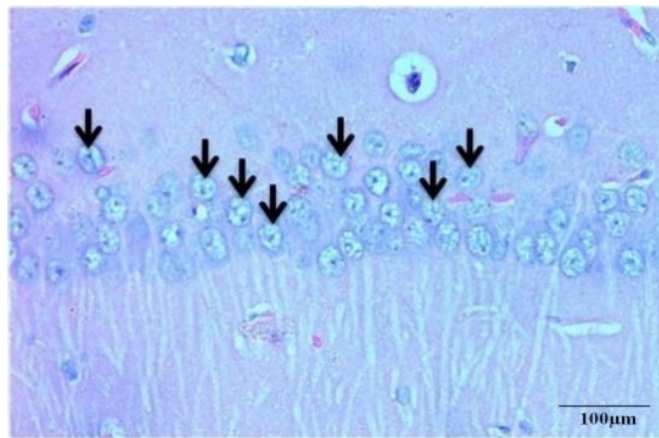
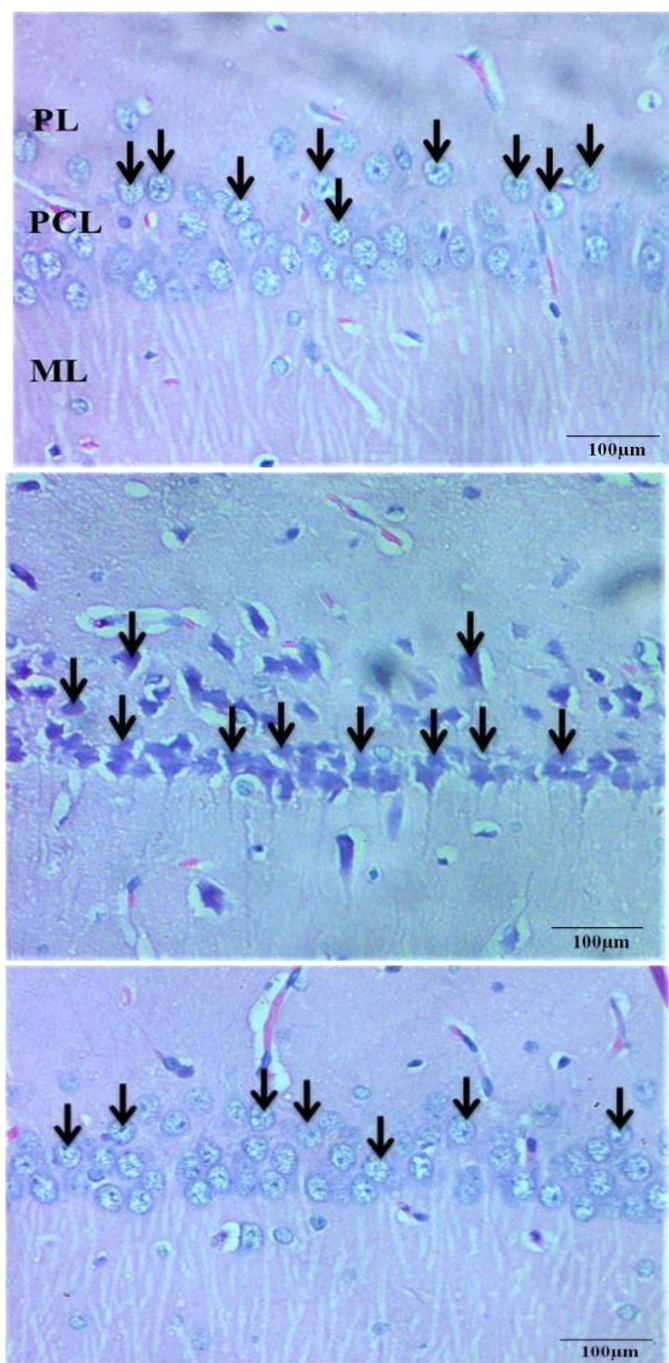


Plate 1: Section of the hippocampus. A show a well-defined polymorphic layer, a pyramidal cell layer with distinct and rounded pyramidal neurons, and a molecular layer. B shows severe pyramidal cell pyknosis in the entire population of the neurons. There were also nuclear shrinkage and condensation in the pyramidal neurons. C shows improvement in the pyramidal cell histo-structure. D shows improved pyramidal cell structure; the round shape of the pyramidal neurons with centrally placed nuclei was also noted. E shows improved and distinct pyramidal cell histo-structure. (Haematoxylin and eosin (H&E), H&E, x400 magnification; Scale Bar: 1 mm = 100 µm).

3.5 Histology of the prefrontal cortex

Plate 2 represents the histology of the prefrontal cortex. The photomicrograph of the prefrontal cortex (PFC) of control rats given water and feed indicated well-defined pyramidal neurons with peripheral processes (**arrows**) and granule cells with rounded vesicular nuclei (**curved arrow**). The photomicrograph of the prefrontal cortex (PFC) of group 2 rats given 2 mg/kg of haloperidol indicated intact pyramidal neurons with peripheral processes (**arrows**) and granule cells with rounded vesicular nuclei (**curved arrow**). The photomicrograph of the prefrontal cortex (PFC) of group 3 rats given 2 mg/kg haloperidol plus 10 mg/kg of vinpocetine indicated distinct peripheral processes (**arrows**). The granule cells are as well preserved (**curved arrow**). The photomicrograph of the prefrontal cortex (PFC) of group 4 rats given 2 mg/kg haloperidol plus 300 mg/kg omega-3 fatty acid indicated distinct peripheral processes (**arrows**). The

photomicrograph of the prefrontal cortex (PFC) of group 5 rats given 2 mg/kg haloperidol plus 300 mg/kg omega-3 fatty acid plus 10 mg/kg of vinpocetine indicated preserved pyramidal neurons (arrows) and granule cells (**curved arrows**).

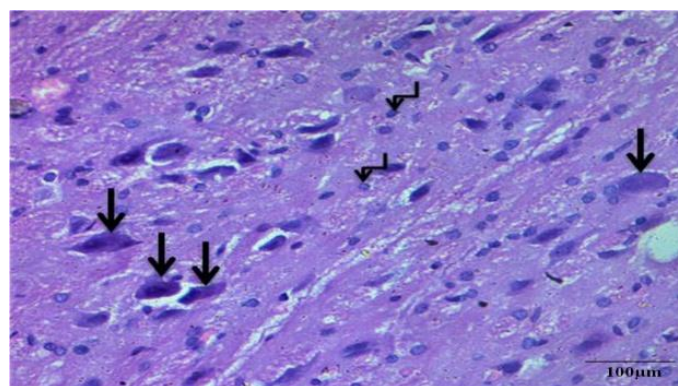
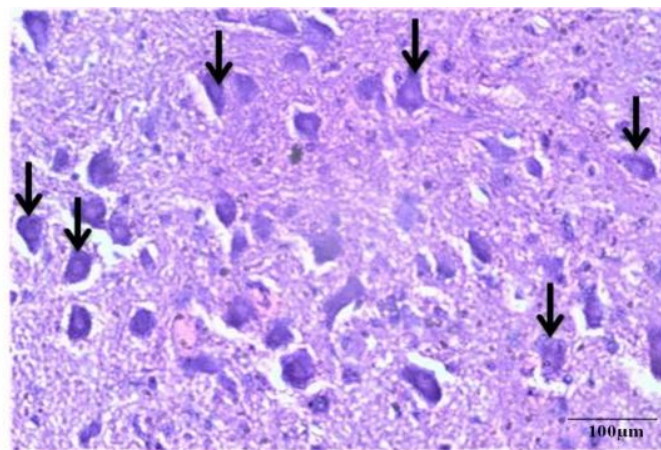
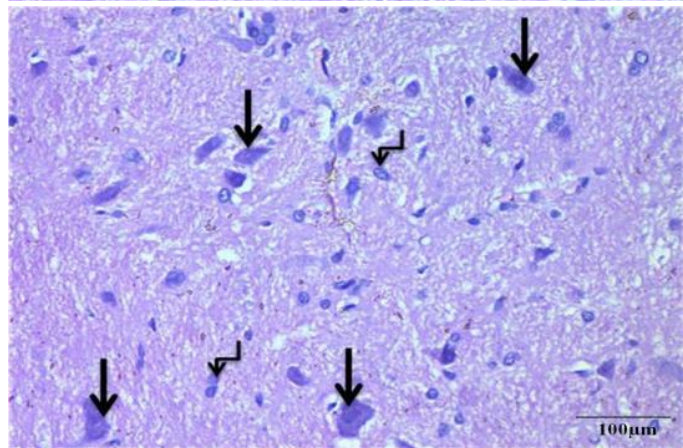
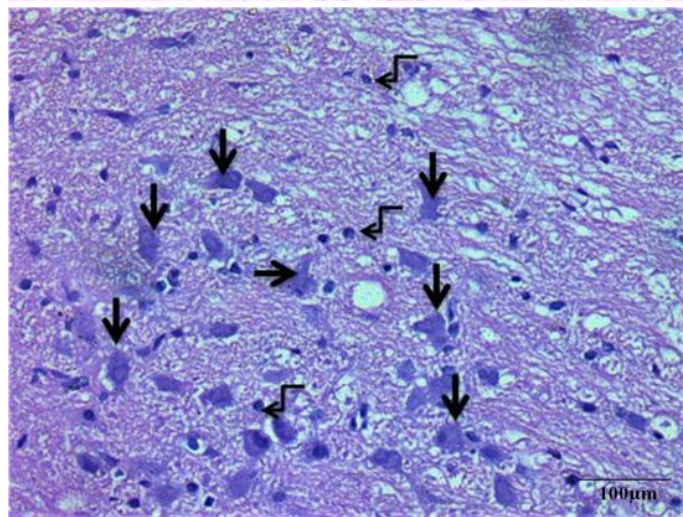
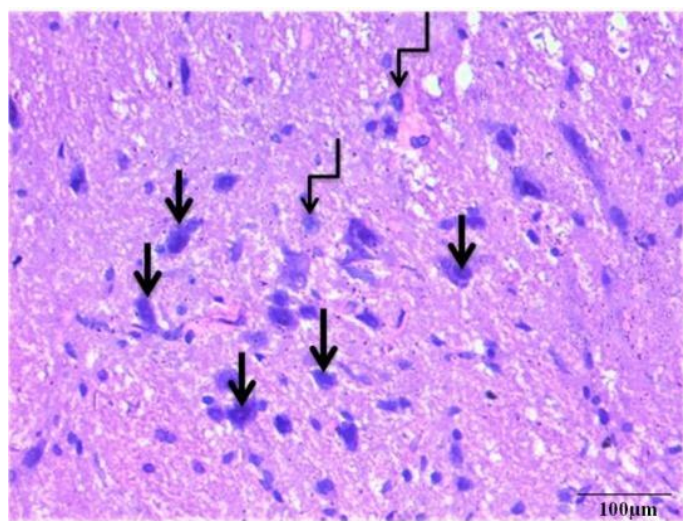


Plate 2: Section of the prefrontal cortex. A shows well-defined pyramidal neuron with peripheral processes and granule cells with rounded vesicular nuclei. B shows intact pyramidal neurons with peripheral processes and granule cells with rounded vesicular nuclei. C shows distinct peripheral processes and well-preserved granule cells. D shows distinct peripheral processes. E shows preserved pyramidal neurons and granule cells. (Haematoxylin and eosin (**H&E**), H&E, x400 magnification; Scale Bar: 1 mm = 100 μm).

3.6 Glial fibrillary acidic Protein (GFAP) analysis of the hippocampus

Plate 3 represents the GFAP analysis of the hippocampus. The photomicrograph of the hippocampus of the control rats showed few astrocytes displayed positive but very weak staining for GFAP (**arrows**). Note the few astrocytes in the polymorphic layer (PL), pyramidal cell layer (**PCL**), and molecular layer (**ML**). The photomicrograph of the hippocampus of group 2 rats given 2 mg/kg of haloperidol displayed positive and intense staining for GFAP. Note the proliferating astrocytes in all the layers of the hippocampus. The photomicrograph of the hippocampus of group 3 rats given 2 mg/kg haloperidol plus 10 mg/kg of vinpocetine showed positive but weak staining for GFAP (**arrows**). Note the few astrocytes in all the layers of the hippocampus. The photomicrograph of the hippocampus of group 4 rats given 2 mg/kg haloperidol plus 300 mg/kg omega-3 fatty acid exhibited positive but weak staining for GFAP (**arrows**). Note the few astrocytes in all the layers of the hippocampus. The photomicrograph of the hippocampus of group 5 rats given 2 mg/kg haloperidol plus 300 mg/kg omega-3 fatty acid plus 10

mg/kg of vinpocetine indicated positive but weak staining for GFAP (**arrows**). Note the few astrocytes in all the layers of the hippocampus. The GFAP immunoreactivity of positive cells in the hippocampus indicated a significant ($p<0.05$) increase in group two given 2 mg/kg haloperidol when compared with groups 3, 4, and 5, respectively (2 mg/kg haloperidol plus 300 mg/kg omega-3 fatty acid plus 10 mg/kg of vinpocetine) (Fig. 3a).

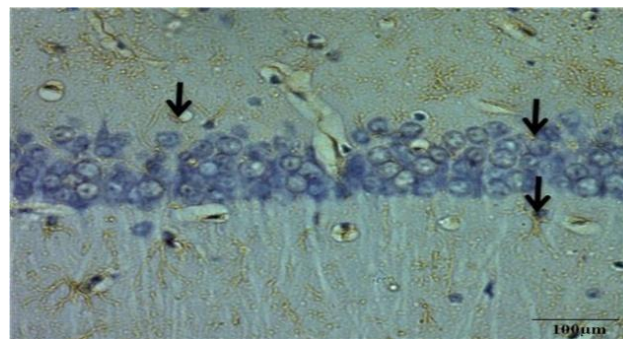
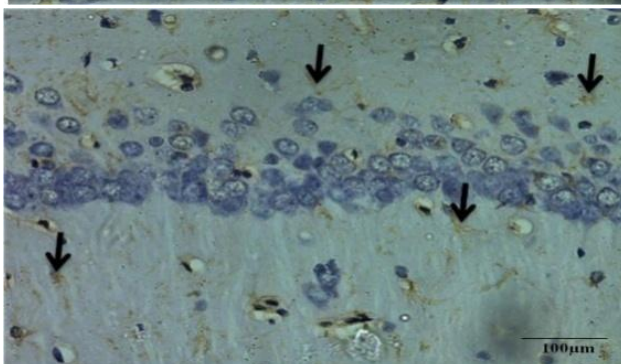
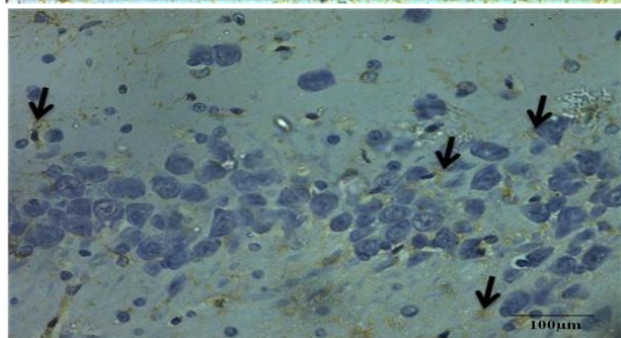
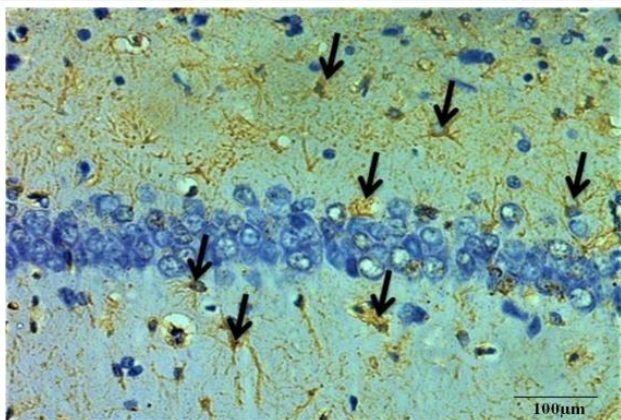
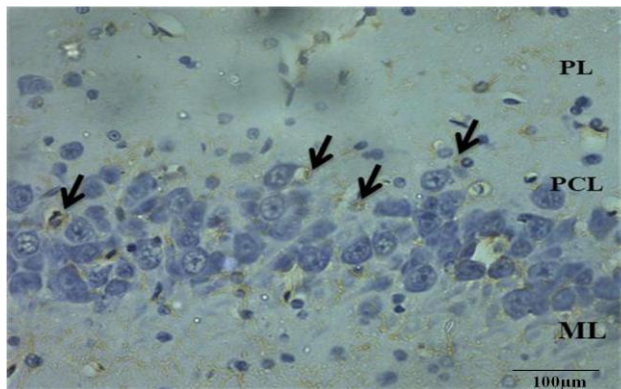


Plate 3: Section of the hippocampus. A few astrocytes displayed positive but very weak staining for GFAP; B shows positive and intense staining for GFAP; C shows positive but weak staining for GFAP; D shows positive but weak staining for GFAP; E shows positive but weak staining for GFAP. (GFAP x400 magnification; Scale Bar: 1 mm = 100 µm).

3.7 Glial fibrillary acidic protein (GFAP) analysis of the prefrontal cortex

Plate 4 represents the GFAP analysis of the prefrontal cortex. The photomicrograph of the prefrontal cortex (PFC) of control rats showed few astrocytes displaying positive but very weak staining for GFAP (**arrows**). Photomicrographs of the prefrontal cortex (PFC) of group 2 rats given 2 mg/kg of haloperidol displayed positive and intense staining for GFAP. The photomicrograph of the prefrontal cortex (PFC) of group 3 rats given 10 mg/kg of vinpocetine plus 2 mg/kg of haloperidol displayed positive but weak staining for GFAP (**arrows**). The photomicrograph of the prefrontal cortex (PFC) of group 4 rats given 300 mg/kg of omega-3 fatty acid plus 2 mg/kg of haloperidol indicated positive but weak staining for GFAP (**arrows**).

The photomicrograph of the prefrontal cortex (PFC) of group 5 rats given 10 mg/kg of vinpocetine plus 300 mg/kg of omega-3 fatty acid plus 2 mg/kg of haloperidol showed positive but weak staining for GFAP (**arrows**). The GFAP immunoreactivity of positive cells in the prefrontal cortex showed a significant ($p<0.05$) increase in group two given 2 mg/kg haloperidol when compared with groups 3, 4, and 5, respectively (2 mg/kg haloperidol plus 300 mg/kg omega-3 fatty acid plus 10 mg/kg of vinpocetine) (Fig. 3b).

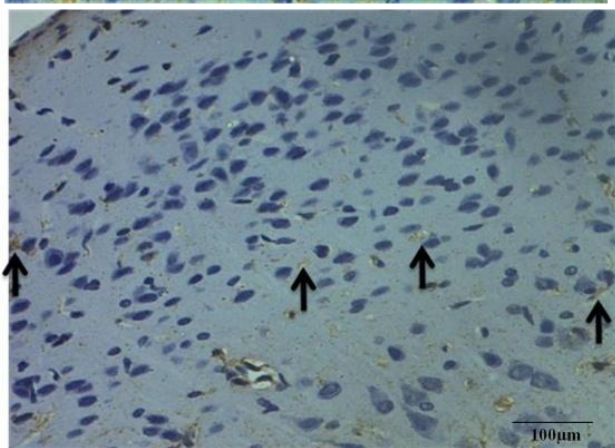
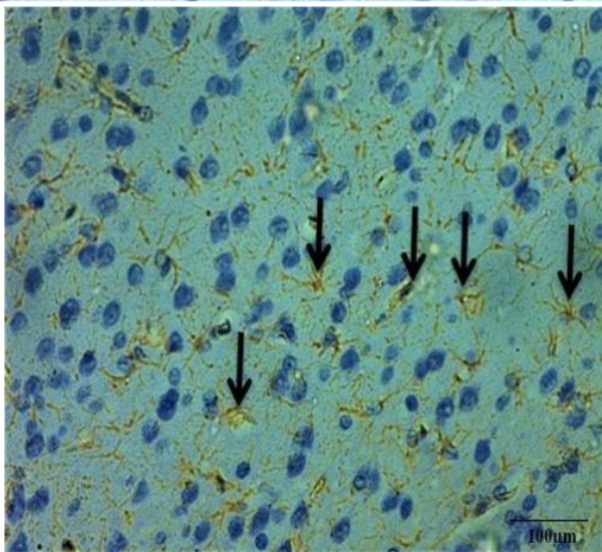
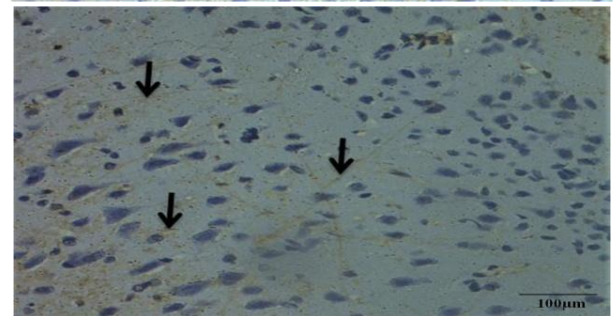
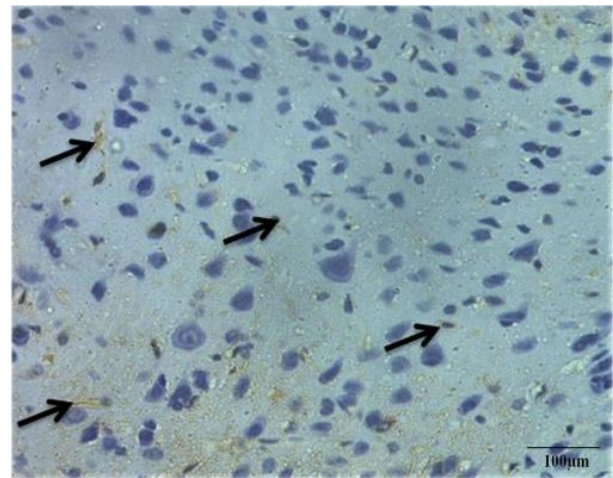
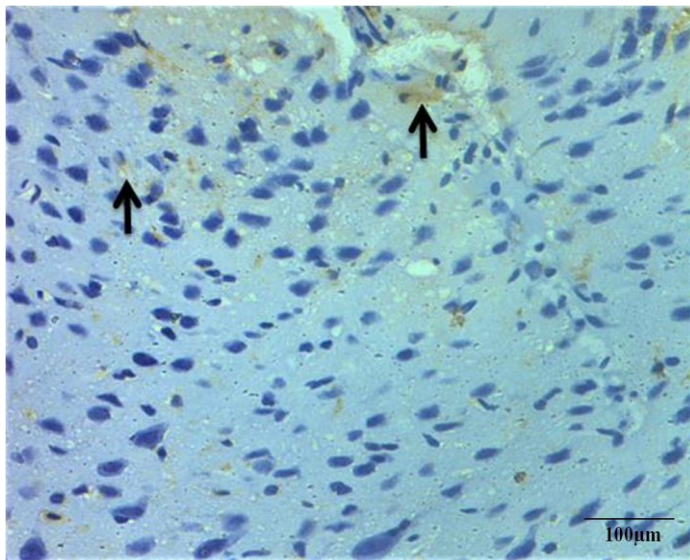
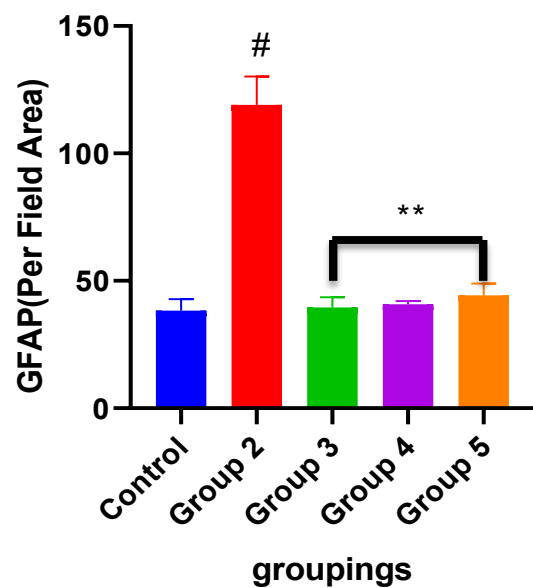


Plate 4: Section of the prefrontal cortex. A few astrocytes displayed positive but very weak staining for GFAP; B shows positive and intense staining for GFAP; C shows positive but weak staining for GFAP; D shows positive but weak staining for GFAP; and E shows positive but weak staining for GFAP. (GFAP x400 magnification; Scale Bar: 1 mm = 100 µm).



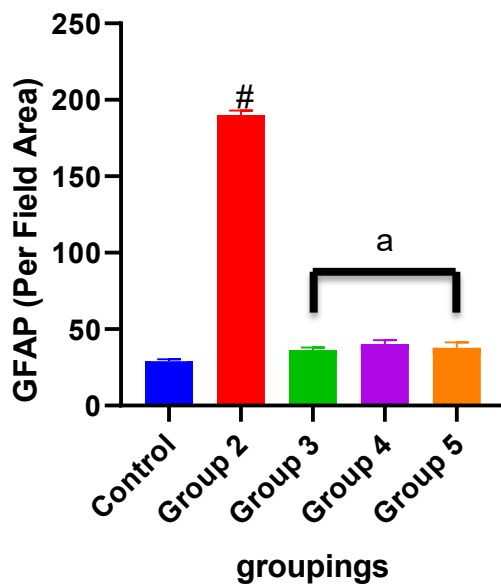


Figure 3: GFAP immunoreactivity of positive cells in a. hippocampus and b. prefrontal cortex.

Values are expressed in mean $\pm$ SD. # = significantly different from control and other groups, $p < 0.05$. ** = significantly different from group 2 at $p < 0.05$. a = significantly different from group 2, $p < 0.05$

4. DISCUSSION

This study evaluated the neuroprotective potential of vinpocetine and omega-3 fatty acids administered alone or together against haloperidol-induced deficits, including cognitive dysfunction, neuroinflammation, oxidative imbalance, and neuronal structural damage. Haloperidol administration led to pronounced spatial memory impairment, activation of inflammatory mediators, suppression of endogenous antioxidants, and glial reactivity in the hippocampal and prefrontal regions. These impairments were alleviated by each treatment, with the combination showing the greatest efficacy. The neuroprotective effects shown by vinpocetine and omega-3 fatty acids corroborate other plant-based interventions. Inwang et al. (2024a) reported that ripe *Musa sapientum* peels offered marked neuroprotection against lead acetate-induced brain damage, reducing oxidative stress and preserving the morphology of neurons. Furthermore, Inwang et al. (2024b) showed that *Talinum triangulare* methanol leaf extract provided both antioxidant and anti-inflammatory properties against cadmium-induced cognitive dysfunction, enhancing spatial learning and memory while mitigating neuroinflammation. Together, these studies elucidate the therapeutic potential of natural products in alleviating neurotoxicity and support the growing body of evidence favouring plant-derived interventions for cognitive protection.

The reduction in T-maze percentage alternation and the increased latency observed in the labyrinth test following haloperidol exposure indicate substantial spatial working memory deficits. This agrees with earlier literature showing that persistent dopamine D2 antagonism interferes with

hippocampal memory processing and prefrontal cortex integrity (Kapur and Seeman, 2001; Rocchetti *et al.*, 2015). The observed deficits may arise from haloperidol-induced reductions in neuroplastic and neurotrophic mechanisms (Park *et al.*, 2018; Jîtcă *et al.*, 2024).

Treatment with vinpocetine or omega-3 fatty acids resulted in marked behavioral improvements, suggesting restoration of brain regions associated with working memory. Vinpocetine facilitates neuronal signaling through increased cerebral blood flow and PDE-1 inhibition (Zhang *et al.*, 2018), whereas DHA-rich omega-3 fatty acids enhance synaptic structure and membrane dynamics (Bazinet and Layé, 2014). Their co-administration yielded the most pronounced effects, supporting a synergistic mechanism.

The significant upregulation of IL-6, IL-1 β , and TNF- α following haloperidol exposure reflects robust neuroinflammatory activation, consistent with literature describing haloperidol-induced cytokine dysregulation and oxidative stress (Raudenska *et al.*, 2013; Valvassori *et al.*, 2021; Gupta and Verma, 2024).

Treatment with vinpocetine and omega-3 fatty acids led to marked decreases in IL-6, IL-1 β , and TNF- α . This effect is attributable to vinpocetine's inhibition of NF- κ B signaling pathways (Jeon *et al.*, 2010; Zhang *et al.*, 2018) and the generation of inflammation-resolving lipid mediators from omega-3 fatty acids (Zhang and Spite, 2012; Weylandt *et al.*, 2012). When combined, these agents produced the most substantial cytokine reductions, implying reinforcement between their anti-inflammatory mechanisms.

Haloperidol markedly suppressed the activities of SOD, CAT, and GPx while increasing MDA concentration, indicating substantial oxidative stress. This is consistent with established evidence that the drug enhances superoxide production and accelerates lipid oxidative damage (Arnaiz *et al.*, 1999; Andreazza *et al.*, 2015).

Vinpocetine and omega-3 fatty acids effectively enhanced antioxidant enzyme activities and attenuated lipid peroxidation, signifying improved cellular oxidative defense. Vinpocetine scavenges free radicals and preserves mitochondrial integrity (Santos *et al.*, 2000; Svab *et al.*, 2019), while omega-3 fatty acids enhance membrane stability and activate antioxidant gene expression (Dyall, 2015). The combination therapy exerted the most substantial protective effect, demonstrating synergistic neuroprotection.

Examination of brain tissue revealed severe pyramidal neuron shrinkage, pyknosis, and structural deterioration following haloperidol administration, consistent with reports of antipsychotic-induced neurotoxicity in hippocampal and prefrontal cortical regions (Roeske *et al.*, 2021).

Treatment with vinpocetine or omega-3 fatty acids attenuated pyramidal neuron degeneration and supported laminar structural preservation, while the combined therapy yielded the greatest restoration of tissue architecture, paralleling enhancements in cognitive and biochemical markers.

GFAP immunoreactivity was markedly upregulated in haloperidol-treated rats, demonstrating strong astrocytic reactivity. Treatment with vinpocetine and omega-3 fatty acids effectively reduced GFAP expression, indicating

decreased glial activation and a more balanced neuroimmune state.

This research represents an innovative approach to targeting haloperidol-induced neuronal damage, demonstrating that the combination of vinpocetine and omega-3 fatty acids can concurrently modulate cognitive function, oxidative stress, and neuroinflammation.

A primary limitation of this study is the limited sample size (n=5), which may affect the robustness and external validity of the results. Acute biochemical assessments were performed, but chronic and longitudinal effects remain unclear. Molecular mechanisms such as NF- κ B, Nrf2, and BDNF signaling were not explored. Only male rats were studied, and behavioral testing was confined to spatial memory, neglecting other cognitive or emotional domains. Future studies should increase sample size, incorporate both sexes, examine long-term treatment outcomes, and explore molecular mechanisms in greater detail.

5. CONCLUSION

In conclusion the present study demonstrates that vinpocetine and omega-3 fatty acids act synergistically to protect against haloperidol-induced neurotoxicity, improving cognitive outcomes such as spatial working memory, suppressing neuroinflammatory mediators, balancing oxidative stress, and maintaining structural integrity of key brain regions. This combined approach may hold clinical potential for mitigating antipsychotic-induced cognitive impairments.

6. DECLARATIONS

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Authors' Contributions

UA, Inwang designed the experiments; UA, Inwang; and CI, Aigboduokhai performed experiments and collected data; EO, Nwuzor discussed the results and strategy; UA, Inwang Supervised, directed and managed the study; EO, Nwuzor, N,P Odunnaya; Emmanuel I. Obi; and UA, Inwang Final approved of the version to be published.

Conflicts of Interest

All authors have approved the final version of the manuscript. The authors declare that they have no conflict of interest.

Declaration

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