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Synergistic Neuroprotective Effects of Omega-3 Fatty Acids and Vitamin E on Sodium Arsenite-Induced Cognitive Dysfunction in Wistar Rats

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Abstract

Background

Sodium arsenite, a widely encountered environmental toxicant, is known to induce cognitive dysfunction through oxidative stress, neuroinflammation, and neuronal degeneration, particularly within brain regions associated with memory. This study investigated the neuroprotective effects of omega-3 fatty acids and vitamin E, administered individually and in combination, on recognition and spatial memory deficits induced by sodium arsenite in Wistar rats.

Results

Twenty-five adult female rats were randomly assigned to five groups: control, sodium arsenite only, sodium arsenite plus omega-3 fatty acids, sodium arsenite plus vitamin E, and sodium arsenite co-treated with omega-3 fatty acids and vitamin E. Cognitive performance was evaluated using the Y-maze and classic labyrinth tests, while oxidative stress status was assessed by measuring glutathione peroxidase, superoxide dismutase, and malondialdehyde levels. Histopathological examination and glial fibrillary acidic protein (GFAP) immunohistochemistry were performed on the hippocampus and prefrontal cortex to assess neuronal integrity and astrocytic activation. Sodium arsenite exposure resulted in significant ($p < 0.05$) impairment of spatial memory, marked reductions in antioxidant enzyme activities ($p < 0.05$), increased lipid peroxidation, pronounced neuronal degeneration, and elevated GFAP immunoreactivity. Treatment with omega-3 fatty acids or vitamin E partially ameliorated these alterations, while their combined administration produced a more pronounced improvement in behavioral performance, antioxidant defense, neuronal morphology, and attenuation of reactive astrogliosis.

Conclusion

In conclusion, the combined supplementation of omega-3 fatty acids and vitamin E exerts synergistic neuroprotective effects against sodium arsenite-induced cognitive dysfunction, likely through complementary antioxidant and anti-inflammatory mechanisms. These findings highlight the potential of combined nutritional antioxidant strategies in mitigating environmentally induced neurotoxicity.

INTRODUCTION

Sodium arsenite, a toxic form of inorganic arsenic, is known to damage the brain by causing oxidative stress, inflammation, and neuronal damage, leading to impaired memory and learning [1, 2]. Sodium arsenite injures brain tissues, especially those in the hippocampus, which manage learning and memory and arsenic pollution in water and food in Nigeria and other places is a continuing health problem for the

public [3, 4, 5]. Earlier studies reported that exposing animals to arsenic results in increased oxidative stress and difficulty in remembering things [2, 6].

Studies in Wistar rats have shown that arsenic exposure disrupts hippocampal function and reduces the activity of important antioxidant enzymes like SOD and catalase [6, 7, 8]. Although, some research suggests that Omega-3 fatty acids and Vitamin E can protect the brain from oxidative damage and improve memory, their combined effect on recognition memory in sodium arsenite-induced cognitive dysfunction has not been fully studied [9, 10, 11]. This study, therefore, aims to investigate whether using Omega-3 fatty acids and Vitamin E together can improve recognition memory in Wistar rats exposed to sodium arsenite.

Studies involving rodents have shown that sodium arsenite impairs memory and ability to recall by putting stress on the brain and damaging nerve cells [7, 12], people exposed to arsenic have reduced levels of Bdnf, c–Fos, and Nmdar1 hippocampal genes and abnormal methylation patterns in histone, which reduces their ability to learn and remember things. Rats exposed to arsenite after birth have trouble remembering locations and faces, and this is connected to spine loss on Wistar rats' dendrites, in the same way [13, 14]. Researchers found that exposing adult rats to arsenite for six weeks brought about less exploring, greater damage to lipids, lower activity of antioxidant enzymes, and memory disturbances [15, 7]. Long term contact with arsenite at a low concentration in pregnant animals i.e, rats living in low dose arsenite exposure displayed white matter disruption and cognitive deficits in the prefrontal cortex of rats [6]. Xiong *et al.* [16] discovered that exposure to sodium arsenite in mice raised levels of oxidative stress in the hippocampus, activated the Nrf2/PPARγ antioxidant route, and seriously impaired the animals' spatial learning ability. All these studies show the reason why it is important to investigate how antioxidants Omega-3 and Vitamin E might protect Wistar rats from arsenite induced memory loss.

Vitamin E acts as a membrane stabilizer, an anti-inflammatory substance, and protects the nervous system [17, 18]. It plays a major role in protecting the cells from stress caused by free radicals by removing them, slowing down oxidation of cell fats, and boosting the activity of many antioxidant enzymes [19]. Studies on animals with neurodegenerative disorders have found that Vitamin E improves cognitive and movement abilities and at the same time lowers levels of the β-amyloid and phosphorylated tau components [20, 21]. In rats with injured spinal cords, α-tocopherol helped by down regulating Alox15 so that there was less ferroptosis and better neural repair and motor coordination [22].

Eating foods rich in omega-3 PUFAs is important for the brain as they limit harmful reactions in the brain, ease inflammation, and improve the working of synapses. Experiments done on aged rats that showed symptoms of postoperative delirium showed that fish oil with EPA and DHA reduced their levels of oxidation and inflammation, which helped their mental performance [9].

While Omega-3 and Vitamin E as antioxidants seem helpful for the brain, few studies have shown the effect of combining them and how they affect recall after exposure to arsenic [9]. For this reason, this research helps by showing that using everyday natural products might be able to treat arsenic's effect on

brain function. The result of this work could lead to better ideas on nutrition for those experiencing memory problems because of their environment

The aim of this study is to evaluate the neuroprotective effects of Omega-3 fatty acid and Vitamin E, individually and in combination, on recognition memory in Wistar rats with sodium arsenite-induced cognitive dysfunction.

METHODS

Animals

The study was done in an animal house of the Alex-Ekwueme Federal University, Ndufu-Alike Ikwo, Ebonyi State. Twenty five (25) adult female Wistar rats, weighing between 180–250 g were procured in the animal house of Alex Ekwueme Federal University, Ndufu Alike Ikwo. The animals were kept in sterile paddy husk-lined clean polypropylene cages in the animal house of the university. They were maintained at a controlled environmental condition of temperature (23 ± 2°C) and humidity (50 ± 5%). The study followed a normal 12 hour/dark cycle. The rats were given the time to acclimatize to the normal laboratory conditions for two weeks before the start of the experiment. The animals were freely given normal rodent chow and clean drinking water (*ad libitum*).

Chemicals and reagents

Chemicals and reagents employed in this study were as follows: Sodium arsenite ($NaAsO_2$) grade - analysis grade (Sigma-Aldrich, USA). Fatty acids, pharmaceutical grade (EPA/DHA) omega-3 and Vitamin E (a-tocopherol) (procured from a licensed pharmaceutical vendor). Biochemical assay kits Reagents were of analytical grade and were prepared fresh according to the instructions of the manufacturer.

Experimental design

The experimental animals (n = 5 per group) were randomly selected and divided into five groups (one control group and four experimental groups), which were given varying treatments and dosage of the sodium arsenite.

A randomized controlled experimental design was adopted. The animals were divided into six groups (n = 5 per group). The experimental grouping is as follows:

Group 1 (Control): 1 mL of distilled water

Group 2: administered with 20 mg/kg $NaAsO_2$ only for 21 days.

Group 3: given 20 mg/kg of $NaAsO_2$ plus 300 mg/kg of omega-3 fatty acid for 21 days.

Group 4: administered with 20 mg/kg $NaAsO_2$ plus 100 mg/kg vitamin E for 21 days.

Group 5: administered with 2 mg/kg $NaAsO_2$ plus 300 mg/kg of omega-3 fatty acid plus 100 mg/kg of vitamin E for 21 days.

Cognitive deficit was induced with sodium arsenite (20 mg/kg, body weight p.o. for 21 days). This dosage was selected based on previous study [23]. Omega-3 fatty acids (300 mg/kg) and vitamin E (100 mg/kg) were administered once daily for 21 days via oral gavage. The dose of omega-3 fatty acid (300 mg/kg) and vitamin E (100 mg/kg) were selected based on previous studies by Meganathan *et al.* [24] and Rafati *et al.* [25] respectively. Drugs were given 30 minutes before the sodium arsenite exposure to ensure systemic absorption.

Neurobehavioral Test

Y-maze test

On the 22nd day of the study, percentage alternation was determined using the Y-maze Test. Y-maze is a common test used to measure spatial and recognition memory. Animals were trained to alternate between the arms, based on the recollection of previously visited arms. The equipment was an acrylic-painted, white-painted Y shape maze. The length of each arm was 30 cm, and the height and width of arm were 14 cm and 8 cm respectively, with the three arms positioned at the same angle. In each trial a rat was put at the end of one arm and given time to move freely in the maze of 8 minutes. Spontaneous alternation behaviour was rated as consecutive entry into the three arms in triplet sequences that overlapped. Each trial was characterized by alternation that was scored with the EthoVision system [26].

Classic Labyrinth Test

On the 23rd day of the study, spatial navigation was evaluated using the Classic Labyrinth Test (CLT) as previously described Inwang *et al.* [27]. This testing took place for 2 days after the Y-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.

Biochemical assays

Euthanasia

The animals were euthanized by cervical dislocation while still under deep anesthesia with isoflurane (1.5-3%, 100 ml), thereafter were perfused with 0.01 mmol/l phosphate-buffered saline [28]. The skull was excised, and the brain harvested and homogenized, and the homogenate used to measure oxidative stress marker and antioxidants activities.

Assay of antioxidant activities

Catalase activity was quantified using the established method of Aebi [29]. 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% H_2O_2. The change in absorbance at 240 nm was recorded spectrophotometrically to calculate the rate of H_2O_2 oxidation. To measure the activity of gluthathione 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 [30].

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) [31].

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.

Quantification of GFAP immunoreactivity

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

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 [32].

Statistical Analysis

Results are displayed as means ± SEM and they were subjected to one-way ANOVA and post-hoc comparison (Tukey). Probability value of *P < 0.05* was taken to be statistically significant. Statistical analyses were done using GraphPad Prism 8.0.

RESULTS

Evaluation of spatial memory

Percentage alternation was investigated in Fig. 1 (a). The result showed that, compared to the control group, percentage alternation increased in decreased significantly ($p < 0.05$) in the $NaAsO_2$, $NaAsO_2$ + ω-3 FA, and $NaAsO_2$ + Vit E groups respectively. Compared to the $NaAsO_2$ group, percentage alternation significantly ($p < 0.05$) increased in $NaAsO_2$ + ω-3 FA, $NaAsO_2$ + Vit E and $NaAsO_2$ + ω-3 FA + Vit E groups respectively. There was a significant ($p < 0.05$) increase in $NaAsO_2$ plus ω-3 FA and $NaAsO_2$ + Vit E groups when compared with $NaAsO_2$ + ω-3 FA + Vit E groups.

The arrival time latency was investigated in Fig. 1 (b). The results showed that, compared to the control group, the arrival time latency increased significantly ($p < 0.05$) in the $NaAsO_2$, $NaAsO_2$ + ω-3 FA, $NaAsO_2$+Vit E and $NaAsO_2$ + ω-3 FA + Vit E groups respectively. Compared to the $NaAsO_2$, the arrival time latency was significantly ($p < 0.05$) shorter in $NaAsO_2$ + ω-3 FA + Vit E group.

Evaluation of antioxidant activity

Glutathione Peroxidase activity (GPx) was evaluated in Fig. 2 (a). The results showed a significantly ($p < 0.05$) decrease in GPx activity in $NaAsO_2$, $NaAsO_2$ + ω-3 FA, and $NaAsO_2$ + Vit E groups respectively when compared with the control group. Compared to the $NaAsO_2$ group, GPx activity increased significantly ($p < 0.05$) in $NaAsO_2$ + ω-3 FA, $NaAsO_2$ + Vit E and $NaAsO_2$ + ω-3 FA + Vit E groups respectively. Also, $NaAsO_2$ + ω-3 FA + Vit E significantly ($p < 0.05$) increased in GPx activity when compared with $NaAsO_2$ + ω-3 FA group.

Superoxide dismutase activity **(**SOD**)** was evaluated in Fig. 2 (b). The results showed a significantly ($p < 0.05$) decrease in SOD activity in $NaAsO_2$, $NaAsO_2$ + ω-3 FA, and $NaAsO_2$ + Vit E groups respectively when compared with the control group. Compared to the $NaAsO_2$ group, SOD activity increased significantly ($p < 0.05$) in $NaAsO_2$ + ω-3 FA, $NaAsO_2$ + Vit E and $NaAsO_2$ + ω-3 FA + Vit E groups respectively. Also, $NaAsO_2$ + Vit E and $NaAsO_2$ + ω-3 FA + Vit E significantly ($p < 0.05$) increased in SOD activity when compared with $NaAsO_2$ + ω-3 FA group. Similarly, $NaAsO_2$ + ω-3 FA + Vit E significantly ($p < 0.05$) increased in SOD activity when compared with $NaAsO_2$ + ω-3 FA group.

Malondialdehyde activity (MDA) was determined in Fig. 2 (c). The results revealed a significantly ($p < 0.05$) increase in MDA activity in $NaAsO_2$, $NaAsO_2$ + ω-3 FA, and $NaAsO_2$ + Vit E groups when compared with the control group. Conversely, there was a significant ($p < 0.05$) decrease in MDA activity in $NaAsO_2$ + ω-3 FA, $NaAsO_2$ + Vit E, and $NaAsO_2$ + ω-3 FA + Vit E groups compared with the $NaAsO_2$ group. Also,

$NaAsO_2$ + Vit E significantly ($p < 0.05$) decreased in MDA activity when compared with $NaAsO_2$ + ω-3 FA group. Similarly, $NaAsO_2$ + ω-3 FA + Vit E significantly ($p < 0.05$) decreased in MDA activity when compared with $NaAsO_2$ + ω-3 FA group.

Histology of the Hippocampus

Plate 1 represents the histology of the hippocampus. The photomicrograph of the hippocampus of the control rats given feed and water revealed a well-defined polymorphic layer (**PL**), pyramidal cell layer (**PCL**) with distinct and rounded pyramidal neurons (**arrows**) as well as well-structured molecular layer (**ML)** The photomicrograph of the hippocampus of group 2 rats given 10 mg/kg of sodium arsenite revealed severe pyknosis in a greater population of the neurons. The photomicrograph of the hippocampus of group 3 rats given 100 mg/kg of omega-3 fatty acid plus 10 mg/kg of sodium arsenite revealed preserved pyramidal neurons. **Plate 4**: The photomicrograph of the hippocampus of group 4 rats given 100 mg/kg of vitamin E plus 10 mg/kg of sodium arsenite revealed preserved as well as improved pyramidal neurons. The photomicrograph of hippocampus of group 5 rats given 100 mg/kg of omega-3 fatty acid plus 100 mg/kg of Vitamin E plus 10 mg/kg of sodium arsenite revealed preserved and improved histo-structure of the pyramidal neurons.

Plate 1

Photomicrograph of hippocampus of control rats revealed well-defined polymorphic layer, pyramidal cell layer with distinct and rounded pyramidal neurons as well as well-structured molecular layer. Photomicrograph of hippocampus of group 2 revealed severe pyknosis in a greater population of the neurons. Note the nuclear shrinkage and condensation in the pyramidal neurons. Photomicrograph of hippocampus of group 3 rats revealed preserved pyramidal neurons. Note the round shape and with the centrally placed nuclei. Photomicrograph of hippocampus of group 4 rats revealed preserved as well as improved pyramidal neurons (**arrows)**. Photomicrograph of hippocampus of group 5 rats revealed preserved and improved histo-structure of the pyramidal neurons (**arrows**). H&E, x400 mag

Histology of the Prefrontal Cortex (PFC)

Plate 2 represents the histology of the hippocampus. The photomicrograph of PFC of control rats given water and feed indicated well-structured pyramidal neurons with peripheral processes. The photomicrograph of PFC of group 2 rats given 10 mg/kg of sodium arsenite indicated necrotized neuron surrounded by halos (double arrows) and neurocytochromatolysis. The photomicrograph of PFC of group 3 rats given 10 mg/kg of omega-3 fatty acid plus 100 mg/kg of sodium arsenite indicated preserved and improved pyramidal neurons. The photomicrograph of PFC of group 4 rats given 100 mg/kg of vitamin E plus 10 mg/kg of sodium arsenite indicated improvement in the histo-structure of the pyramidal neurons. The photomicrograph of PFC of group 5 rats given 100 mg/kg of omega-3 fatty acid plus 100 mg/kg of Vitamin E plus 10 mg/kg of sodium arsenite indicated improved/preservedhisto-structure of the pyramidal cells.

Plate 2

A.Photomicrograph of PFC of control rats indicated well-structured pyramidal neurons with peripheral processes. B.Photomicrograph of PFC of group 2 rats indicated necrotized neuron surrounded by halos and neurocytochromatolysis. C.Photomicrograph of PFC of group 3 rats indicated preserved and improved pyramidal neurons. D.Photomicrograph of PFC of group 4 rats indicated improvement in the histo-structure of the pyramidal neurons. E.Photomicrograph of PFC of group 5 rats indicated improved/preservedhisto-structure of the pyramidal cells. H&E, x400 mag

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 given water and feed showed few astrocytes displaying positive but weak staining for GFAP (**arrows**). The photomicrograph of the hippocampus of group 2 rats given 20 mg/kg of sodium arsenite indicated 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 300 mg/kg of omega-3 fatty acid plus 20 mg/kg of sodium arsenite indicated positive but weak staining for GFAP (**arrows**). Note the proliferating astrocytes in all the layers of the hippocampus. The photomicrograph of the hippocampus of group 4 rats given 100 mg/kg of vitamin E plus 20 mg/kg of sodium arsenite indicated positive but weak staining for GFAP (**arrows**). The photomicrograph of the hippocampus of group 5 rats given 300 mg/kg of omega-3 fatty acid plus 100 mg/kg of vitamin E plus 20 mg/kg of sodium arsenite indicated positive but weak staining for GFAP (**arrows**). The GFAP immunoreactivity of positive cells in the hippocampus indicated a significant ($p < 0.05$) increase in group 2 and 3 respectively given when compared with the control. There is a significant ($p < 0.05$) decrease in group 2 and 3 respectively when compared with the group 2.

Plate 3: Section of the hippocampus. A. showed few astrocytes displaying positive but weak staining for GFAP. B. indicated positive and intense staining for GFAP. C. indicated positive but weak staining for GFAP. D. indicated positive but weak staining for GFAP. E. indicated positive but weak staining for GFAP. F. GFAP immunoreactivity of positive cells in the hippocampus,Values are expressed in mean ± standard error of mean, # **=** significantly different from control and other groups @$p < 0.05$, ** = significantly different from group 2 but non-significant with control@$p < 0.05$. (GFAP x400 magnification; Scale Bar: 1 mm = 100 µm).

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

Plate 4 represents the GFAP analysis of the prefrontal cortex. The photomicrograph of the of PFC of control rats given water and feed indicated showing few astrocytes displaying positive but very weak staining for GFAP (**arrows**). The photomicrograph of PFC of group 2 rats given 20 mg/kg of sodium arsenite indicated positive and intense staining for GFAP. The photomicrograph of the PFC of group 3

rats given 300 mg/kg of omega-3 fatty acid plus 20 mg/kg of sodium arsenite indicated positive but weak staining for GFAP (**arrows**). The photomicrograph of the PFC of group 4 rats given 100 mg/kg of vitamin E plus 20 mg/kg of sodium arsenite indicated positive but weak staining for GFAP (**arrows**). The photomicrograph of PFC of group 5 rats given 300 mg/kg of omega-3 fatty acid plus 100 mg/kg of Vitamin E plus 20 mg/kg of sodium arsenite indicated positive but weak staining for GFAP (**arrows**). The GFAP immunoreactivity of positive cells in the PFC showed a significant ($p < 0.05$) decrease in group 3,4 and 5 respectively compared to the control group.

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; E shows positive but weak staining for GFAP. F. GFAP immunoreactivity of positive cells in the PFC. Values are expressed in mean ± standard error of mean # **=** significantly different from control and other groups @$p < 0.05$,** = significantly different from group 2 @$p < 0.05$. (GFAP x400 magnification; Scale Bar: 1 mm = 100 µm).

DISCUSSION

Impairment of spatial memory is a well-established outcome of arsenic neurotoxicity, primarily achieved by oxidative stress, neuroinflammation, and the dysfunctional relationship between the hippocampus and the prefrontal cortex. In the current investigation, treatment with sodium arsenite ($NaAsO_2$) resulted in a substantial impairment of spatial working memory, antioxidant function, and neuronal integrity, but treatment with omega-3 fatty acids (ω-3 FA) as well as vitamin E resulted in a substantial protective effect.

The marked decrease in the percentage alternation and the prolongation of the arrival time latency in $NaAsO_2$-treated rats are clear indicators of the impairment of spatial working memory and exploratory efficacy. The Y-maze test is known to be sensitive to the integrity of the hippocampus and the prefrontal cortex, both of which are targets of oxidative and inflammatory challenges [33]. Arsenic has been shown to affect synaptic plasticity and cholinergetic function, both of which are necessary for the processing of spatial memory [12] (Tyler & Allan, 2014).

Supplementation with ω-3 FA or vitamin E caused significant improvement in percentage alternation relative to administration of $NaAsO_2$, indicating a reversal in cognitive deficit. It is important to mention that co-administration of ω-3 FA and vitamin E resulted in an improvement in arrival time latency, indicating a synergistic effect. Omega-3 fatty acids, particularly docosahexaenoic acid (DHA), have been known to increase neural fluidity, neurotransmission, and neurogenesis, whereas vitamin E has been shown to prevent lipid peroxidation, thereby protecting neural membranes from oxidative damage [34, 35].

Arsenic-induced neurotoxicity is closely linked to oxidative stress. The marked reduction in GPx and SOD activity and the increase in MDA level in the $NaAsO_2$-treated rat confirm that there was a substantial inhibition of the endogenous antioxidant defense system. Arsenic increases the level of ROS beyond

what the endogenous system can handle and also enhances lipid peroxide accumulation, causing damage to the neuronal membrane and resulting in malfunctioning of signal transmission [36].

Administration of ω-3 FA and vitamin E caused a significant improvement in GPx and SOD activity and a decrease in MDA contents, with a more prominent effect noticed with the combination therapy. Vitamin E, an antioxidant with high lipid solubility, directly decreases peroxyl radicals and mitigates lipid peroxidation chain reactions, thus limiting the formation of MDA [37]. Omega-3 fatty acids, despite having high unsaturations, have the paradoxical effect of lowering oxidative stress through modulation of redox-regulated transcription factors and increased expression of antioxidants [38]. These biochemical changes can explain the observed behavioral improvement in the Y-maze test.

Histopathological analysis further confirmed the results obtained from behavioral and biochemical analyses. The extent of neuronal pyknosis in the hippocampus and necrosis in the prefrontal cortex induced by $NaAsO_2$ treatment suggest arsenic-induced neuronal degeneration [39]. The nuclear contraction and chromatin condensation in these cells indicate neuronal damage, especially in the pyramidal cells that play a pivotal role in the processing of memories.

On the other hand, pyramidal neurons were maintained in ω-3 FA or vitamin E group rat brains, with a superior maintenance of cytoarchitecture offered by the combination of the two, with the latter having the most effective structural maintenance. This supports existing literature that omega-3 fatty acids increase pyramidal neuron survival as well as dendritic integrity, and vitamin E acts as an antioxidant agent in protecting neurons from oxidative lipid damage [34, 40]. This is could have led to the cognitive function improvements.

Reactive astrogliosis, evidenced by the high immunoreactivity of GFAP, is known to be a manifestation of neuroinflammation and injury. The high intensity of the GFAP stain in the hippocampus and prefrontal cortex of $NaAsO_2$-treated rats is, in fact, an indicator of high levels of activation of these astrocytes due to arsenic-induced injury mediated by oxidative damage. At the beginning, activation of the astrocytes is actually defensive, but overactivation has also been known to contribute to the pathophysiology of the injury together with the production of pro-inflammatory cytokines.

ω-3 FA and vitamin E treatment resulted in a substantial decrease in GFAP gene expression, signifying a reduced neuroinflammatory response. Omega-3 fatty acids are known to promote the conversion of astrocytes to an anti-inflammatory phenotype through the generation of pro-resolution mediators, while vitamin E inhibits redox-mediated inflammatory signaling [38, 37]. The normalization of GFAP gene expression, especially in the combined treatment, signifies a successful modulation of glial responsiveness.

Taken together, these results establish that sodium arsenite causes impairment in cognition due to oxidative damage, neurodegeneration, and activation of astrocytes in the memory-associated regions of the brain. Omega-3 fatty acids and vitamin E, when used adjunctively, lessened these changes, though the combination always had more significant benefits. These findings are likely due to the additive

effects of the three components, which act through membrane stabilization, antioxidants, and anti-inflammatory pathways.

Notably, the research emphasizes the possible benefits of a combination of nutritional antioxidants in countering neurotoxicity provoked by the environment. The impact of arsenic poisoning in the developing world, for instance, could be greatly alleviated based on these discoveries to offer a complementary approach in maintaining neuroprotection.

This research indicates an innovative approach to targeting sodium arsenite -induced neuronal damage, demonstrating that the combination of vinpocetine and vitamin E can concommitantly modulate cognitive function, oxidative stress, and neuroinflammation.

Limitations

This study has limited sample size, which may affect the validity of the results. Acute biochemical assessments were performed, but chronic and longitudinal effects remain unclear. Molecular mechanisms such as NF-κB, Nrf2, and BDNF signaling were not explored.

CONCLUSION

The combination of omega-3 fatty acids and Vitamin E has a strong, synergistic neuroprotective effect on working memory impairment by sodium arsenite, probably via a combination of antioxidant and anti-inflammatory effects. Such a combination could be useful in the management of environmentally induced neurotoxicity.

Abbreviations

α-tocopherol – Alpha-tocopherol

AE-FUNAI – Alex Ekwueme Federal University, Ndufu-Alike Ikwo

ANOVA – Analysis of Variance

BDNF – Brain-Derived Neurotrophic Factor

CLT – Classic Labyrinth Test

CREB – cAMP Response Element-Binding Protein

DHA – Docosahexaenoic Acid

DTT – Dithiothreitol

EDTA – Ethylenediaminetetraacetic Acid

EPA – Eicosapentaenoic Acid

FCS – Fetal Calf Serum

GFAP – Glial Fibrillary Acidic Protein

GPx – Glutathione Peroxidase

H&E – Hematoxylin and Eosin

HRP – Horseradish Peroxidase

MDA – Malondialdehyde

ML – Molecular Layer

$NaAsO_2$ – Sodium Arsenite

NF-κB – Nuclear Factor Kappa-B

NMDA – N-Methyl-D-Aspartate

NMDAR1 – N-Methyl-D-Aspartate Receptor Subunit 1

Nrf2 – Nuclear Factor Erythroid 2–Related Factor 2

PCL – Pyramidal Cell Layer

PFA – Paraformaldehyde

PFC – Prefrontal Cortex

PL – Polymorphic Layer

PPARγ – Peroxisome Proliferator-Activated Receptor Gamma

PUFAs – Polyunsaturated Fatty Acids

ROS – Reactive Oxygen Species

SEM – Standard Error of the Mean

SOD – Superoxide Dismutase

Vit E – Vitamin E

ω-3 FA – Omega-3 Fatty Acids

Declarations

Ethical approval

All procedures complied with ethical guidelines for animal experimentation and were approved by the Faculty of Basic Medical Sciences Research Ethics Committee at AE-FUNAI, referencing AE-FUNAI-2024/00967. Humane handling practices were strictly followed to minimize distress.

Consent for publication

Not applicable.

Competing Interest

The authors declared that there are no conflicts of interests.

Authors contribution

We declare that this work was done by the author(s) named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Uduak A. Inwang wrote the first draft and coordinated the research. The protocol was designed by Uduak A. Inwang and Elisha U Ogwo, Kubiat T. Umana, Emmanuel O. Nwuzor , Ekakitie O. Obruche, and Bakumor S. Yolo to facilitate sample collection. Charles I. Aigbuduokhai and Martins K. Agogo carried out statistical analysis. Uduak A. Inwang, Elisha U Ogwo, Obinna O. Uchewa, Tochukwu N. Nnama, and Monday O. Aleke supervised the work.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

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Plates

Plates are available in the Supplementary Files section.

Figures

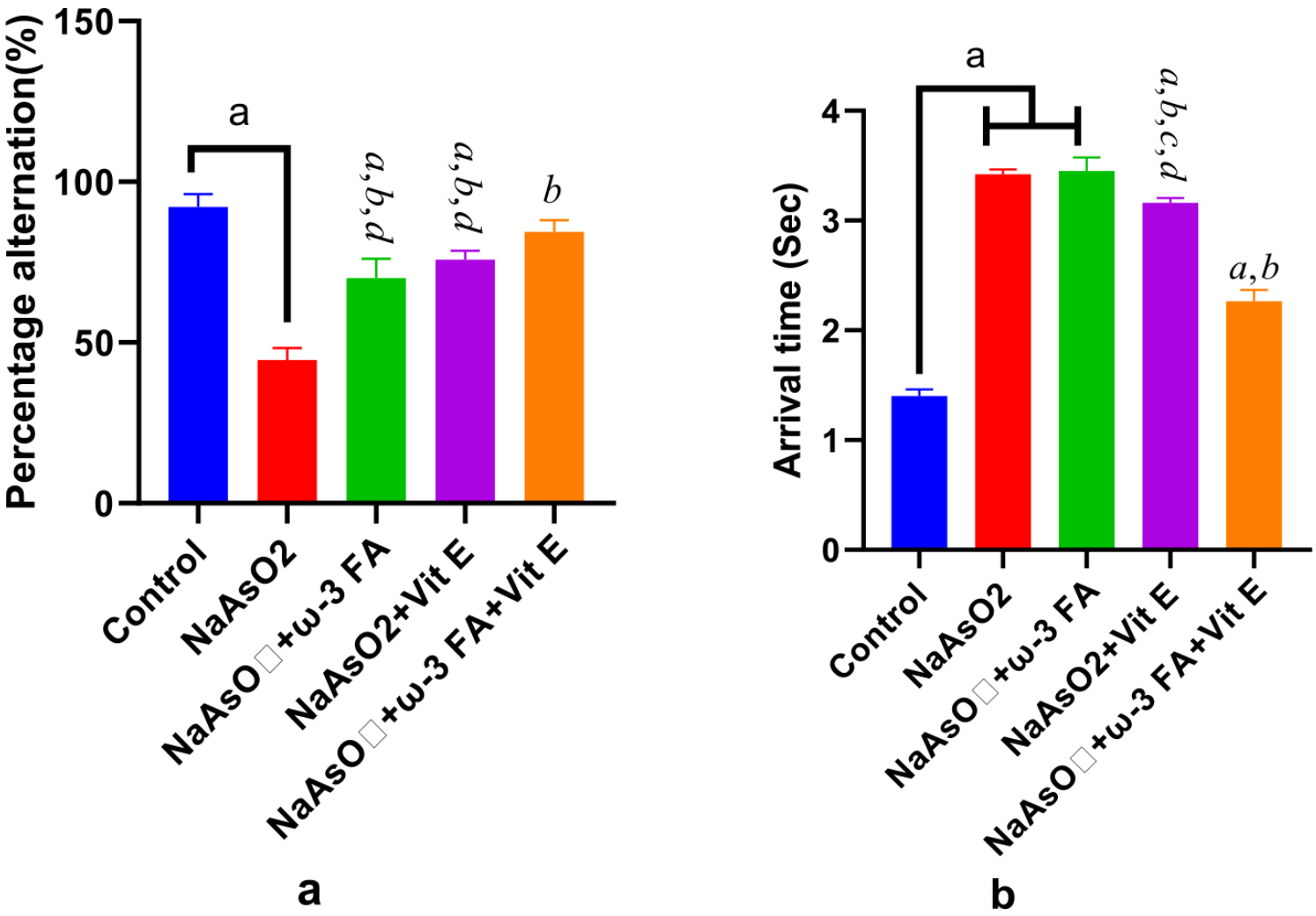


Figure 1

Effect of dietary omega-3 fatty acid monotherapy and synergy on. A. Percentage Alternation (%) in Y-Maze. B. Arrival time. a =($p<0.05$, Positive Control); b=($p<0.05$,$NaAsO_2$ group);c= ($p<0.05$, $NaAsO_2$ + Vit E group), d= ($p<0.05$, $NaAsO_2$ + ω-3 FA + Vit E group) using one-way ANOVA followed by Turkey post-test. Each data represented mean ± SEM (n=5)

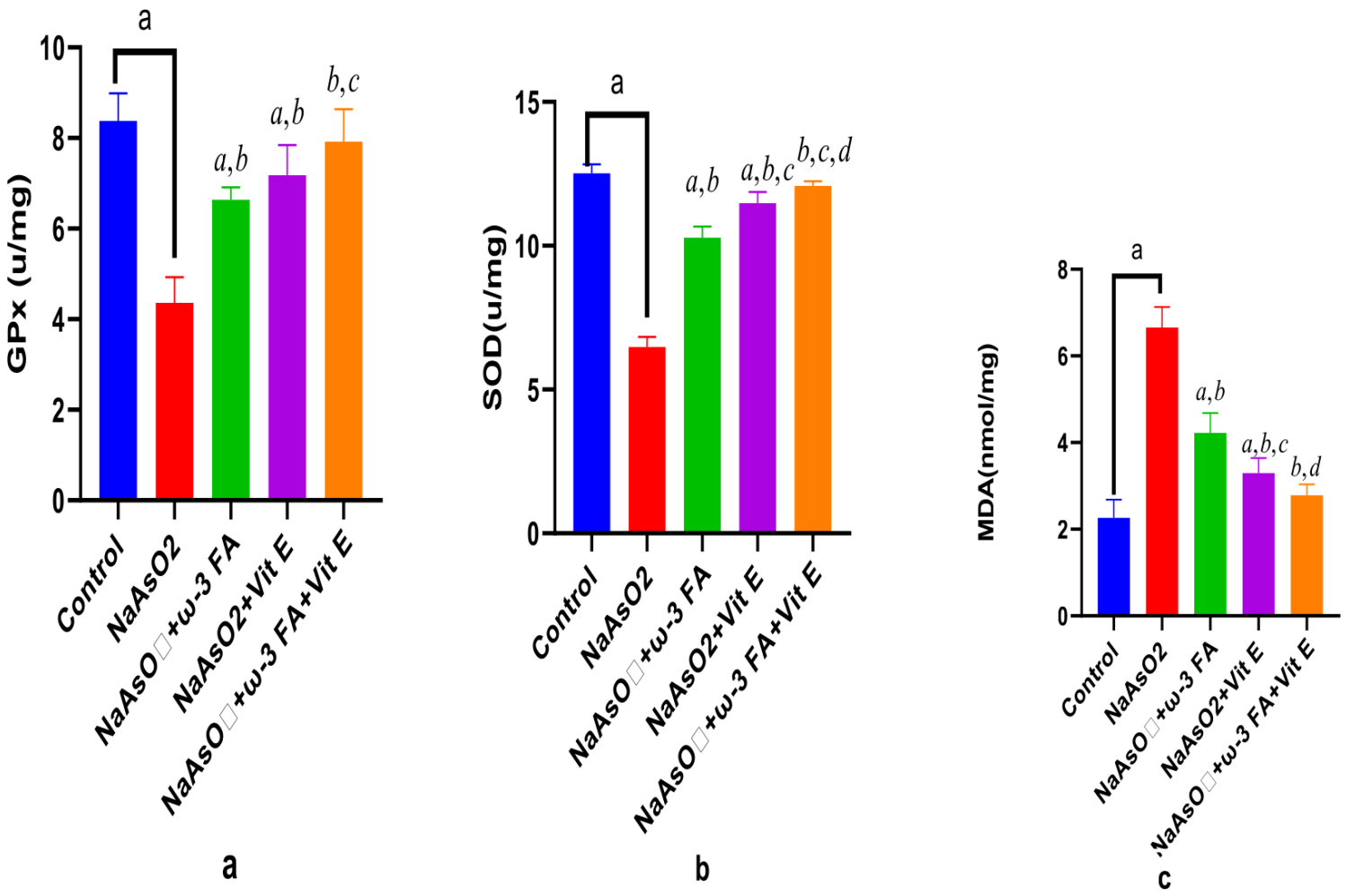


Figure 2

Effect of dietary omega-3 fatty acid monotherapy and synergy on a. GPx activity, b. SOD activity, and c. MDA activity. Results are expressed as mean±SD (n=5). The results were analyzed by one-way ANOVA followed by Turkey post hoc tests. a =($p<0.05$, Positive Control); b=($p<0.05$,$NaAsO_2$ group);c= ($p<0.05$, $NaAsO_2$ + Vit E group), d= ($p<0.05$, $NaAsO_2$ + ω-3 FA + Vit E group).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Plate1.png
- Plate2.png
- Plate3.png
- Plate4.png