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Omega-3 Fatty Acids Protects Against Sleep Deprivation- Induced Stress, Anxiogenic Behavior and Histoarchitectural Changes in Wistar Rats' Basolateral Amygdala (BLA)

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ABSTRACT

Introduction: Chronic sleep deprivation is linked to deleterious changes in several brain regions. However, omega-3 fatty acids (OFA) have been reported for its anti-inflammatory, antioxidant and possible neuroprotective properties.

Aim: The study aimed to evaluate the neuroprotective effect of omega-3 fatty acids on sleep deprivation-induced basolateral amygdala (BLA) toxicity.

Methods: Twenty male Wistar rats (150-200g) divided into four groups of five rats each were used. Group (I) received 2ml/kg body weight of distilled water, while groups (II-IV) were sleep deprived for 18 hours daily. Additionally, group (III & IV) were administered different dosage of OFA (200 & 400 mg/kg body weight respectively) orally for 21 days. Elevated plus maze (EPM) test was conducted to assess anxiety-like behavior. Blood for biochemical assay was collected, and the harvested brains were processed for histological and immunohistochemical studies.

Results: The sleep-deprived group showed no significant difference ($p < 0.05$) in anxiety-like behavior compared to the control. However, the group administered OFA (200 mg/kg body weight) showed anxiolytic behavior in the EPM compared to the SD and control groups. Sleep deprivation significantly increased corticosterone level compared to the control. However, a significant reduction in corticosterone level was recorded in the OFA treated groups compared to the sleep deprived only group. Histoarchitectural distortion as well as astrogliosis were observed in the BLA of SD. However, preservation of normal histoarchitecture of the BLA and normal astrocytic morphology were observed in the treated groups.

Conclusion: Taken together, this study revealed that omega-3 fatty acids protect against stress response and BLA alterations in the sleep-deprived rat model.

Keywords: Omega-3 fatty acids; Sleep deprivation; Basolateral Amygdala; Anxiety-like behavior; Corticosterone; Histopathology

INTRODUCTION

Sleep has survived ubiquitously throughout of mammalian history, emphasizing a key functional core to its existence (Rechtschaffen, 1998). Humans spend approximately one-third of their lives sleeping, existing in two alternating phasic pattern of non-rapid eye movement (NREM) and rapid eye movement (REM) sleep (Kroeger *et al.*, 2016; Ingiosi *et al.*, 2020). However, increasing demand of modern technological society for vocational or lifestyle reasons leave about one-third of the world population sleep-deprived, such that sleep deprivation is now a major public health concern (Que *et al.*, 2023).

According to the National Institute of Health, a typical adult need about 7- 8 hours of sleep daily to maintain peak physical and mental health (Ohayon

et al., 2017). Getting less than the required amount of sleep in terms of duration and quality, relative to one's physiological or developmental need, which may occur as a total lack of sleep over a short period of time (acute) or persist as partial lack of sleep over an extended period of time (chronic) is termed sleep deprivation (Medic *et al.*, 2017; Bandyopadhyay & Sigua, 2019).

Chronic sleep deprivation is pervasive in modern society, with about 40% of United States population reporting less than 7 hours of sleep most night (National Sleep Foundation, 2015). Similarly, more than 25% of Nigerians are sleep-deprived according to a study conducted by Ogunsemi and colleagues (Ogunsemi *et al.*, 2020). Sleep deprivation results in impaired functions in the brain regions involved in learning, cognition and

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emotion, such as the prefrontal cortex, hippocampus and the amygdala (McEwen, 2006; Penev, 2007). The amygdala is the center of emotional circuit, regulating emotional memory consolidation and reactivity especially relating to stress and fear (Rexrode *et al.*, 2023). Dendritic spine remodeling and synaptic downscaling has been reported in the neurons of the amygdala during sleep, thereby regulating emotional behavioral outcome. As a consequence, worsening mood such as induced anxiety state has been reported to be one of the most consistent behavioral depiction of sleep deprivation, and may be a risk factor for prevailing neuropsychiatric behaviors in individuals (Krystal, 2012; Lo *et al.*, 2016).

Omega-3 fatty acids have been reported for its numerous roles in brain functions and structure (Wu *et al.*, 2004; Scorza *et al.*, 2013). These dietary lipids existing naturally as fish oil and consisting mainly of docosahexanoic acid (DHA) and eicosapentanoic acid (EPA), carry out several modulatory roles by integrating into the phospholipid membranes structurally and functionally (Kidd, 2007; Alzoubi, & Aldomi, 2017). By optimizing the membrane fluidity, omega-3 fatty acid has been reported to elicit antioxidant, anti-inflammatory and antiapoptotic cellular processes in the brain, thereby enhancing neuroplasticity, cognition and protection against neurological lesions (Sullivan *et al.*, 2018; Shi *et al.*, 2019; Liu *et al.*, 2021). Specifically, DHA involvement in emotional regulation, exploratory and cognitive activities, and the correlation of its deficiency with hyperactivity-impulsivity, anxiety-like behavior and learning difficulty both in humans and animals, has been reported (Fedorova & Salem, 2006; Kidd, 2007; Song *et al.*, 2016).

The present study therefore, aim to evaluate the neuroprotective effect of omega-3 fatty acid on anxiety-like behavior as well as structural and functional changes in the basolateral amygdala of adult male Wistar rats, as related to stress response, mood changes as well as neurological lesions following sleep deprivation.

MATERIALS AND METHODS

Ethical approval

Ethical approval for the study was obtained from the Ahmadu Bello University Ethics Committee on Animal Use and Care (ABUCAUC/2024/034).

Experimental animal maintenance

A total of twenty (N=20) apparently healthy male Wistar rats weighing between 150-200g, were randomly selected into groups by blocking technique and used for this research. The rats were acquired from the Animal House, Department of Human Anatomy, Faculty of Basic Medical Sciences, Ahmadu Bello University, Zaria. They were housed in clean cages and provided with a standard pellet diet (rat chow-vital feed) and unrestricted access to clean drinking water.

Sample Size Determination

In order to obtain the sample size, the resource equation ($E = N/k$) was used, where E is the sample size, N represent the total animals and k is the number of groups.

Experimental design

The animals were divided into four groups of five rats ($k=4$; $n=5$). Group 1 rats served as the control group and received 2ml/kg body weight of distilled water throughout the experiment. Group II rats were subjected to 18 hours of sleep deprivation from 2:00 pm to 8:00 am daily. Group III were administered 200mg/kg body weight (4 % of LD₅₀) of Omega-3 fatty acids following sleep deprivation for 18hours (2pm-8am) daily. Likewise, Group IV received 400 mg/kg body weight (8 % of LD₅₀) of Omega-3 fatty acids following sleep deprivation for 18 hours (2 pm-8 am) daily. All administrations were done orally using a gavage, and the experiment lasted for 21 days. In order to avoid bias, research assistants who were masked from the identity of the groups were employed to carry out administration, neurobehavioral observation and data collection as well as data analysis under the guidance of the investigator.

REM-sleep deprivation procedure

In this study, the modified multiple platform method targeting the rapid eye movement (REM) sleep was employed to induce sleep deprivation in the rats. The setup consisted of a water tank made of Plexiglas material, measuring 127.0 cm in length, 44.0 cm in width, and 45.0 cm in height. Inside the tank, there were 10 cylindrical platforms, each 6 cm high and 6.5 cm in diameter. These platforms were spaced horizontally and vertically at distances of 13 cm and 10 cm, respectively. The tank was filled with water up to 1 cm of below the platform. Five Wistar rats, from the same home cage, were placed in each tank for a duration of 18 hours daily. During this time, they had access to water and food as desired. The loss of muscle tone associated with REM-sleep cause the rats to wake rapidly on touching the water once they enter this sleep phase. Notably, this model did not socially restrict the rats' movement or isolate them (Javad-Moosavi *et al.*, 2017).

Neurobehavioral Study

The elevated plus maze; a cross-like maze with two open and two closed arms (20-cm-tall walls on the closed arms) which is elevated 50cm above the ground was used for this test. On the last (21st) day of the experiment, after the acclimatization period of 15 minutes in the neurobehavioral study room, the test was conducted under dim light conditions. Each rat was placed in the central square, with the head facing the open arm, opposite to where the investigator was and its exploratory behavior was observed for 5 min. During that time, the number of open- and closed-arm entries as well as the time spent in each arm was recorded with an overhead camera, placed at the top of the maze. Entry into an arm was defined as having occurred when the animal placed all four limbs onto the arm. The maze was cleaned with a 70% ethanol solution in between rats to avoid possible interferences caused by the previous animal's odor or residues.

Frequenting the closed arm as well as spending more time the closed arm suggests anxiogenic behavior while frequenting the open arm as well as spending more time in the open arm suggest anxiolytic behavior (Walf & Frye, 2007).

Animal Sacrifice

At the end of the experiment, the animals were humanely euthanized using an intraperitoneal injection of 75 mg/kg body weight of ketamine hydrochloride. Once asleep, the animals were decapitated and the skulls carefully opened along the midline and the brains were removed and fixed by immersion in 10% formal saline solution for 24 hours to ensure proper fixation. Following fixation, the prepared tissue samples were taken to the histology laboratory at the Department of Human Anatomy, Ahmadu Bello University in Zaria for further processing.

Blood samples were also collected, aliquoted and the sera were stored at -20 degrees for hormonal assay of corticosterone using the ELISA kit as directed by Fine Test.

Determination of Corticosterone Level

The blood sample was centrifuged at 3,000 rpm, for 15 min. The obtained blood serum was aliquoted and stored at -20°C. The determination of cortisol level as performed using the ELISA kit as directed by manufacturer. Quantitative test results were obtained by measuring and comparing the absorbance reading of the wells of the samples against the standards with a microplate reader at 650 nm. The values of the samples were interpolated on the standard curve to obtain the corresponding values of the concentrations expressed in ng/ml.

Tissue Processing

This was done by passing the tissue through ascending grades of alcohol of 70%, 90% and 95% and 100% for dehydration for two changes of (50 mins) each respectively. It was then passed into xylene for 40 minutes to clear the alcohol and make it mixable with molten paraffin wax. After this, tissue was transferred into molten paraffin wax for infiltration. Embedding commenced with the use of molten paraffin as the embedding media at a temperature of 60 degrees for 40 minutes. After the tissue were embedded in paraffin wax, they were mounted on wooden blocks and then sectioned as follows; the blocked tissues were affixed to a rotating microtome and trimmed to get an even surface prior to sectioning. Thin section 8 - 10 microns each were then cut and the sections were floated on warm water bath to spread the tissue properly prior to mounting on a glass slide. The tissues were then passed through changes of xylene for dewaxing and then taken to water by passing the slide with the tissue intact through descending grades of alcohol-absolute, 100%, 95%, 90%, and 70%, two changes for each and finally water. The tissues were stained with hematoxylin for twenty-five minutes, the excess of which was rinsed off under running tap water. Counter staining for hematoxylin was then done using eosin, the excess of which was also rinsed off under tap water. After proper staining, the sections were dehydrated through ascending grades of alcohol,

followed by clearing with xylene. DPX was used as mountant and the slide were covered with cover slip (Feldman & Wolfe, 2014).

Immunohistochemistry

Immunohistochemistry was done at histopathology laboratory, Ahmadu Bello University teaching hospital Zaria. Prepared brain slides were labelled with a pencil and placed in plastic slide racks. They were incubated for 20-30mins at 60 degrees to dewax. Sections were deparaffinized in two changes of xylene for 5 minutes each and were hydrated by passing through ascending grades of alcohol and finally distilled water. Antigen retrieval was done by incubating sections in 10mM citrate buffer at a PH of 6, for 20minutes in a microwave; this was done to open the binding site of tissues for stain adherence. The section was incubated in methanol/H₂O₂ solution for 25minutes to inactivate endogenous peroxidase activity. Sections are rinsed in 1x PBS three times for 5 minutes each after which a Peroxidase-Anti-Peroxidase pen is used to circle the tissue to prevent overflowing of antibodies and was further incubated in 2% Phosphate buffered solution milk for 1 hour. Incubation with primary antibody specific for GFAP followed and secondary chromogenic antibody for visualization under light microscope.

Micrography and Photomicrography

Histological and immune-histochemical examinations for pathological changes were carried out on the stained brain tissues using Rats Brain Atlas by Paxinos and Watson (Paxinos & Watson, 2007) as a guide to locate the basolateral amygdala with the aid of light microscopy (Optical Microscope; HM-LUX, Leitz Wetzlar, Germany). Photomicrographs of the region of interest were obtained using Digital Microscopic Camera, MA 500 AmScope®, USA. These were conducted in the Microscopy and Stereology Research Laboratory section of the Department of Human Anatomy, Ahmadu Bello University, Zaria.

Staining Intensity

The Images were imported into the Image J software and converted to gray scale. "Thresholding" was done via the image tool-bar, the regions of interest were mapped out sufficiently pick stained areas. The images were quantified using mean gray value. The areas of staining interest were analyzed and result was then exported to graph-pad for further analysis.

Data Analysis

The data analysis was conducted using GraphPad Prism software (version 9.3.1), and IBM SPSS (version 23). The results were reported as the mean $\pm$ standard error of the mean (SEM). Statistical comparisons between means were performed using one-way analysis of variance (ANOVA), and Turkey post hoc test was carried out as appropriate. Value of $p < 0.05$ was considered statistically significant. The results were appropriately presented in graphs.

RESULTS

Omega-3 Fatty Acids Reduces Anxiety-like Behavior Following Sleep-Deprivation

The result revealed no significant change in anxiety-like behavior in the SD group compared to the control. However, a significant increase ($p < 0.05$) in both frequency of entry as well as time spent in the open arm (anxiolytic behavior) of the EPM in the group administered 200mg/kg bodyweight (bw) of omega-3 fatty acids following SD when compared to the SD only and control groups (Figures 1a & 1b). Similarly, there was significant ($p < 0.05$) decrease in the time spent in the closed arm of the EPM in the Wistar rats administered 200mg/kg.bw of Omega-3 fatty acids following SD when compared with the control (Figure 1d). Conversely, there was no significant ($p < 0.05$) difference in the frequency of entry into the closed arm across all groups (Figure 1c). Similarly, no significant ($p < 0.05$) difference in grooming and rearing of rats across all groups in the EPM, although, grooming was decreased in both groups administered Omega-3 fatty acids following SD with the 200mg/kg fatty acids group showing the least grooming frequency compared to the SD group, while rearing frequency was also found to be decreased in rats administered omega-3 fatty acids following SD when compared to the SD group (Figure 1e and 1f).

Omega-3 Fatty Acids Attenuate Serum Corticosterone Levels in Wistar Rats Following SD.

Biochemical assessment of the serum corticosterone level in Wistar rats following SD revealed a significant ($p < 0.05$) increase in serum corticosterone level in SD only group when compared to the control. However, the group that was administered 200mg/kg.bw of omega-3 fatty acids showed significant decrease ($p < 0.05$) in serum corticosteroid level when compared to the SD only group (Figure 2).

Omega-3 Fatty acids preserve the General Histoarchitecture of the BLA Following SD-Induced Distortion

Histological (H&E) examination of Wistar rats' basolateral amygdala (BLA) of the control group demonstrated normal histoarchitecture of the region with the basic pattern of the nuclei consisting of large neurons (pyramidal and granule cells) loosely packed in a cortical-like manner (Figure 3). However, the examination of BLA of Wistar rats subjected to sleep deprivation only showed extensive pathological changes in the normal histoarchitecture of the region such as condensation of neuronal nuclear materials (pyknosis), vacuolations around the neurons (perineuronal vacuolations) as well as dead neurons (necrosis) (Figure 3). Conversely, the BLA of Wistar rats treated with omega-3 fatty acids (200mg/kg) following sleep deprivation showed preservation of normal histology of the region as compared to the control group (Figure 3), while the group treated with omega-3 fatty acids (400mg) following sleep deprivation demonstrated considerable restoration, however mild distortion in the histology of the region could be noticed, including vacuolations and karyorrhexis.

Omega-3 Fatty Acids Regulates Astrocytes Activation in the BLA of Wistar Rats Following Sleep Deprivation

Immunohistochemical examination of the BLA of Wistar rats in the present study revealed normal expression of GFAP-positive astrocytes in the control group (Figure 4A). Conversely, an increased expression of GFAP-positive astrocytes indicative of reactive astrocytes was observed in the sleep-deprived only rats (Figure 4B). However, the rats administered 200mg/kg.bw of Omega-3 fatty acids showed limited invasion of GFAP-positive astrocytes indicating a regulated astrocytic function in the BLA (Figure 4C). On the other hand, the group administered 400mg/kg.bw of Omega-3 Fatty acids revealed similar expression of GFAP-positive astrocytes to that observed in the SD only group characterized by increased expression of reactive astrocytes (Figure 4D).

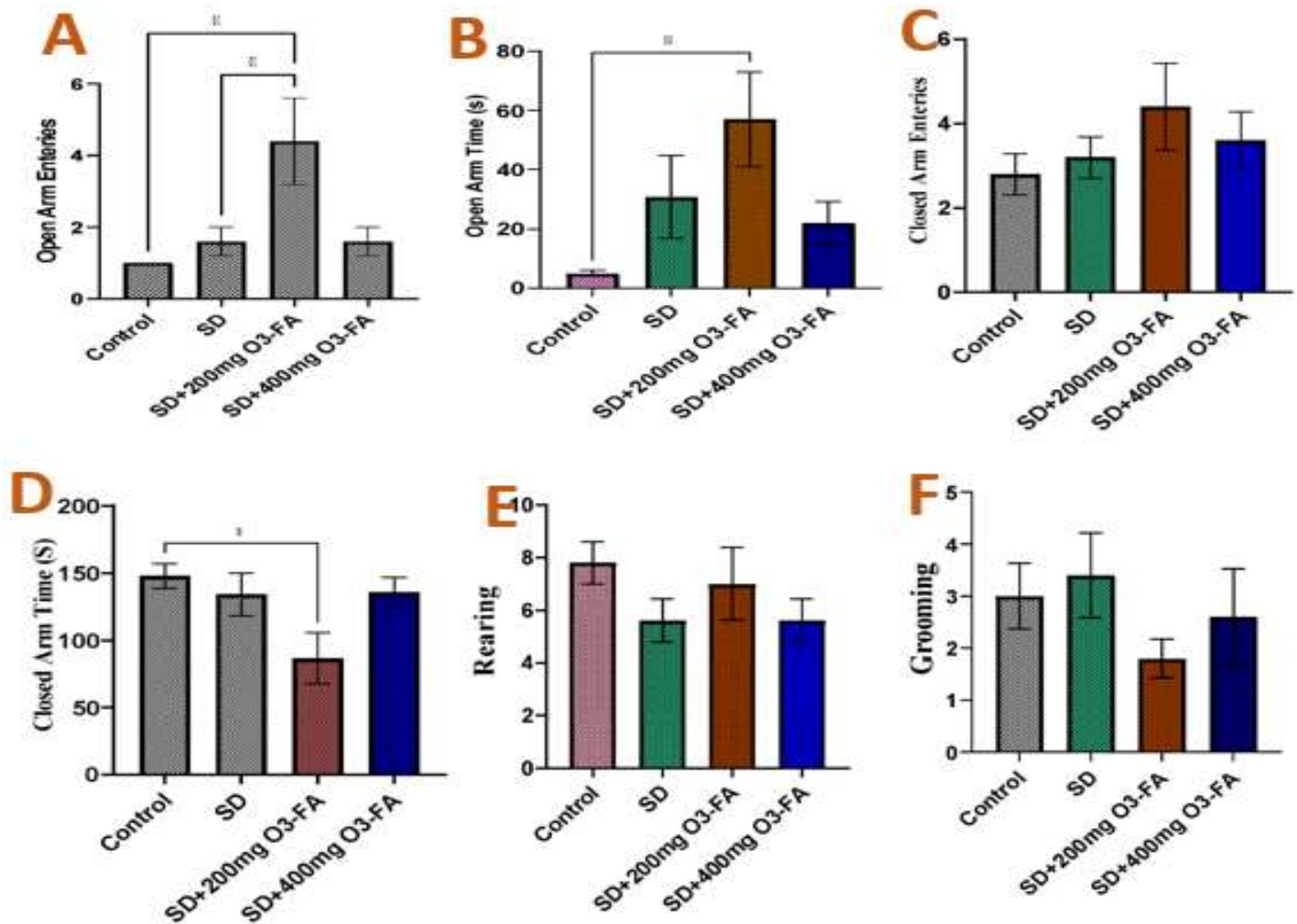


Figure 1: Effect of Omega-3 fatty acids on anxiety-like behavior of adult male Wistar rats following sleep deprivation in the elevated plus maze. (A) Frequency of entries into the open arm of the elevated plus maze. (B) Time spent in the open arm of the elevated plus maze. (C) Frequency of entries into the closed arm of the elevated plus maze. (D) Time spent in the closed arm of the elevated plus maze. (E) Rearing of rats in the elevated plus maze. (F) Grooming frequency of rats in the elevated plus maze. SD = Sleep deprivation; O3-FA = Omega 3 fatty acid. (*= $p < 0.05$; one-way ANOVA followed by Tukey's *post hoc* test).

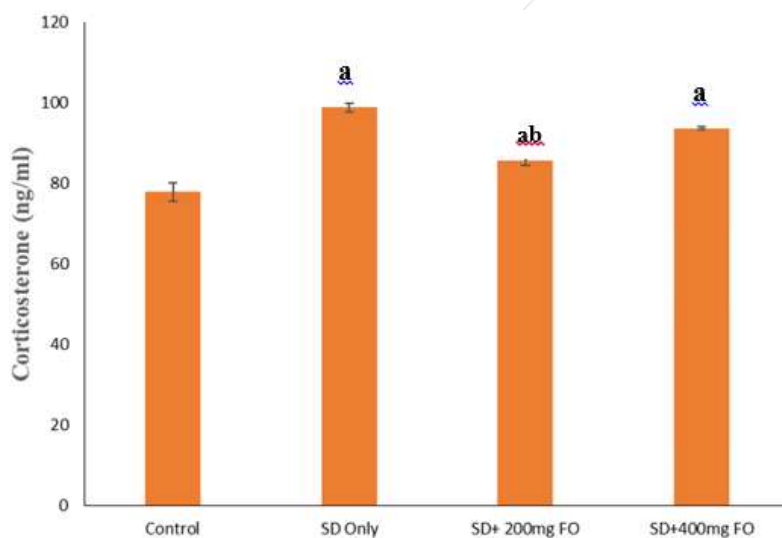


Figure 2: Effect of Omega-3 fatty acids (fish oil) on Serum Corticosterone of Adult Male Wistar rats Following Sleep Deprivation. $n = 5$, mean $\pm$ SEM, One-way ANOVA, $a = p < 0.05$ significance difference when compared to control; $b = p < 0.05$ significance difference when compared to SD only. SD= Sleep-Deprived; FO= Fish Oil (Omega-3 fatty acids).

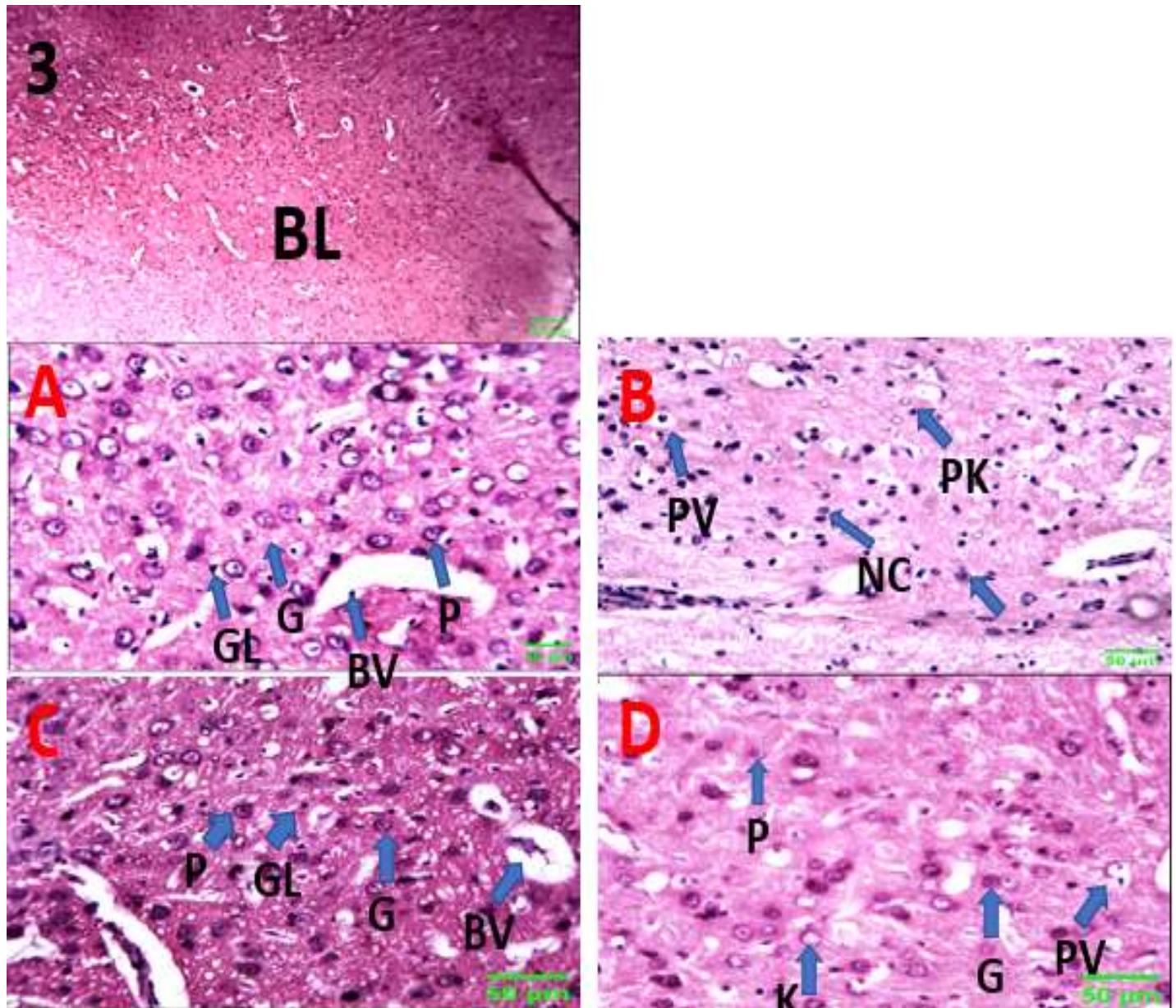


Figure 3: Representative Photomicrograph of the basolateral amygdala (BLA) region. (3) Coronal section of adult male Wistar rats' cerebral hemisphere of the control group showing the location of the basolateral amygdala (BL) (H&E; 50µm). (A) Coronal section through the BLA of control (sleep-rested) group showing normal histoarchitecture of the BLA consisting of pyramidal neurons (P), granule cells (G), glial cells (GL) and blood vessels (BV) (H &E; 50µm). (B) Section through the BLA of sleep deprived (SD only) group showing pathological changes such as perineuronal vacuolations (PV), pyknosis (PK) and necrosis (NK) and pyramidal neuron (P) (H&E; 50µm). (C) Coronal section through the BLA of 200mg/kg Omega-3 fatty acids treated group following sleep deprivation showing preserved histoarchitecture of the BLA similar to the control with pyramidal neuron, granule cells, glial cells and blood vessels (P; G; GL; BV) (H&E; 50µm). (D) Coronal section through the BLA of 400mg/kg of Omega-3 fatty acids following sleep deprivation showing normal histological features together with mild distortion such as Karyorrhhexis (K) (H&E; 50µm).

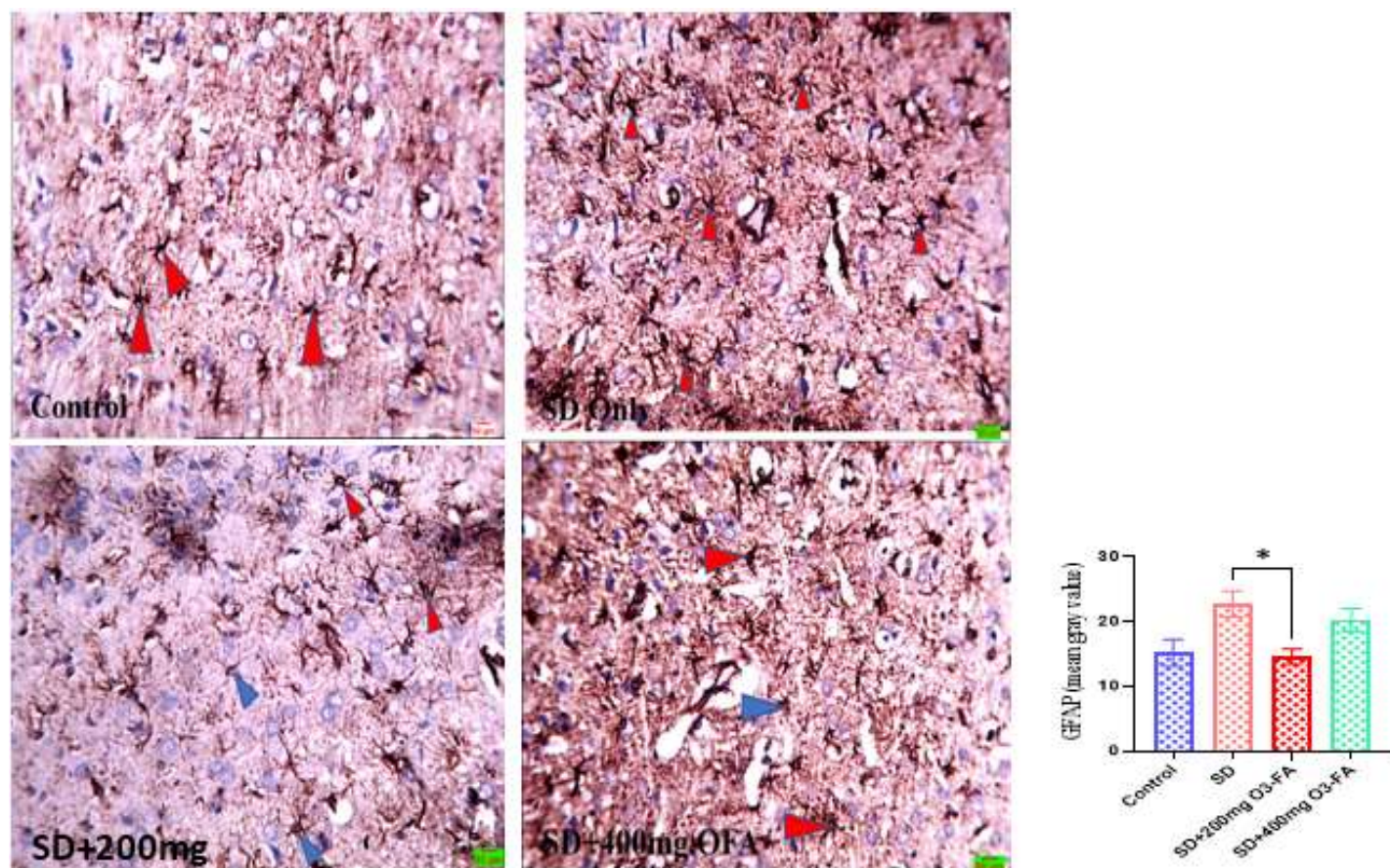


Figure 4: Representative photomicrograph of the basolateral amygdala (BLA) region, showing; Coronal section through the BLA of **control** (sleep-rested) group showing presence of numerous resting astrocytes (red arrow); Coronal section through the BLA of sleep deprived (**SD only**) group showing increased expression of activated astrocytes (red arrow); Coronal sections through the BLA of Omega-3 fatty acids treated groups (**SD+200mg** and **SD+400mg OFA**) showing reduced expression of activated astrocytes and increase expression of activated astrocytes respectively (GFAP stain; 50 μ m). The graph shows the mean grey value of GFAP staining intensity in the groups. SD = Sleep deprivation; OFA = Omega-3 fatty acid. (*= $p < 0.05$; one-way ANOVA followed by Tukey's *post hoc* test).

DISCUSSION

In the present study, the neuroprotective effect of Omega-3 fatty acids on the BLA of Wistar rats following SD was evaluated using neurobehavioral, biochemical, and microscopic assessments.

The regulatory effect of Omega-3 fatty acids and their derivatives such as DHA, EPA and ALA on the central nervous system (CNS) processes such as neuroinflammation, neurotransmission, synaptic plasticity, neurogenesis, neurodegeneration and thereby cognition, mood and behavior has been reported (Lange, 2020). These numerous neuro-modulatory functions of omega-3 fatty acids have been attributed to its occurrence as an integral component of the cell membrane and its potential to integrate into the membrane phospholipid bilayer, thereby increasing the membrane fluidity, and mediating numerous inter, and intracellular signal transduction (DiNicolantonio & O'Keefe, 2020). Consequently, Omega-3 fatty acids deficiency was associated with many neurological disorders, including Alzheimer's disease, major depression and anxiety disorder (Lange, 2020).

The results from the elevated plus maze test in this study revealed no significant difference in all the variables i.e frequencies and time spent in the open and closed arms, as well as grooming and rearing in the sleep deprived (SD) group, when compared to the control group. The similarity in behaviors observed in the SD groups in this study in comparison to the control is in disparity with previous studies reporting anxiogenic behavior in animals after sleep deprivation (Silva *et al.*, 2004, Nair *et al.*, 2011). However, this result is line with the study of Zareian and Nategh, (2020), who also reported no change in anxiety-like behavior in adult male Wistar rats following sleep deprivation. According to the study, the result may be due to reduced sleep requirement in adult animal, which may result in increased resistance to SD effect in adult rats compared to the young rats which were found to be anxiogenic in the same study. Furthermore, even though human research has produced consistent results, animal studies on anxiety-like effect of sleep deprivation using EPM have produced inconsistent results (Silva *et al.*, 2004; Pires *et al.*, 2016), while some produced anxiogenic effects (Silva *et al.*, 2004; Nair *et al.*, 2011), others have produced anxiolytic effect (Suchecky *et al.*, 2002, Zielinski *et al.*, 2013), leading to questions about the translational possibility of animal model of anxiety to human.

However, the group treated with 200mg/kg of omega-3 fatty acids following SD showed anxiolytic behavior in the elevated plus maze by significantly ($p < 0.05$) frequenting and spending more time in the open arm when compared to the SD and control rats, as well as spending significantly ($p < 0.05$) less time in the closed arm when compared to the control, with no difference found in their grooming and rearing across all the groups. The anxiolytic behavior exhibited by the rats treated with 200mg/kg omega-3 fatty acids could be due to its ability to prevent hyperactivation of the hypothalamic-pituitary-adrenal axis induced by sleep deprivation, by attenuating the effects of stress hormone (corticosterone) on dendritic structure and neuronal activity of the basolateral amygdala. Additionally, Omega-3 fatty acids have been reported to increase serotonin levels in the brain of stressed rats (Vancassel *et al.*, 2008), which in turn may reduce neuronal excitability in the bed nucleus of stria terminalis by activation of the 5-HT-1A and 5-HT-1B. Thus, reducing anxiogenic behavior in stressed rats. This agrees with works of Wu *et al.* (2020) and Neto *et al.* (2022) who both reported anxiolytic behaviors in stressed rats following omega-3 fatty acids supplementation.

Several studies carried out on human and animals have reported an effect of both acute and chronic sleep deprivation on cortisol/corticosteroid levels as the end product of hypothalamic-pituitary-adrenal axis activity (Omisade *et al.*, 2010; Thorsley *et al.*, 2012; Song *et al.*, 2015; Wright *et al.*, 2015).

Similarly, result obtained from this study showed a significant increase in serum corticosterone level in all the groups that were subjected to sleep deprivation (SD) when compared to the control. This suggests a stress effect of sleep deprivation on the experimental rats. The elevated level of corticosterone hormone observed in all the groups that underwent sleep deprivation may be due to the possibilities of sleep deprivations induced hyperactivation of the basolateral amygdala and/or bed nucleus of stria terminalis by plastic neuronal changes, which significantly increased hypothalamic-pituitary-adrenal (HPA) axis activity, and thus, plasma corticosterone levels (Pérez *et al.*, 2013). This is in agreement with the work of Xing *et al.*, (2021), which found that the SD rats showed higher levels of corticosterone than the control rats did, indicating that SD may be an activator of the HPA axis responses.

On the other hand, supplementation with 200mg/kg omega-3 fatty acids significantly reduce the corticosterone hormone level when compared with the sleep deprived (SD) group without omega-3 supplementation. This is in agreement with the work of Oravcová *et al.* (2022), which reported a significant reduction in cortisol level in children with depression following long term supplementation with omega-3 fatty acids. This could be due to omega-3 fatty acids supplementation increasing DHA content in the phospholipid membranes of excitatory glutamatergic neurons of the amygdala. This in turn decreases arachidonic acid (AA) levels in the cytoplasm of these neurons. Thus, inhibitory transmission could be enhanced in the amygdala decreasing the serum corticosterone levels in the sleep deprived rats. Additionally, inflammation has been reported to have endocrine

and metabolic effects (Agorastos *et al.*, 2014). Elevated level of various pro-inflammatory cytokines has been reported in sleep deprivation. Therefore, Omega-3 fatty acids ability to inhibit toll-like receptor-4 (TLR4) and tumor necrosis factor receptor (TNFR), thereby inhibiting the expression of pro-inflammatory factors, which may be regulatory to reducing plasma level of stress hormone in SD rats (Cao *et al.*, 2020). Furthermore, Omega-3 fatty acids can also increase the expression of microglia M2 phenotype, thereby promoting the resolution of inflammation (Gutiérrez *et al.*, 2019; Liu *et al.*, 2021).

Histopathological changes indicative of perineuronal vacuolations and pyknosis was observed in the cytoarchitecture of the basolateral amygdala of sleep-deprived rats when compared to the control. This may be due to SD-induced neuroinflammatory processes and microglia-activation during sleep deprivation, which have been reported to have numerous effects on the structure and function of neurons in the brain, particularly the amygdala which is a key region mediating stress response (Liu *et al.*, 2014; Li & Barres, 2018). Increased glucocorticoid (corticosterone) release during sleep deprivation has been related to upregulation of pro-inflammatory cytokines such as NLRP3 and IL-1 β expressions in the BLA region of rats due to increase, which also leads to structural deformation such as neuronal plasticity and various damages to the neurons (neuritis). This is in agreement with the work of Xing *et al.*, (2021), who reported that the activation of the sympathetic nervous system and HPA axis after SD leads to a heightened proinflammatory state in the brain.

Omega-3 fatty acids (200mg/kg) in the present study showed neuroprotective function by preserving the normal histoarchitecture of the BLA, when compared with the control. This may be due to the multifunctional regulatory effect of omega-3 fatty acid on inflammation (Larrieu & Layé, 2018). Numerous functions have been reported, including the regulation of nuclear transcription factors (NF- κ B) to inhibit the expression of inflammatory factors as well as downregulation of the proinflammatory signal pathways, such as NF- κ B and c-Jun N-terminal kinases (JNK)-related pathways by binding to the specific receptor such as GPR120 (Huang *et al.*, 2017). These altogether may lead to the reversal of inflammatory processes and therefore, prevention of neuronal damages by inflammation and preservation of normal histoarchitecture of the brain regions following the noxious effect of sleep deprivation. This is in agreement with the work of Wu *et al.*, (2020), which reported anti-inflammatory effect of omega-3 fatty acid following inflammation induced by ovariectomy.

Immunohistochemical examination of GFAP positive astrocytes in the present study revealed limited invasion of immunoreactive astrocytes in the BLA of Wistar rats with majority of the astrocytes in their resting phase in the control group. This is in line with the normal ramified structure of astrocytes, with intricate arborization in the absence of injury and/or assault, as stated by Que and colleagues (2023). However, immunohistochemical examination of the BLA of Wistar rats exposed to sleep

deprivation without treatment (SD only) revealed widespread presence of immunoreactive astrocytes, although image-J quantification of the GFAP mean-gray value revealed an increase but insignificant staining intensity when compared to the control. This result agrees with the work of Bellesi *et al.* (2017) who examined the effects of 6–8 h of sleep, spontaneous awakening, sleep deprivation, and chronic sleep deprivation on brain cells. They discovered that chronic SD potentially induced microglial activation and astrocytic transformation to A1-neurotoxic phenotype, leading to an impairment in neuronal synaptogenesis and phagocytosis, and consequently neuronal death, and this may be the cause of the neurodegenerative changes observed in the H&E result reported in this study. According to Liddelow *et al.* (2017), and Fan and Huo, (2021), reactive astrocytes can be generally divided into two subtypes; a neuroprotective phenotype (A2) and a neurotoxic phenotype (A1). In the context of SD, diverse activation of microglia and astrocytes, due to imbalance homeostasis caused by SD, and through astrocyte-microglia crosstalk can contribute to tissue repair and promote CNS pathology or may exacerbate inflammatory reactions and tissue damage. It has been reported by Atrooz *et al.* (2019) that a rise in inflammatory markers seen in response to chronic SD scenarios, points toward a possible function for glia in aggravating the injury and prolonging its consequences. Studies have reported several ways by which astrocytes act to cause neuroinflammation and subsequent neuronal death (Waxman, 2003; Calabrese *et al.*, 2007; Colombo *et al.*, 2012). This could be by facilitating the activation and translocation of NF- κ B into the nucleus (Qian *et al.*, 2007) or by secreting IL-1, TNF α , and complement component 1q, microglia-induced neurotoxic A1 astrocytes causes neuronal death and worsen the progression of neurodegeneration (Liddelow *et al.*, 2017; Xu *et al.*, 2018). Administration of omega-3 fatty acids (200mg/kg.bw & 400mg/kg.bw), following sleep deprivation however showed limited invasion of immunoreactive astrocytes with resting phase morphology similar to the control, with a significant ($p < 0.05$) decrease in GFAP mean gray value when compared to the sleep deprived only group.

This may be due to the mitigative effect of omega-3 fatty acid on inflammation (Larrieu & Layé, 2018). Numerous protective effects of omega-3 fatty acids stems from its modulatory functions, including the regulation of nuclear transcription factors (NF- κ B) to inhibit the expression of inflammatory factors as well as downregulation of the proinflammatory signal pathways, such as NF- κ B and c-Jun N-terminal kinases (JNK)-related pathways by binding to the specific receptor such as GPR120 (Huang *et al.*, 2017). Omega-3 fatty acids can also inhibit TLR4 and tumor necrosis factor receptor (TNFR), which is a major pathway for astrocyte-induced neuroinflammation (Cao *et al.*, 2020).

Conclusion: The present study suggests that omega-3 fatty acids showed neuroprotective efficacy against sleep deprivation-induced stress, basolateral amygdala-neurological lesions as well as anxiogenic behavior in Wistar rats.

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