

# EFFECT OF OMEGA-3 SUPPLEMENT ON OXIDATIVE STRESS MARKERS IN BRAIN AND HEART TISSUE HOMOGENATES OF SHOCK INDUCED ALBINO RATS.

## ABSTRACT

The study evaluates the effect of omega-3 supplement on oxidative stress markers in the brain and heart homogenates in albino rats induced with shock stress using the electroconvulsive device. 25 rats weighing averagely 110g were divided into 5 groups of five rats each with different treatment regimens. Group I served as the negative control, Group II as shock control, Group III as omega-3 control, Group IV given omega-3 supplement for the first one week and induced stress by shock for 5 days, Group V was induced stress by shock for 5 days and then administered omega-3 supplement for one week. 1 ml Omega-3 supplement equivalent to 123mg/kg body weight was given to the rats through oral gavage method. The results shows that omega-3 supplement significantly increased ( $P < 0.05$ ) the superoxide dismutase, catalase and glutathione values while the malonaldehyde and nitric oxide values were reduced after being exposed to shock stress, then pretreatment and post treatment with omega 3 supplement in both the brain and heart tissues of the albino rats when compared to the shock control group whose values were reduced except for the value of malonaldehyde and nitric oxide which was increased. Oxidative stress is implicated in the brain homogenates than in the heart of the albino rats induced with shock stress. The study reveals that Omega-3 supplementation shows more effective protection for these organs notwithstanding the time of administration though pretreatment shows more ameliorating effect than the post treatment. Hence intake of omega 3 might protect our organs against environmental induced stress.

**KEY WORDS:** *Omega 3 supplement, Oxidative stress, Heart and Brain homogenates, albino rats.*

## 1. INTRODUCTION

The brain and heart are among the important organs in all living things. Their functions cannot be underestimated and very much indispensable in the human body. The brain dictates all functions of the body, translates the information from the outside world and embodies the essence of the mind and soul [1]. The heart, a muscular organ pumps blood through the blood vessels of the circulatory system [2]. The pumped blood transports oxygen, nutrients to the body and also metabolic waste such as carbon dioxide to the lungs [3]. Due to massive activities of these organs, they are prone to complications that might result to cardiovascular and neurological diseases which can lead to death. Oxidative stress is a state in which oxidation overwhelms the antioxidants capacity in the body system. It is best described as a loss of balance between them. This condition does not only result to hazardous events such as lipids peroxidation, protein damage or degradation and change in DNA but also has a role in physiological adaptation phenomenon and regulation of intracellular signal transduction [4]. Oxidative stress can be an excessive formation of free radicals (free radicals are chemical species that contain unpaired electrons which increases the chemical reactivity of an atom or molecule) and insufficient removal of highly reactive molecules such as reactive oxygen species, (ROS) and reactive nitrogen species (RNS) [5]. The reactive oxygen species include free radicals like superoxide radical, hydroxyl ion and non-radical species such as

hydrogen peroxide. The reactive nitrogen species include the free radicals such as nitric oxide, nitrogen dioxide.

Under physiological conditions, molecular oxygen undergoes a series of reactions that ultimately lead to generation of superoxide anion, hydrogen peroxide and water. Oxidative stress is said to be responsible for some various diseases and characterized by an imbalance between oxidants and antioxidants in favour of oxidants. This leads to disruption of redox signaling and physiological function [6].

Oxidative stress is known to be the chief factor in the pathogenesis of lifestyle related diseases such as atherosclerosis, heart failure, hypertension, diabetes mellitus and malignancies. Oxidative stress is caused by accumulation of reactive oxygen species (ROS) produced as the usual by-products of cellular metabolism and from the exposure to some environmental pollutant [7]. In fact, accumulation of reactive oxygen species beyond immediate needs of the cell is said to be detrimental to the cellular structure and functional integrity resulting to oxidative degradation of critical molecules such as DNA, proteins and lipids leading to the loss of controlled by the appropriate antioxidant scavenger at the cellular levels [5,7]. It is worthy to note that some cells possess an intricate network of defense mechanisms to neutralize excess reactive oxygen species and reduce oxidative stress. However, some tissues like the brain, are much more prone to oxidative stress because of their increase consumption of oxygen and the consequent generation of large amounts of reactive oxygen species [4]. It is a common knowledge that stress is a common phenomenon that is experienced by every individual and this can lead to overproduction of reactive oxygen species which is a leading factor in the development of oxidative stress.

The polyunsaturated fatty acids (PUFA) which include eicosapentanoic acid (EPA) and Docosahexanoic acid (DHA) are dietary fats beneficial to health in numerous ways. They are in many parts of the body, including cell membrane [8] and play a role in anti-inflammatory and ageing process. They are known to be utilized well in the viscosity of cell membrane. EPA and the DHA are necessary for proper fetal development, including neural, retinal, and immune function. Omega-3 PUFAs are, apart from being building blocks in cell membranes, bio transformed to eicosanoids such as prostaglandins, thromboxanes, and leukotrienes, as well as endocannabinoids and other lipid-based signaling substances that are involved in many biochemical processes in the body such as inflammation and immune responses [9]. Also, they have been seen to have effect on so many aspects of cardiovascular function such as inflammation, peripheral artery disease, and anticoagulation [10]. They have promising results in prevention and weight management, and cognitive function in people with mild Alzheimer's disease [8]. Nevertheless, because our bodies do not efficiently produce some omega-3 fatty acids, it is recommended that we obtain some of it from supplements at least 600mg to 1200mg per day from sources like fatty fish and fish oil. Adequate consumption of food rich in omega-3 fatty acids are recommended to prevent essential fatty acids deficiency. Omega-3 is well tolerated and has been proven safe hence recommended for consumption and its consumption are not influenced by sex, race or ethnicity [11]. Omega-3 FAs supplementation increases total antioxidant capacity levels [12], the possible mechanism of the effect of omega-3 fatty acids on increase of total antioxidant capacity may be due to decreased production of reactive oxygen species. Omega-3 fatty acids significantly increased superoxide dismutase activity and glutathione levels, decreased TBARS levels, elevated resistance to ROS damages, decreased lipid peroxidation, and improved antioxidant defense in erythrocytes, which increases total antioxidant capacity in the body [12]. The possible mechanism of omega-3 FAs supplementation effect on GSH may be due to omega-3's effects on the mitochondrial oxidant state [13]. There is evidence that omega-3 FAs supplementation changes mitochondrial membrane phospholipid fatty acid composition. Among the plenty of detoxifying enzymes and antioxidants existing in mitochondria, GSH has the main role of defense for maintaining adequate mitochondrial redox circumference [14].

## **2. Materials and Methods**

### **2.1 Experimental Animals**

A total of twenty-five (25) albino rats aged five weeks weighing averagely 110g were obtained from the animal house of the Department of Human Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, University of Port Harcourt, in Rivers State of Nigeria. The rats were kept in clean disinfected wooden cages with saw dust as beddings in the animal house with 12 hours light/dark cycle at a temperature of about 25 °C -26 °C. The animals were allowed for seven days to acclimatize to the new

environment with the access to clean water and animal feed. All these cares were in accordance with international guidelines for the care and use of laboratory animals [15] as regulated by the National Research Council [16]. The animal dose of 123mg of the omega 3 supplement was calculated according to the method of Nair and Jacob [17].

**Table 1: Treatment Regimens for the Animal**

Groups	No of Animals	Treatments/Administration
I. Negative control	5	Distilled water and Feed
II. Omega-3 control	5	Received 123mg omega-3 supplement, Not induced
III. Shock Only	5	Induced with Shock stress by exposure to 5MA shock using electro-convulsive therapy for 10 minutes daily for five (5) days
IV. Omega-3 / Shock (Pretreatment)	5	Received 123mg omega-3 supplement for 1 week and then induced with shock stress by exposure to 5MA shock using electro-convulsive therapy (ECT) for 10mins daily for 5 days.
V. Shock / omega-3 (Post Treatment)	5	Induced with shock stress by exposure to 5MA shock using electro-convulsive therapy for 10 minutes daily for 5 days and then treated with 123mg Omega-3 supplement for 1 week.

## 2.2 SAMPLE COLLECTION AND TISSUE HOMOGENIZATION

At the end of the treatment period, the animals were sacrificed under chloroform anesthesia. The brain and heart tissues were separately removed and kept in an ice-cold condition. The tissues (brain & heart) were immediately homogenized using a Sigma-Aldrich tissue homogenizer with phosphate buffer saline and (P.B.S) and protease inhibitor. This was centrifuged in a cold centrifuge and supernatant collected for analysis respectively, and stored at -20 °C until analysis is done. Determination of oxidative stress biomarkers such as superoxide dismutase (SOD), catalase (CAT), Glutathione (GSH), malondialdehyde (MDA), and nitric oxide (NO) were analyzed using colorimetric methods and animal ELISA kits, product of solar bio life sciences (China). The data obtained were analyzed using GraphPad prism 8.02 version and the results expressed as mean  $\pm$  standard deviation (SD). P value = 0.05 was accepted as statistically significant.

### 3. RESULTS AND DISCUSSION

The results of the analysis done in the brain and heart tissue homogenates collected from albino rats treated with omega 3 supplement before and after shock induced stress are shown in Table 2 and 3 respectively. In Table 4 and 5, these oxidative parameters were compared to check out significant differences in the organs when omega 3 supplements were given before and after shock stress was induced.

**Table 2: Results of Oxidative Stress Parameters in Heart Tissue Homogenates of Albino Rats Treated with Omega 3 Supplement Before and After Shock induced Stress.**

	SOD u/ml	CAT(u/mg protein)	MDA(nmol/mg protein)	GSH mg/ml	NO $\mu$ mol/ml
Negative Control	5.58 $\pm$ 0.08 <sup>a</sup>	0.33 $\pm$ 0.02 <sup>a</sup>	0.07 $\pm$ 0.01 <sup>a</sup>	16.48 $\pm$ 0.03 <sup>a</sup>	14.04 $\pm$ 0.02 <sup>a</sup>
Shock only	3.27 $\pm$ 0.07 <sup>b</sup>	0.24 $\pm$ 0.01 <sup>b</sup>	0.17 $\pm$ 0.01 <sup>b</sup>	14.26 $\pm$ 0.01 <sup>b</sup>	32.26 $\pm$ 0.01 <sup>b</sup>
Omega-3 Control	6.06 $\pm$ 0.02 <sup>c</sup>	0.58 $\pm$ 0.01 <sup>c</sup>	0.07 $\pm$ 0.01 <sup>a</sup>	21.02 $\pm$ 0.01 <sup>c</sup>	13.72 $\pm$ 0.01 <sup>a</sup>
Omega-3/Shock	6.52 $\pm$ 0.02 <sup>d</sup>	0.50 $\pm$ 0.01 <sup>d</sup>	0.06 $\pm$ 0.01 <sup>a</sup>	17.78 $\pm$ 0.01 <sup>d</sup>	13.86 $\pm$ 0.12 <sup>a</sup>
Shock/Omega-3	4.12 $\pm$ 0.01 <sup>e</sup>	0.42 $\pm$ 0.02 <sup>e</sup>	0.07 $\pm$ 0.01 <sup>a</sup>	15.66 $\pm$ 0.01 <sup>e</sup>	14.01 $\pm$ 0.01 <sup>a</sup>
F value	2078	436.8	168.1	133582	2089315
P value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Remarks	S	S	S	S	S

*S - Values with different superscript in the same column are significantly different from each other.*

**Table 3: Results of Oxidative Stress Parameters in Brain Tissue Homogenates of Albino Rats Treated with Omega 3 Supplement Before and After Shock Induced Stress.**

	SOD u/ml	CATu/mg protein	MDA(nmol/mg protein)	GSH mg/ml	NO $\mu$ mol/ml
Negative Control	8.45 $\pm$ 0.29 <sup>a</sup>	0.75 $\pm$ 0.02 <sup>a</sup>	0.81 $\pm$ 0.01 <sup>a</sup>	19.27 $\pm$ 0.01 <sup>a</sup>	16.93 $\pm$ 0.03 <sup>a</sup>
Shock only	3.14 $\pm$ 0.06 <sup>b</sup>	0.31 $\pm$ 0.01 <sup>b</sup>	1.22 $\pm$ 0.01 <sup>b</sup>	14.20 $\pm$ 0.02 <sup>b</sup>	27.85 $\pm$ 0.01 <sup>b</sup>
Omega-3 Control	7.73 $\pm$ 0.27 <sup>c</sup>	0.68 $\pm$ 0.01 <sup>a</sup>	0.76 $\pm$ 0.01 <sup>a</sup>	19.34 $\pm$ 0.01 <sup>a</sup>	14.51 $\pm$ 0.01 <sup>c</sup>
Omega-3/Shock	10.45 $\pm$ 0.05 <sup>d</sup>	0.72 $\pm$ 0.01 <sup>a</sup>	0.68 $\pm$ 0.02 <sup>c</sup>	18.29 $\pm$ 0.02 <sup>c</sup>	17.34 $\pm$ 0.01 <sup>a</sup>
Shock/Omega-3	7.82 $\pm$ 0.08 <sup>ce</sup>	0.70 $\pm$ 0.02 <sup>a</sup>	0.83 $\pm$ 0.02 <sup>ad</sup>	18.33 $\pm$ 0.03 <sup>cd</sup>	19.48 $\pm$ 0.01 <sup>d</sup>
F value	1049	1285	689.1	67262	583807
P value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Remarks	S	S	S	S	S

*S - Values with different superscript in the same column are significantly different from each other.*

**Table 4: Comparison of the Effect of Omega-3 Supplement on Oxidative Stress Parameters in Heart Tissues Homogenates Before and After Shock Induced Stress in Albino Rats.**

	SOD u/ml	CAT(u/mg protein)	MDA(nmol/mg protein)	GSH mg/ml	NO $\mu$ mol/ml
Omega-3/Shock	6.52 $\pm$ 0.02	0.50 $\pm$ 0.01	0.06 $\pm$ 0.01	17.78 $\pm$ 0.01	13.86 $\pm$ 0.01
Shock/Omega-3	4.12 $\pm$ 0.01	0.42 $\pm$ 0.02	0.07 $\pm$ 0.01	15.66 $\pm$ 0.01	14.01 $\pm$ 0.01
T value	26.28	17.79	1.04	294.01	1.25
P value	<0.0001	<0.0001	0.33	<0.0001	0.25
Remarks	S	S	NS	S	NS

*S – Values are significantly different from each other.*

**Table 5: Comparison of the Effect of Omega-3 Supplement on Oxidative Stress Parameters in Brain Tissues Homogenates Before and After Shock Induced Stress in Albino Rats.**

	SOD u/ml	CAT(u/mg protein)	MDA(nmol/mg protein)	GSH mg/ml	NO $\mu$ mol/ml
Omega-3/Shock	10.45 $\pm$ 0.05	0.72 $\pm$ 0.01	0.68 $\pm$ 0.01	17.96 $\pm$ 0.02	17.34 $\pm$ 0.01
Shock/Omega-3	7.82 $\pm$ 0.08	0.70 $\pm$ 0.01	0.83 $\pm$ 0.01	18.33 $\pm$ 0.03	19.48 $\pm$ 0.01
T value	319.81	0.53	11.84	1.58	125.62
P value	<0.0001	0.61	<0.0001	0.15	<0.0001
Remarks	S	NS	S	NS	S

*S - Values in the same column are significantly different from each other.*

The Oxidative Stress markers such as glutathione (GSH), Catalase (CAT), superoxide dismutase (SOD), Malondialdehyde (MDA), and nitric oxide (NO), were assayed on the brain and heart tissue homogenates to estimate the severity of oxidative damages potentially caused by this method of induction. This is in agreement with the reports of Yoshikawa & Natio [18], that the assessment of the extent of oxidative stress using biomarkers found in blood, urine, and other biological fluid and tissues may provide information of diagnostic value. The study showed that the antioxidant enzymes such as the SOD, CAT & GSH were all reduced when the animals were exposed to shock induction but increased with the administration of omega-3 supplement. This means that omega-3 supplement has a positive effect on improving SOD activities. This is in agreement with the reports of Avramovic *et al.*, [19] who reported that omega-3 fatty acids increase the activity of SOD and reduce Lipid Peroxidation in brain tissues of aged albino rats.

Catalase, an antioxidant defense enzyme found in all living organisms that are exposed to oxygen catalyzes the decomposition of H<sub>2</sub>O<sub>2</sub> to H<sub>2</sub>O and O<sub>2</sub>. According to Zheng *et al.*, [20], it is a very important enzyme that protects the cell from oxidative damage by reactive oxygen species. In this study, the catalase activity was reduced after shock stress induction. It was showed to be increased as a result of omega-3 supplement administration. This corroborates the reports of Tabei *et al.*, [21] who reported that omega-3 fatty acids supplementation was found to increase heart catalase activity. Also, the report of Ali *et al.*, [22] states that activity of catalase is increased after the administration of omega-3 supplement though in aluminum chloride stress induced animals. On the contrary, Garrel *et al.*, [23] reported that catalase activity was not altered by the administration of Omega-3 supplementation while Kusunoki *et al.*, [24] stated that Omega-3 supplement has no significant effect on catalase activity, all these disagrees with the findings in this study.

Glutathione an antioxidant capable of preventing damage to important cellular components caused by free radicals, peroxides, lipid peroxides and heavy metals [25] was significantly reduced after stress induction by shock method and it was observed to be improved after the administration of omega-3 supplement hence in consonance with the reports of Sorto-Gomez *et al.*, [26] who reported that fish oil supplementation resulted in increase in GSH values. This is because fish oil is known to contain high amount of omega-3 fatty acid. The result of this study also corroborates the finding of Patten *et al.*, [27] who reported that omega-3 supplementation increased GSH levels and the possible mechanism of this action might be due to its Omega-3 effect on the mitochondrial oxidant state as there is evidence that Omega-3 fatty acid's supplementation changes mitochondrial membrane phospholipid fatty acid composition.

Malondialdehyde (MDA), an oxidative stress marker that results from lipid peroxidation of polyunsaturated fatty acids (PUFAs) [28] from this study was significantly increased after stress induction by shock and was observed to be reduced by the administration of Omega-3 supplement. This implies that omega-3 supplement has a positive effect in reducing MDA concentration. This observation is in agreement with the report of Heshmati *et al.*, [12] who reported that Omega-3 fatty acids supplementation significantly decreased the concentration of MDA. Several mechanisms can be adduced for the observation. Omega-3 can affect syntaxin-3 which is a single effector in cell membrane expansion, and may also play a role in reducing the lipid peroxidation by changing cellular membrane structures by stimulating syntaxin-3 [29]. Studies by Egan *et al.*, [30], revealed that omega-3 fatty acids have cyclooxygenase-2 (cox-2) enzyme inhibitor activity which may describe the effect of omega-3 fatty acids on reducing MDA because Cox-2 produce inflammatory and oxidant prostaglandins which may cause lipid peroxidation.

Nitric oxide is known as the main mediators in chronic inflammatory process. It could either be protective or cytotoxic when there is uncontrolled, prolonged and / or massive production of NO by inducible nitrogen oxygen species (iNOS). It may cause liver damage, inflammation, and even tumor [31].

This study has revealed a statistically significant increase in the concentration of NO after stress induction by shock and was found to be reduced by the action of pre and post administration of omega-3 supplement. This means that omega-3 has a positive effect on the reduction of NO concentration. This is in consonance with reports of [32] who reported that omega-3 fatty acids reduce NO levels in diethylnitrosamine toxicity induced rats. The possible mechanism is by the reaction of omega-3 with free radicals acting as a free radical scavenger which may lead to reduce NO concentration. In addition, supplements rich in omega-3 PUFAs reduce the reactive oxygen species and NO production [33]. Furthermore, Cardoso *et al.*, [34] reported that omega-3 deficiency can result in increasing nitric oxide (NO) levels while supplementations with Omega-3 reversed the situation.

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