The Cerebellum under Stress: Dietary African Walnut (*Tetracarpidium conophorum*) Abrogates Oxidative Stress-driven Neuropathology induced by Chronic Unpredictable Stress

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Received 4th March 2021; Revised 28th March 2021; Accepted 4th April 2021

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**Abstract**

**Background:** Consumption of a healthy diet abundant in antioxidant and anti-inflammatory phytochemicals, offers an effective and least expensive way to prevent neurodegeneration. Herein, the role of *Tetracarpidium conophorum* (African walnut) enriched diet in chronic stress-induced cerebellar neuropathology was investigated.

**Methodology:** Twenty-one male Wistar rats were used for this investigation. Rats were randomly assigned into three groups (A, B, and C), each consisting of 7 rats (*n* = 7). Group A (Control group) were fed control diet; group B rats were subjected to different chronic unpredictable stressors (CUS) + control diet for 21 days, while group C rats were subjected to CUS + Walnut-enriched diet for 21 days. Serum corticosterone levels, the expression level of antioxidant and inflammatory markers, and cytoarchitectural changes in cerebellum were assessed by enzyme-linked immunosorbent assay (ELISA) immunohistochemistry methods.

**Results:** The walnut-enriched diet prevented astrogliosis, modulated serum corticosterone expression, and tumor necrotic factor-α in the cerebellum. The walnut-enriched diet also caused an improvement in the antioxidant profile, indicating that it suppressed chronic unpredictable stress-induced perturbations.

**Conclusion:** Our results suggest that African walnut exerts protective effects against oxidative stress-driven dysfunction by reducing serum corticosterone levels, modulating oxidative stress pathways, and preventing neuronal morphological damage in the cerebellum.

**Keywords:** African Walnut, Oxidative stress, Cerebellum, Chronic unpredictable stress
1. INTRODUCTION

Chronic stress is implicated in the development of many psychopathological syndromes in humans, including major depression and anxiety disorders which is a risk factor for developing a variety of human ailments. Specifically, anxiety and depressive disorders have been frequently associated with stressful life events [1,2]. Activation of the stress system leads to behavioral and peripheral changes that adjust homeostasis and improve coping with stressful situations. On the other hand, a lack of adaptation to excessive demands can lead to pathological syndromes, such as depression and anxiety [2].

Chronic stress affects brain areas such as hippocampus, cerebellum, amygdala and prefrontal cortex, involved in anxiety and affective disorder, evidenced in postmortem and brain imaging studies of depressed and anxious patients [3]. The nervous system's neurochemical pathways play a vital role in regulating stress responses [4], such as diminished serotonergic transmission in the prefrontal cortex [5] postulated to be involved in the pathogenesis of anxiety and depression. Several lines of evidence suggest that depletion of monoamine: serotonin [6], noradrenalin [7], and dopamine [8] sustained stress could be the reason for anxiety and behavioral depression [9, 10]. In support of the behavioral consequences of post -stress, animal studies have revealed that, chronic or acute exposure to stress can modify the activity of neuroendocrine and neurotransmitters system that affect behaviour profile indicative of human psychopathology [10].

The cerebellum functions in controlling various motor activities in the brain. It also controls the range, distance, and amplitude of voluntary muscle activity. It is the integrating center for willed muscular movements and posture. By implication, the cerebellum's main function is to coordinate the sequence and the strength of muscular contractions during posture and voluntary movements [11,12]. It controls proprioceptive impulses from joint pain and Golgi tendons. Because the cerebellum has connections with the vestibular apparatus, it functions in the maintenance of balance. It plays an essential role in producing smooth words and for precise and intrinsic movements [13].

Nutrition is able to alter the health status of the general population. The World Health Organization strongly emphasized the role of unhealthy eating habits, sedentary lifestyle, and cigarette smoking as risk factors for the onset of chronic diseases, including neurodegenerative diseases, cardiovascular diseases, cancer, respiratory and metabolic disorders [14]. Plants are a valuable source of a wide range of secondary metabolites used to treat and prevent diseases. Many medicinal plants possessed anti-convulsant, anti-depressant, anti-anxiety, and memory enhancement effects [15,16].

The common walnuts (Juglans regia) contain several phytochemicals, including high amounts of polyunsaturated fatty acids, vital for a healthy brain [17]. Walnuts also contain polyphenolic compounds that reduces oxidation and inflammation in nervous cells boost interneuronal signaling, improve neurogenesis, and promote sequestration of insoluble toxic protein aggregates [17].

Our day-to-day activities are encompassed with stress. Inability to rest or combat these stressful conditions with therapeutic interventions such as drugs, micronutrients, and foods containing antioxidants can later result in neurodegenerative disease, which has become endemic in the world, especially sub-Saharan Africa. Although extensive research has been carried out on walnut, little or no work has been done to understand the therapeutic potential of proper walnut dieting in the management of neurodegenerative disease, especially those related to the cerebellum. Therefore this research is designed to understand the effect of the African walnut (Tetracarpidium conophorum) diet on cerebellar architecture of male Wistar rat exposed to chronic unpredictable stress.

2. METHODS AND MATERIALS

2.1 Animal Grouping and Treatment

Twenty one male Wistar rats weighing 170-180g were procured and maintained under standard laboratory conditions at the College of Health Sciences, Osun State University, Nigeria, where they had liberal access to rat chow and water. After one week of acclimatization, the rats were randomly assigned into three groups (A, B and C), each consisting of 7 rats (n = 7). Group A (Control group) were fed control diet; group B rats were exposed to Chronic Unpredictable Stress (CUS) + control diet for 21 days, while group C rats were exposed to CUS + Walnut-enriched diet for 21 days. Rats in groups A and B were fed with the control diet, while group C rats were fed with a custom-made diet containing a control diet + 9% African walnut. Table 1 shows the composition of
these diets. Experimental animals were exposed to chronic unpredictable stress for 21 days using different paradigms like force swim test, tail pinch, food deprivation, damp sawdust, sawdust free cage, cage tilting, water deprivation, and cage change mate for varying time duration. Animals were utilized in accordance with laws and regulations outlined in the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals as approved by the College of Health Sciences Ethics Review Committee, Osun State University, Nigeria (URC Publication No. 13:978-0-309-15401-7, 2019).

**Table 1:** Composition of control diet and walnut-rich diet for control and experimental animals.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Percentage of weight</th>
<th>Control Diet</th>
<th>9% Walnut Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groundnut cake</td>
<td>25%</td>
<td>25g</td>
<td>24.4g</td>
</tr>
<tr>
<td>Palm kernel cake</td>
<td>25%</td>
<td>25g</td>
<td>24.4g</td>
</tr>
<tr>
<td>Corn starch</td>
<td>15%</td>
<td>15g</td>
<td>13.5g</td>
</tr>
<tr>
<td>Industrial soya</td>
<td>10%</td>
<td>10%</td>
<td>9.4g</td>
</tr>
<tr>
<td>Bone meal</td>
<td>5%</td>
<td>5g</td>
<td>5g</td>
</tr>
<tr>
<td>Wheat fibre</td>
<td>5%</td>
<td>5g</td>
<td>4.8g</td>
</tr>
<tr>
<td>Lysine</td>
<td>0.2%</td>
<td>0.2g</td>
<td>0.2g</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.3%</td>
<td>0.3g</td>
<td>0.3g</td>
</tr>
<tr>
<td>Concentrate</td>
<td>0.3%</td>
<td>0.3g</td>
<td>0.3g</td>
</tr>
<tr>
<td>Ground walnut</td>
<td>9%</td>
<td>0</td>
<td>9g</td>
</tr>
<tr>
<td>Soy bean oil</td>
<td>10%</td>
<td>10g</td>
<td>2.63g</td>
</tr>
<tr>
<td>Total</td>
<td>100%</td>
<td>100g</td>
<td>100g</td>
</tr>
</tbody>
</table>

**2.2. Chronic unpredictable stress procedures**

During the experiment, rats in the control group were fed with normal diet and left undisturbed (except regular cage cleaning and feeding) in a separate room. The rats in the other groups exposed to CUS were housed individually. The CUS procedure was carried out according to the method as described previously by Losey, [18] and Fan et al. [19], but with slight modifications.

**2.3. Animal Sacrifice, Histology and Immunohistochemistry**

At the end of 21 days, the animals were sacrificed under Isoflurane anesthesia and decapitated. Skull was opened with brain forceps and cerebellar tissue samples were collected. Cerebellar tissues for histology and immunohistochemistry were fixed in 10% formal saline for 48 hours, dehydrated in ascending grades of alcohol, cleared in xylene, infiltrated and embedded with paraffin wax, sectioned and mounted on slides. Sections were routinely stained with Hematoxylin and Eosin stain to demonstrate the general morphology of the cerebellar cortex. Glial fibrillary acidic protein (GFAP) immunoreactivity and expression in astrocytes was demonstrated using commercial Anti-Glial Fibrillar Acidic Protein (GFAP) (Cat. # AB5804) procured from EMD Millipore, according to the manufacturer’s guide.

**2.4 Colorimetric estimation of stress levels**

**2.4.1 Corticosterone assay**

Blood was carefully drawn from a large vein in the posterior paws and collected into serum bottles; allowed to coagulate for 1 hour and then centrifuged for 10000 rpm for 10 min. Serum aliquot was obtained using disposable plastic pipette tips and stored at -20°C for corticosterone analysis. Corticosterone ELISA kit (ab108821) procured from Abcam, USA was used to determine the corticosterone stress levels in both control and experimental rats. This assay procedure was carried out according to the manufacturers’ guide. Absorbance was read at a wavelength of 450nm using the microplate reader.

**2.4.2 Enzymatic determination of antioxidants and inflammatory markers**

Cerebellar tissues for colorimetric estimation of oxidative damage were washed in phosphate buffered solution (PBS), homogenized on ice and centrifuged at 12,000 rpm in a centrifuge for 15mins. Tissue supernatants was collected into new vials, and stored on ice for immediate assay of enzymatic antioxidants.

**2.5 Gluthathione peroxidase (GPx) assay**

Glutathione peroxidase colorimetric estimation in cerebellar tissue homogenate was determined according to the method of Flohé and Gündler [20] as described by Soudani et al. [21]. Herein, the samples were incubated with hydrogen peroxide in the presence of glutathione for a particular time period and utilized hydrogen peroxide was determined by directly estimating GSH content using Ellman’s reagent, 5,5-dithiobisnitrobenzoic acid (DTNB). Absorbance was read at a wavelength of 405-415 nm using the microplate reader.
2.6 Lipid peroxidation

The Malondialdehyde (MDA), one of the by-products of peroxidation of polyunsaturated fatty acid was used to determine the oxidative stress levels in the cerebellum. This assay was performed according to the method of Okawa et al., [22] in which MDA reacted with Thiobarbituric acid reactive substances (TBARS) in an acidic medium at 100°C to produce a pink/red-colored product extracted with butanol and measured using a spectrophotometer at an absorbance of 520-535 nm.

2.7 Lactate dehydrogenase

To quantify the lactate dehydrogenase activity in the cerebellar tissue of the experimental animals, lactate dehydrogenase activity assay kit, Catalog Number MAK066 procured from Sigma, USA was used. Prior assay, cerebellar tissue was rinsed in PBS (pH 7.4), homogenized in 5 mL buffer containing 100 mM potassium phosphate (pH 7.0) and 2 mM EDTA and centrifuged at 10,000 rpm for 15 mins. Supernatant were assayed immediately. Lactate dehydrogenase present in tissue reduced nicotinamide adenine dinucleotide (NAD) to NAD + hydrogen (NADH) and its absorbance was measured at a wavelength of 450 nm with a spectrophotometer.

2.8 Total protein

The cerebellar total protein was determined according to the method of Bradford [23] using the Pierce Coomassie (Bradford) Protein Assay Kit, a commercial and ready to use kit with catalogue number 23200 procured from Sigma, USA. When mixed with a protein solution, the acidic Coomassie-dye reagent changed color from brown to blue in proportion to the amount of protein present in the sample. Absorbance was read with a spectrophotometer at 595 nm.

2.9 Tumor necrotic factor α

The mouse TNF-Alpha ELISA Assay kit with catalogue number SKU: MTF19-K0, procured from Eagle Biosciences, USA, was used to estimate the level of inflammation in the cerebellar homogenate of control and experimental groups. The assay was performed according to the manufacturers’ instruction and absorbance was read at 450 nm.

2.10 Light microscopy

Histological, histochemical and immunohistochemical analyses of the cerebellar cortex was captured using Olympus binocular research microscope (Olympus, New Jersey, USA) which was connected to a 5.0 MP Amscope Camera (Amscope Inc, USA).

2.11 Data Analysis

Results obtained from quantitative studies were analysed using GraphPad Prism® software (Version 8.0) and tested for analysis of variance (ANOVA) with Tukey’s multiple comparisons test. Significance was set at p<0.05.

3 RESULTS

3.1 Serum corticosterone profile increased following exposure to chronic unpredictable stress

In the present study, serum corticosterone was assayed to examine the stress response of experimental animals. It was observed that animals in group B that underwent CUS presented with a significantly high level of serum corticosterone when compared to the control rats (p < 0.05). Walnut-enriched diet was able to prevent the over-expression of serum corticosterone in the Stress+Walnut group which was significant (p < 0.05) when compared to rats in group B. (Figure 1)

![Figure 1 Chart showing serum corticosterone levels in the experimental animals. Data were presented as mean and standard error of mean (Mean ± SEM). P value <0.05 was considered to be statistically significant; (n=7). * is the significant level of difference in comparison with group A; + is the significant level of difference in comparison with group B.](image)

3.2 African walnut-enriched diet associated with increased cerebellar glutathione peroxidase level

Chronic unpredicted stress (group B) induced a significant depletion in the levels of Gpx when compared to the control animals (group A) and animals treated with walnut-enriched diet (group C) (p<0.05) as seen in Figure 2.

3.3 Chronic unpredictable stress exposure altered cerebellar lipid peroxidation profile

MDA was assayed to quantify the level of lipid peroxidation. CUS induced a high level of lipid peroxidation as
observed in group B animals with a significantly high level of MDA when compared to rats treated with walnut-enriched diet (group C) and the control group (p<0.05) (Figure 3).

3.4 African walnut-enriched diet reduces lactate dehydrogenase activity

Wistar rats in group B (animals exposed to CUS) had increased activity of LDH which was significant when compared with the control group (p<0.05). African walnut-enriched diet significantly reduced the LDH levels in the cerebellum (group C) when compared with animals in group B (Figure 4).

3.5 Dietary African walnut attenuates CUS-induced total protein depletion in cerebellar neuronal cells

Animals exposed to chronic unpredictable stress (group B) had significantly (p<0.05) reduced total protein count when compared with the control animals (group A). Rats fed with walnut-enriched diet maintained a total protein count which was not significantly different when compared to the control group (Figure 5).

3.6 Chronic unpredictable stress upregulated the expression of TNF-α

TNF-α, an inflammatory cytokine produced during acute inflammation is responsible for a diverse range of signal-
ing events within cells, leading to necrosis and apoptosis. In this study, CUS significantly (p<0.05) increased the activity of TNF-α as observed in group B rats when compared to group C and the control group (Figure 6).

![Graph showing tumor necrotic factor activity in experimental animals](image)

**Figure 6:** Chart showing tumor necrotic factor activity in the experimental animals. Data were presented as mean and standard error of mean (Mean ± SEM). P value <0.05 was considered to be statistically significant; (n=7). * is the significant level of difference in comparison with group A; + is the significant level of difference in comparison with group B.

### 3.7 Qualitative Evaluation

The characteristic presentations of histological properties of the cerebellum in this study were demonstrated using H&E staining technique (Figure 7). The transitional regions between the layers of the cerebellar cortex (molecular (M); purkinje (P) and granule (G) layers) was focused at higher-power magnification to study neuronal arrangements and layer-specific micro-architectural disparity across experimental groups. The control rats presented with fine assortment of cells across the three layers. The well-arranged cerebellar layers and neuronal morphology (green arrows) is seen in the aforementioned group.

Conversely, the cerebellar cortical sections of animals that underwent CUS were characterized by fragmented granule cell layers and clustered Purkinje cells that have pyknotic cell bodies and damaged dendritic processes that are sparsely distributed around the vaguely demarcated cerebellar layers. In addition to these degenerative changes, the neuropil appeared fragmented with irregularly shaped and sized neurons.

African walnut-enriched diet regime was able to confer protection on the morphological integrity of the cerebellar cortical layers. The representative photomicrograph of cerebellar cortex of rats in this group presented with large Purkinje cells with noticeable cell bodies and dendrites that venture deeply into the molecular layer. Also, the granule cell layers in these groups consist of small granule neurons with regular clusters formed across the laconic architecture (green arrows).

![Photomicrographs demonstrating cellular morphology and anatomy in the molecular (M), purkinje (P) and granule cell (G) layers of the cerebellar cortex in Wistar rats across the various study groups.](image)

**Figure 7:** Photomicrographs demonstrating cellular morphology and anatomy in the molecular (M), purkinje (P) and granule cell (G) layers of the cerebellar cortex in Wistar rats across the various study groups. Hematoxylin and Eosin stain (Scale bars: 25 μm). A= control, B= Stress, C= Stress+Walnut diet.

### 3.8 Chronic unpredictable stress caused astrocyte activation in the cerebellum

Astrocytes expression in the cerebellar cortex (molecular (M); purkinje (P) and granule (G) layers) were focused across the different study groups. An increased expression of reactive astrocytes (red dotted circle) in the cerebellum was observed in animals that underwent chronic unpredictable stress (group B). Also, the astrocytes in this group appear to have increased size and are presented with characteristics of astrogliosis (neurodegeneration-related glia activation). Walnut-enriched diet regime on the other hand, prevented astrogliosis. Animals in group C (Stress+walnut diet) presented with regular distribution of astrocytes similar to the control group (red arrows) (Figure 8).

### 4 DISCUSSION

Chronic stress is a risk factor for the development of many psychopathological conditions in humans, including cognitive dysfunction and anxiety disorders. Numerous studies have shown cerebellar responsivity to stress. Moreover, the cerebellum is connected with stress-related brain areas and expresses the machinery required
Figure 8: Photomicrographs showing immunohistochemical demonstration of astrocytes using anti Rat-GFAP across the main layers of the cerebellar cortex in Wistar rats from the study groups. (Scale bars: 25 μm). The molecular layer (M), purkinje cell layer (P), and granule cell layer (G) are demonstrated across study groups. A= control, B= Stress, C= Stress+Walnut diet.

to process stress-related neurochemical mediators [24]. In the present study, it has been demonstrated that dietary walnut can produce protective effects against chronic stress-induced cerebellar dysfunction in rat models by modulating oxidative and inflammatory pathways. Serum corticosterone concentration increased significantly in the stressed animals while dietary walnut modulated the serum levels of corticosterone in this study.

Serum corticosteroid elevation is a highly conserved response to stressor exposure in vertebrates, and is essential to adapting animals to stress and overcoming the stressor [25,26]. It has been suggested that the activation of the hypothalamus-pituitary-adrenal (HPA) axis by different stressors results in the release of glucocorticoid hormones (corticosterone) [25]. Studies have shown that at baseline concentrations, corticosterone helps animals mobilize energy stores, enhances certain immune components and promotes escape and self-maintenance behaviors [27,28]. However, sustained high levels of corticosterone due to frequent or prolonged stressors can result in a number of stress-related pathologies, including suppression of reproduction and the immune systems, metabolic dysregulation and cognitive impairment [29,30]. Findings from this study show that dietary walnut maintained serum corticosterone levels at the baseline. This can be associated with important nutrients and phytochemicals like polyphenols, vitamin E, folate, ellagitannins, ellagic acid monomers, polymeric tannins, melatonin, pectin, flavonoids, carotenoids, alkaloids, nitrogen-containing or organosulfur compounds, and a variety of minerals [31], which may have improved the coping mechanisms of the animals and modulated the HPA axis via its antioxidant, antidepressant, anti-inflammatory and anxiolytic properties.

The brain is a major high energy consuming tissue and its energy requirements are dependent on mitochondria function. It is important to note that mitochondria uses oxygen to manufacture biological energy at probable self-risk [32]. Gradual oxidative damage to biomolecules in mitochondria lead to increased ROS/RNS production thereby overworking the intrinsic antioxidant defense system. Metabolic processes become increasingly less effective conferring severe challenges to neuronal survival [33,34]. Major forms of oxidative stress include protein oxidation, lipid peroxidation, and oxidative damage to DNA and RNA [35,36]. There are more evidences that support the fact that oxidative stress plays a crucial role in the cascade of events that leads to neurodegenerative diseases such as Alzheimer’s Alzheimer’s [37,38]. ROS are highly reactive molecules that oxidize cellular components such as DNA, proteins and lipids and eventually result in the degradation of cell membrane, mitochondrial apparatus and DNA consequently leading to apoptosis [39].

Our findings in the present study revealed that CUS induced oxidative stress, as we noted depletion in the levels of GPx and increased MDA levels in animals that underwent CUS. Flores et al., [40] also reported an upsurge in ROS generation occasioned by exposure to CUS. The superfluous free radical generated as a result of CUS cannot be catered for by intrinsic antioxidants, as a result these ROS will exacerbate the rate of lipid peroxidation that occurs normally in cells and tissues. Lipid peroxidation results from oxidative degradation of lipids which if not controlled can result into cellular and DNA damage [41].

African walnut-enriched diet suppressed oxidative stress-driven cerebellar pathology induced by CUS in this study. This may not be unconnected with the antioxidant property of African Walnut. *Tetracarpidium conophorum* have been shown to contain phytonutrients that provide direct neuroprotection [42,43] as well as indirect protection through improved lipid profiles and endothelial function and increased plasma antioxidant capacity [44]. The use of plants as food as well as for herbal remedies against diseases in Africa has become an accepted practice. Herb-
al and natural plant extracts have gained wide attention for their use in the treatment or management of neurological, psychiatric, and degenerative diseases based on their few or lack of side effects [45]. Furthermore, the use of complementary and alternative medicine by individuals with stress-induced psychopathological dysfunction is gaining widespread acceptance on account of its greater safety and fewer side-effects when compared to orthodox medicine [46].

Perturbation of central and peripheral glucose bioenergetics has also been implicated in neurodegenerative diseases. Lactate dehydrogenase (LDH) is involved in glucose metabolism and ultimately making available to tissues the biological energy required for physiological processes. LDH is an enzyme in the energy producing glycolytic pathway which may be affected by oxidative stress and may contribute to the alteration in glucose metabolism documented in AD [47]. Determining tissue levels of LDH serve as a marker for metabolic diseases because this enzyme is massively synthesized and abnormally released into the cellular environment during toxic insult and damage to tissues. In the present study, rats that underwent CUS showed significant overexpression of LDH compared to the control group. It has been documented that exacerbated free radical production, impairment in neuronal bioenergetics and alteration in mitochondrial functions are implicated as early pathogenesis of Alzheimer’s Alzheimer’s disease [48]. LDH catalyzes the conversion of pyruvates to lactate when oxygen is absent or in short supply, a phenomenon known as oxygen debt. This may account for the up-regulation of cerebellar LDH in the CUS group. CUS may have induced chemical hypoxia, low energy generation in the mitochondria with increased release of ROS. Interestingly, walnut-enriched diet conferred a level of protection against cellular hypoxia in animals fed with African walnut-enriched diet as the levels of LDH in these groups were similar to those of the control groups.

The inter-relationship between the innate immune system and the central nervous system (CNS) has moved to the forefront of biomedical research, with the discovery that these two physiological systems modulate each other by a steady mutual interaction [49].

Inflammation is a key signature in the etiology of several neurodegenerative disorders. Microglia, the immune cells of the central nervous system, are activated early in neuropathological conditions and can both trigger and propagate early disease processes via innate and adaptive immune mechanisms such as upregulated immune cells and antibody-mediated inflammation [50].

In this study, CUS caused an increased in TNF-α activity in the cerebellum. Neurons when inflamed, produced TNF-α, a signaling protein involved in systemic inflammation. Perturbations in TNF production has been implicated in a variety of neurodegenerative diseases. Our findings show that dietary walnut attenuated inflammatory responses induced by CUS by modulating the expression of TNFα in the cerebellum (Figure 6).

The inhibition of proinflammatory cytokine expression in the brain may be dependent upon the presence of omega-3 polyunsaturated fatty acids (PUFAs) in African walnut. They contain large amounts of polyunsaturated fatty acids (PUFAs) such as α-linoleic acid (ALA) and linoleic acid (LA), which have been shown to boost brain health and function even with an increase in age. Even though PUFAs play an important role in brain health, the presence of other phytochemical components contributes to healthy neuronal processes which ensures the efficient working of the immune system and provide cellular defense against chronic inflammation [51].

More so, result from this study shows that CUS elicited the activation of reactive astrocyte. The robust increases in GFAP expression in animals subjected to CUS suggest that increases in the expression of TN-α resulted from classically activated astrocytes and these findings are consistent with findings from previous studies [52,53]. Neural health indices are linked with the activities of astrocytes because they provide structural and functional support for neurons. The function of glia cells, which are major sources of proinflammatory molecules in the CNS are affected by CUS and can display a wide spectrum of immune and inflammatory mediators in the microenvironment that may eventually result in neurotoxic outcomes [54]. It was observed that dietary walnut prevented astrocyte activation which is a common signature in brain pathological conditions. Vitamin C, which is present in African walnut may be involved in ascorbic acid recycling in the brain which is mediated by an interplay between astrocytes and neurons for the maintenance of normal brain function. In pathological conditions where inflammatory biomarkers are increased, these recycling mechanisms may be compromised, eliciting neuronal toxicity. Hence, African walnut through its antioxidant and anti-inflammatory actions can mediate cellular toxicity resulting from CUS [55].

The morphological demonstration of the cerebellum of
animals exposed to different stress paradigms in this study showed pathological changes characterized by necrotic and pyknotic neurons. Poor interjection between Purkinje cell and other layers (molecular and granular layer) is suggestive of the degenerative propensity of CUS on Purkinje cells. This kind of alteration in cellular morphology may result into disruption in the signaling processing, neuronal timing and synaptic efficacy in the cerebellum (red dotted circle). Uncontrolled generation of ROS is a known cause of necrotic neuronal death which is the hallmark of neurodegenerative diseases [56]. African walnut-enriched diet proved to be protective against CUS-induced neuronal damage by maintaining the structural integrity of the hippocampus.

4.1 Conclusion

In this study, histological, immunohistochemistry and biochemical analysis have been employed to demonstrate the neuroprotective effects of African walnut-enriched diet with respect to suppression of inflammatory and oxidative stress-driven perturbations in the cerebellum induced by chronic unpredictable stress (CUS) in rats.

Our results suggest that African walnut exerts protective effects against oxidative stress-driven dysfunction by modulating serum corticosterone levels, boosting the levels of glutathione peroxidase, and preventing abnormal lipid peroxidation. Furthermore, African walnut-enriched diet prevented astrogliosis and neuronal damage in the cerebellum due to its anti-inflammatory properties. These results provide a new vista for exploiting *Tetra-carpidium conophorum* as an alternative neuroprotective agent against stress-induced pathological dysfunction.

Conflict of Interest

The authors declare that no conflict of interest is associated with this work

Authors Contribution

TAA conceived and designed the study and wrote the manuscript; OST contributed to study design, and manuscript writing; OAA, and EOY performed the data analysis and interpretation; EO and YDO were involved in data verification and immunohistochemical investigation. All authors approved the final version of the manuscript

References


