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Neuroprotective Roles of Oleic Acid: An Antioxidant Status and Cerebellar Characterization in Rat Models of Aluminium Chloride-Induced Alzheimer Disease

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Cerebellum; Wistar rats; Oleic acid; Aluminum chloride; Alzheimer disease

1. Abstract

1.1. Background: Previous studies have linked Alzheimer's disease (AD) with cerebellar dysfunction. However, these studies lack substantial cerebellar characterization and features during AD progression.

1.2. Purpose: This study is aimed at investigating cellular and metabolic changes within the cerebellum in rat models of AD, while assessing the neuroprotective mechanisms of oleic acid (OA) on the corresponding pathology.

1.3. Methods: 40 Male Wistar rats were grouped into 8 consisting of 5 rats in each. Group 1 received 1 ml of distilled water, 2 received 50 mg/ kgBw/day of AlCl3, 3 received 1mL/ kgBw/day of Oleic acid, 4 received 1.5 mL/kgBw/day of Oleic acid, 5 received 2mL/ kgBw/day of Oleic acid, 6 received 1mL/ kgBw/day of Oleic acid + 50 mg/ kgBw/day of AlCl3, 7 received 1.5 mL/ kgBw/day of Oleic acid + 50 mg/ kgBw/day of AlCl3, 8 received 2mL/ kgBw/day of Oleic acid + 50 mg/ kgBw/day of AlCl3. Following treatments, cerebellar cortices were processed for biochemical and histological analysis.

1.4. Results: Our data revealed the presence of neural spaces, distorted and irregular cerebellar layer and membranes vacuolations, pyknotic and hypertrophied Purkinje cell bodies and granule neurons characterized by short axons and dendrites in rats treated with AlCl3. These features were in line with the decreased expression of antioxidant defense systems– shown by the expressed superoxide dismutase, catalase, glutathione peroxidase, glutathione, and cerebellar lipid peroxidation which was overexpressed in the group administered AlCl3. We further showed that OA reverses cerebellar degeneration through modulation of oxidative stress molecules and neurochemical signaling pathways that causes AD.

1.5. Conclusion: AlCl3 induction led to metabolic and cellular degeneration of the cerebellum while the efficacious OA inhibited the corresponding pathobiology attributing this to its neuroprotective properties.

2. Introduction

Aluminum (Al) has the potential to be neurotoxic in human and animals. It presents in many manufactured foods and medicines and is also added to drinking water for purification purposes [1]. The brain is a potential target for aluminium toxicity [2]. Aluminium can easily enter the blood-brain barrier (BBB) via its definitive high affinity to the receptors of tranferrin thereby accrete in the brain [3]. In the brain aluminium mostly accumulates in most being in a frontal cortex and hippocampus portions [4]. Previous types of animal investigations observed that the extended contact to the aluminium may lead to the neurobehavioral, neuropathological, and neurochemical alterations in a brain that eventually

weaken the memory and learning capacities of the investigational rats [5].

In biological membranes, fatty acids constitute major components that play important roles in intracellular signaling pathways. An unsaturated fatty acid present in nature which is widely distributed and abundant is called the oleic acid. It possesses antibacterial properties that are used in varieties of pharmaceutical products. It is used a pharmaceutical solvent used in the commercial preparation of lotions and oleates. Oleic acid (OA), constitute of 75% of olive oils which is beneficial against cardiovascular disease and liver steatosis [6]. Its effect on cancer cells are not completely understood although they differ based on it cancer cell types [7]. Reports by Guilitti et al. [8] observed that OA reduced lipid accumulation, cell death and autophagy in different HCC cell lines compared to immortalized healthy hepatocytes

There is paucity of scientific literatures concerning the neurologic effect of oleic acid (OA) on aluminum chloride induced neurdegeneration and its anti-oxidant changes. However, the potentials of oleic acid in reversing cerebellar degeneration following Al -induced AD are not well known. In line with this, the study aimed at investigating cellular and metabolic changes within the cerebellum in rat models of AD, while assessing the neuroprotective mechanisms of oleic acid on the corresponding pathology.

3. Methods

A total of 40 male rats (*Rattus norvegicus*) of Wistar strain weighing 100-120 g were collected. The rats were kept in clean separate cages where they were served rat feed and water ad-libitum. Crystalline salts of aluminum chloride ($AlCl_3$) and oleic acid were procured from Sigma-Aldrich (USA). Ethical approval was sought from the College of Medicine Ethical Committee, Madonna University Elele Campus, Rivers State, Nigeria. All protocols and treatment procedures complied strictly with the Institutional Animal Care and Use Committee guidelines (IACUC/MUC/45/2021).

The animals were allowed to acclimatize for 2 weeks and then weighed weekly using a digital scale with accuracy of 0.001g. The rats were divided into 7 groups of 5 rats each. Treatment duration for the project was for 42 days.

3.1. Rat Grouping and Treatment

- Control group-1 ml of distilled water.
- Group 2 received 50 mg/kg body weight of $AlCl_3$
- Group 3 received 1mL/kg body weight of Oleic acid
- Group 4 received 1.5mL/kg body weight of Oleic acid
- Group 5 received 2mL/kg body weight of Oleic acid
- Group 6 received Oleic acid 1mL/kg body weight + 50mg/kg body weight of $AlCl_3$
- Group 7 received Oleic acid 1.5mL/kg body weight + 50mg/kg body weight of $AlCl_3$
- Group 8 received Oleic acid 2mL/kg body weight + 50mg/kg body weight of $AlCl_3$

3.2. Administration of the Extract

The animals were housed in clean cages in a room temperature of 23 to 25^{0}C. They were allowed to roam freely. Their feeding depended on the study design. No animal was subjected to induced pain, suffering or restraint, the extract was administered to the rats using an orogastic gavage, once daily (9am-10am), throughout the period of the study. At the expiration of administration period, the rats were weighed, and sacrificed.

3.3. Tissue Sample Collection

The animals were anaesthetized with diethyl ether, blood (2ml) was collected from each animal via the retro-orbital sinus with 70µl heparinized capillary tube and put into plain sample bottle for the biochemical analysis, an incision was made in the cranial region, the skull was opened and the brain was carefully removed and placed on ice before homogenization. The hearts were washed with ice-cold physiological saline solution (0.9%), blotted dry and weighed. The tissues were homogenized for 30 seconds in 10 volumes of ice-cold 10mmol/l phosphate-buffered saline (pH 7.4) containing 1.15% KCl. The homogenate was subjected to a 3000 rpm of centrifugation at 4°C for 15 minutes. The supernatant fractions were collected and stored at -80°C until analysis. The protein content of supernatant fractions was determined according to Bradford.

3.4. Colorimetric Assay for Biochemical Studies

All animals were anaesthetized with chloroform (0.5 ml i.p.) and transcardially perfused with saline (0.9% NaCl) followed by 4% paraformaldehyde (PFA) in phosphate buffer (PB; 0.1 M; pH 7.4). Enzymatic assay for GPx, GSH, MDA, CAT, and SOD activities were carried out on carefully dissected cerebellar cortices of rats using spectrophotometric techniques. Equal weighing brain tissues (0.090g) were homogenized in 0.25 M sucrose (Sigma) with an automated homogenizer at 4°C. The tissue homogenate was centrifuged for 15 min in a microfuge at 12,000 rpm to obtain the supernatant containing organelle fragments and synaptosomes. The supernatants were aspirated into plain labeled glass curvette placed in ice. GPx, GSH, MDA, CAT and SOD activities were assayed according to the manufacturer's instruction in the assay.

3.5. Histopathological Examinations

All the animals were sacrificed immediately after 24 hours fasting, and then the brain was perfused with phosphate buffered saline and 10% buffered formalin and then was fixed in 10% buffered formalin. The brain tissue was processed with different grades of alcohol and xylene, infiltrated with wax and then paraffin blocks were made. 3µ thick paraffin sections were taken from the blocks, sections were processed with different grades of alcohol, xylene and finally stained with hematoxylin and eosin. Studies on light microscopy of the cerebellar sections on glass slides were captured

using Olympus binocular research microscope (Olympus, New Jersey, USA) connected to a 5.0 MP Amscope Camera (Amscope Inc, USA.).

3.6. Statistical Analysis

All quantitative data were analyzed using the GraphPad Prism ® software (version 9.3.1). Biochemical outcomes using one way ANOVA with Tukey's multiple comparisons test. Significance was set at *p<0.05. The outcomes were represented in tables showing the mean and standard error of mean respectively.

4. Results

• *Oleic acid ameliorates changes in brain SOD levels*

Results showed a significant increase (p<0.05) in SOD levels in groups 4, 5 and group 8 when compared with the control (Table 1) while a significant reduction (p<0.05) in cerebellar SOD levels in groups 2 was observed when compared with control (Table 1).

• *Normoregulatory effects of Oleic acid on brain Catalase (CAT) levels*

A significant increase (p<0.05) in catalase levels was observed in groups 3, 4, 5, 7and 8 when compared to the control group while a significant decrease (p<0.05) in catalase levels in groups 2 was observed when compared with control group 1 (Table 1).

• *Oleic acid potentiates Glutathione peroxidase (GPx) levels*

A significant increase (p<0.05) in Glutathione peroxidase (GPx) levels was observed in groups 3, 4, 5, 7 and 8 when compared with control group (Table 1). A significant reduction (p<0.05) in GPx levels in groups 2 was observed when compared with control (Table 1).

• *Oleic acid improved Brain Glutathione (GSH) levels*

A significant increase (p<0.05) in Glutathione (GSH) levels was observed in groups 3, 4, 5 and 8 when compared to control group while a significant decrease (p<0.05) in brain GSH levels in groups 2 was observed when compared with control (Table 1).

• *Anti lipid peroxidative effects of Oleic acid on Brain Malonialdehdye (MDA) levels*

A significant decrease (p<0.05) in Malonialdehdye (MDA) levels was observed in groups 4, 5, 7 and 8 when compared to the control group (Table 1) while a significant increase (p<0.05) in brain MDA levels in groups 2 was observed when compared with control (Table 1).

Table 1: Showing the serum biochemical analysis of across the groups.

Groups	SOD (U/mg)	CAT U/mg)	GPx (U/mg)	GSH (U/mg)	MDA (U/mg)
Control	2.52±0.21	3.13±0.43	1.10±1.52	2.21±0.71	3.31±1.18
2	2.28±0.25*	1.83±0.63*	0.86±0.52*	2.01±0.11*	3.78±0.51*
3	2.68±0.63	3.91±1.61*	1.61±1.30*	2.80±1.63*	3.10±0.15
4	3.29±1.91*	4.43±1.54*	1.73±1.94*	3.12±1.10*	2.53±0.11*
5	3.81±1.84*	5.15±1.25*	2.20±0.62*	3.93±1.24*	2.20±0.24*
6	2.63±1.50	2.93±0.18	1.21±0.44	2.36±0.31	3.46±0.61
7	2.67±1.71	3.42±1.51*	1.58±0.39*	2.51±1.74	2.92±1.10*
8	3.13±1.48*	3.83±0.65*	1.83±0.16*	3.14±0.12*	2.73±1.14*

*(p<0.05) - significantly different compared to control group.

5. Discussion

The most common cause of dementia is Alzheimer's disease (AD). It is a debilitating and progressive type of neurodegenerative disorder (NDD), classically characterized by neurofibrillary tangles and β-amyloid plaque deposition in the brain [9]. The devastating aspect of this disease leads to impaired behavioural phenotypic manifestations, like increased anxiety, loss of memory and reduced cognition [10] that often result in a low quality of life [11]. It is so debilitating that sufferers have less than a decade to live after being diagnosed and it currently has no cure [12]. Consequently, the ultimate goal of finding a drug therapy against AD is to significantly delay the onset (or progression) or prevent the disease development by averting the deterioration in memory and cognitive functions in people diagnosed with the disease. The observations seen in AD patients can be attributed to the neurochemical imbalances [13] that culminate in compromised functional neuronal and neuroglia integrity and histomorphology in vital brain areas like the hippocampus and prefrontal cortex (PFC) [14, 15]. Sustained neural oxidative stress, for example, activates beta-secretase (BACE) activities [16] inhibits cytochrome oxidase enzyme [17], thereby transforming amyloid precursor protein (APP) processing to an amyloidogenic derivative [18]

In the present study, by assessing oxidative stress markers and histopathological changes in the cerebellum cortex of Wistar rats, we explored the therapeutic benefits of oleic acid as a treatment regimen in aluminium-induced toxicity on a dose dependent level.

In this study, significantly depleted SOD levels in the cerebellar cortex was observed highlighting the detrimental effects of the neurotoxic substance $AlCl_3$.The mechanism of neurotoxic substances were thought to be due to the oxidative stress involved in

the production of reactive oxygen species (ROS), including superoxide anion, hydrogen peroxide, superoxide radical and hydroxyl radical. It has been widely reported that the degree of oxidative damage is dependent on the balance between oxidative stress and the efficiency of the endogenous antioxidant system that is found in the majority of cells Olajide *et al*. [19].

In this study, a significant increase in brain SOD levels in the groups administered oleic acid was observed when compared to the $AlCl_3$ group. One of the brains enzymatic defense systems is the superoxide dismutase (SOD) which changes the superoxide radical anion to H_2O_2 via oxidative decay. Another importance of SOD is to protect dehydratase from free radical superoxide inactivation. In a normal physical and chemical state, SOD catalyzes superoxide (O^{2-}) neutralization, which is a biologically toxic molecule that contributes to neuron pathogenesis via oxidative toxicity. Therefore, the observed cerebellar degeneration in rats treated with $AlCl_3$ may be due to increased neural O^{2-} shown by depleted SOD profiles in this group.

Oxidative impairment within the cerebellum may be due to mitochondrial impairment because $AlCl_3$ selectively destroys neuronal mitochondrial complexes. Impressively, the antioxidant and free radical scavenging activities of oleic acid (OA) was pronounced in groups treated with it alongside and $AlCl_3$ (Group 8). This was in line with Giampietri *et al.* [8] report on oleic acid antioxidant potentials where they observed that OA had effects on lipid accumulation, autophagy and cell death in different HCC cell lines compared to immortalized healthy hepatocytes. Note that the hyper-expression of SOD profiles observed in the cerebellum of rats in this study is a hallmark for OA's neuroprotective mechanisms.

Increased levels of GPx within cerebellar homogenates (Table 1) of rats in the treatment groups showed significant differences. This result was quite interesting as GPx is an effective antioxidant enzyme found in neurons and its decrease has been associated with astrogliosis [20, 21], particularly those leading to neuronal cell death [22] such as those observed in this study. A significant ($p< 0.05$) reduction in GPx activity was seen in the group treated with $AlCl_3$ in our study. This decreased neural GPx levels have been shown to result in increased tissue H_2O_2 content, hence leading to cellular death and damage [23, 24]. Thus, we can say that cerebellar damage is associated with diminished activities of antioxidant enzyme systems; and that oleic acid, via its antioxidant properties, confers protection against cerebellar neurodegeneration.

Catalase (CAT) a heme-containing tetrameric protein naturally produced by the body when exposed to O^{2-} consists of the brain's antioxidant system. In neural cells, catalase and peroxidase activities catabolize H_2O_2 enzymatically in aerobic organisms. This enzyme cannot be saturated at any concentration by H_2O_2 but it reacts with H_2O_2 to form oxygen and alcohol/water using heme as its cofactor [24]. CAT protects cells via detoxification of the generated H_2O_2 and plays a pertinent role in the acquisition of tolerance to oxidative stress as an adaptive response [25]. This was in line with our study while the group administered $AlCl_3$ showed that CAT inhibition led to the increased H_2O_2. It is interesting to outline that, this correlated with reports by Terlecky *et al.* [26]. Thus, CAT deficiency may be related to the pathogenesis of neurodegenerative diseases like AD. CAT treatment reduced H_2O_2 levels and improved neuronal survival following Al-induced toxicity in neuronal culture [27]. This was seen in the groups treated with OA at different dosages in the study.

Generally, brain tissues are highly susceptible to attacks from free radicals due to its high unsaturated lipid contents [28]. Studies have shown that exposure to Al results in the impairment of mitochondrial functions *in vivo* and *in vitro*, as well as it destroys the antioxidant defense system by decreasing the antioxidant enzyme status [29]. Lipid oxidative products are one of the primary outcomes associated with oxidative stress; the significant decrease in brain MDA observed in our study (Table 1) showcased the neuroprotective effects of OA against the induction of lipid production by the neurotoxicant, $AlCl_3$. This result corresponded with other studies following intraperitoneal treatment with Aluminium [30].

Aluminium-induced corticohippocampal histopathology can be attributed to the roles it plays in exacerbating neural levels of reactive oxygen species (ROS) which culminate to neuroinflammation [31]. Al has been implicated in the induction of neuronal excessive iron uptake resulting in the distortion of the intracellular iron pool which drive cascades of chemical events that accelerate ROS production, beyond what endogenous antioxidant enzymes can cope with [32]. Results from our study showed significant decrease ($p<0.05$) in brain MDA levels in the treatment groups when compared with control (Table 1) while a significant increase ($p<0.05$) in the levels of MDA was observed in group 2. These adverse changes in the MDA profiles was restored to near normal levels in groups administered both $AlCl_3$ + OA on a dose dependent level. Therefore, this study reported that aluminium-induced oxidative stress which culminated into several neurotoxic events, such as lipid peroxidation [33] and neuroinflammation [34] that ultimately compromised structural and functional integrity of neural cells, hence the manifestation of the decline seen in the animals exposed to aluminium toxicity.

This study demonstrated the cytological make up of cells revealing pertinent mechanisms-arising from cytoarchitectural alterations that precipitate neurodegenerative deficits (Figure 1). Photomicrographs showed well-arranged and normal cerebellar cytoarchitecture and layers in groups treated with 1ml OA, 1.5ml OA, 2ml OA and control (Figure 1 a, c, d, e). The following observed features were; well delineated purkinje cell neurons present in the purkinje layers and organized cellular mass within the layers of these groups. Cytological features observed in group 2 include

perineural spaces within granular cells, distorted cerebellar cytoarchitecture characterized by increase in cerebellar mass, hypertrophied purkinje cells, cellular vacuolations, distorted neuropil and fragmented granule cell layers. These findings were in consonance with the observed neuronal cell loss, pyknotic nerve cells and dendritic thickening, which are features associated with AD pathogenesis [35]. Furthermore, such alterations resulted to neuronal loss of signal processing, deficient synaptic efficacy and loss of timing of neurons of the cerebellum, and are often seen within cerebellar cortices of patients with dementia [36].

Studies have reported the cholinotoxic effects of $AlCl_3$ to neurons. It was reported that Al disrupted the glutamate- NO-cyclic guanosine monophosphate signaling pathway, leading to apoptosis in neurons [37]. In this study, the $AlCl_3$ degenerative processes accounted for the mechanisms through which cerebellar degeneration was initiated and further mapped out how the cerebellum degenerates during AD progression. However, rat treatment with oleic acid following $AlCl_3$-induced toxicity in groups 6-8 partially normalized cerebellar cytoarchitecture with few degenerative changes but well-structured and delineated cerebellar layers. This study suggest that OA protected against $AlCl_3$- activated neurotoxic cascades which triggered proteases that help terminate toxic molecules required for neuronal homeostasis and survival. It also inhibited lipid peroxidation and thus accelerated the inflammation [38**].** Therefore, preventing the peroxidation of cerebellar cell membranes further delineates its neuroprotective roles as observed in our study.

The cerebellar cytoarchitecture features observed in the groups aforementioned above (Figure 1a, c, d and e) corresponded with reports by Major *et al.* [39] who reported that normal dendritic morphology of the cerebellum showed appropriate synapticity that supported synaptic processes of neuron thereby contributing to the free flow of information in neurons. In line with this, the observed cytological features seen across cerebellar layers in these groups are such that Purkinje neurons possess dendrites with numerous spiny branches that penetrate the molecular layers while Parallel fibers formed by spiny dendritic trees of the Purkinje neurons of the granule cells stimulating GABAergic responses with cerebellar nuclei. It is noteworthy to state that cerebellar granule cells of rats treated with $AlCl_3$ in our study had cryptic and loose appearance; pyknosis of Purkinje cells bodies and damaged dendritic processes that were sparsely distributed around the indistinctly demarcated cerebellar layers. Similarly, rats groups treated with OA before exposure to $AlCl_3$ (F-H) were characterized by Purkinje neurons with sparse neural spaces. Previous studies have reported the bipartite connection between cerebellar nuclei outputs and cerebral areas such as the prefrontal cortex. For example, the cerebello-cerebral connection controls cognitive abilities and movement through the action of the cerebellum. Therefore, the loss of Purkinje neuronal projections as a result Al induced toxicity may lead to cognitive and motor deficits seen in late stages of the disease. These findings were in consonance with by Strick *et al*. [40],

Finally, this study showed also that $AlCl_3$ induction interfered with normal oxidative redox within the mitochondrial machinery of cerebellar cells which increased ROS via the overwhelming of intrinsic antioxidant defense system and oxidized lipid cellular components by causing damage to the neuronal membrane as seen in the histopathology. In our study, oleic acid may serve as a treatment regimen due to its numerous advantages because it reduced aluminium-induced corticohippocampal degeneration on a dose dependent level, significantly improved antioxidant defense system and further enhanced cerebellar histomorphology.

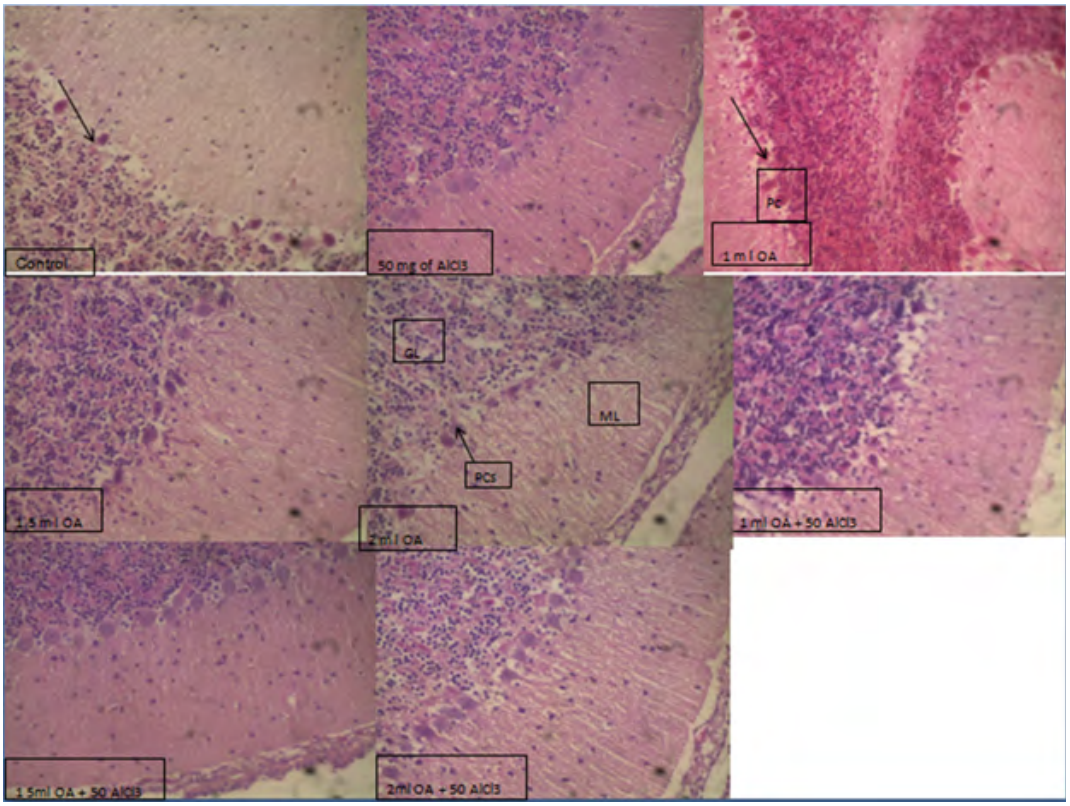

Figure 1: Showing the magnified views of the cerebellar cortex general micromorphological presentations in Wistar rats across the study groups 1-8. The granular layer (GL), Purkinje layer (PL) and molecular layers (ML) are demonstrated across study groups. Normal cerebellar histological features (black arrows) are seen in rats treated with either Control, 1ml OA, 1.5ml OA, 2ml OA. AlCl3 treated group showed an increase in cellular density and mass, distorted cell membrane, fragmented neuropil and granule cell layers and neuropil and significant pyknotic hypertrophied Purkinje cells with indistinct cell membranes (PCs) (black arrows). Groups administered OA + AlCl3 showed normal structured and demarcated cerebellar layers which were similar to those in control and OA groups. (scale bar- 400µm).

6. Conclusion

We conclude that structural, cellular and metabolic degeneration of the cerebellum occurs during AD progression and this was well manifested by neuropathological features associated with AD. In addition, oleic acid exhibited inhibitory and neuroprotective potentials in response to $AlCl_3$-induced cerebellar degeneration. It is therefore important to note that OA (2mL dose) was more efficacious among the dosages administered.

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8. Author Contributions

A.F.C and I.O initiated the research. I.O. and A.F.C. participated in the design and implementation of the experiments. I.O., A.F.C., O.K.K participated in the implementation of the experiment, analysis of results and manuscript writing. O.K.K., O.K.O., and A.G.E. proof read the article for final corrections.

9. Disclosure Statement

The authors declare no conflict of interest. The manuscript is complied with International Committee of Medical Journal Editor's guidelines**.**

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