



The Effects of Aqueous Leave Extract of *Moringa oleifera* on the Hippocampal Histology of Aluminium Chloride-Induced Alzheimer's Disease in Adult Wistar Rats

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Abstract: *Introduction:* Consumption of antioxidant-rich foods and polyphenol treatment can enhance cognitive performance in elderly subjects. Hence, this study was aimed at investigating the effects of aqueous extract of *Moringa oleifera* leaves on the hippocampus of aluminium chloride-induced alzheimer's disease in adult wistar rats. *Method:* Twenty (20) healthy adult male wistar rats (*Rattus norvegicus*), weighing 180-240g were divided into five (5) groups of four (4) animals each. Group one served as normal control group and was given free access to normal saline for 21days, group 2 was given 400 mg/kg of *Moringa* extract only for 21 days, group 3 was given 200 mg/kg of AlCl₃ for 21days. Group 4 which served as treatment group was given co-treatment of *Moringa* extract and AlCl₃ for 21 days while groups 5 which served as standard drug group received co-treatment of Simvastatin and AlCl₃ for 21 days. Parameters studied include; hippocampal CA3 histopathology, and CA3 histochemical changes after H&E and Congo red stains. *Results:* This study revealed that aqueous leaves extract of *Moringa oleifera* and standard drug-Simvastatin- at both Histopathological and histochemical parameter was effective. *Moringa* aqueous extracts ameliorated the reported histochemical and histopathological effects of AlCl₃ in treated rats. *Conclusion:* This study reveals that the tested extracts provided efficiently a neuroprotective effect against Alzheimer's disease. The *Moringa* extract group and Simvastatin group showed effectiveness in treatment of neurodegenerative disease. This shows that *Moringa* extract is an alternative remedy to Simvastatin in the treatment of neurodegenerative disease.

Keywords: Alzheimer's Disease, Hippocampus, *Moringa oleifera*

1. Introduction

Dementia is a clinical syndrome characterized with memory, attention, language, and problem solving impairments affecting the daily activity living of patients. Alzheimer's disease (AD) is the most common neurodegenerative disease affecting more than 40 million people worldwide [1]. Alzheimer's disease is the most common cause of progressive dementia in the elderly population. It is a chronic neurodegenerative disorder that leads to progressive disturbances of cognitive functions

including memory, judgement, decision-making, orientation to physical surroundings and language [2]. Alzheimer's Disease (AD) has been postulated to result from oxidative stress elevation in the brain [3], extracellular formation of amyloid plaques and intracellular deposits of neurofibrillary tangles in the hippocampus, cerebral cortex [4] Acetylcholinesterase (AChE) inhibitors are the most common class of drugs for AD, such as galantamine, rivastigmine, and donepezil, which temporarily enhance the availability of acetylcholine at cholinergic synapses [5].

The hippocampus is a small, curved formation in the brain that plays an important role in the limbic system [6]. It is found in the temporal lobe below the cerebral cortex [7, 8]. In adult humans, the volume of the hippocampus on each side of the brain is about 3-3.5 cm³ as compared to 320-420 cm³ for the volume of the neocortex [6]. The hippocampal formation is a key site of pathology in Alzheimer's disease [9] and its clinical consequence has been extensively studied [10, 11].

Aluminum (Al) is the third most common, and one of the most widely distributed metallic elements in the earth's crust [12, 13]. Although most Al exists as insoluble aluminosilicates and Al oxides, it is a known neurotoxin which presents an opportunity for human exposure and provides ubiquitous contamination [14]. Many reports implicate Al with Alzheimer's Disease (AD), Amyotrophic Lateral Sclerosis (ALS), Parkinsonism Dementia Complex, etc. [15-17]. Al is capable of producing much damage to the nervous system, including the impairment of learning and memory [18].

Statins are a class of medications that reduce cholesterol by inhibiting 3-hydroxy-3-methylglutaryl-coenzyme A reductase [19]. Statins is commonly known as 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors, and is the first-line drug therapy for the treatment of hyperlipidemia and the first choice for the prevention of coronary heart disease [20]. Its clinical application include reducing blood lipids however prevention and treatment of dementia is gradually drawing people's attention [21]. Statins not only lower serum cholesterol levels, but also inhibit pivotal enzymatic reactions (e.g. the isoprenylation of a subset of GTPases) [22] that lead to amyloid deposition and plaque formation; both are considered cornerstone pathways underpinning the development of Alzheimer's disease (AD) [23].

Moringa oleifera is a miracle tree species [24] with so many nutrients, consumption of *M. oleifera* leaf reinforces neural response, stimulates immune functions, and improves health because of the large amounts of microelements and polyphenol antioxidants [25]. Accumulative lines of evidence have demonstrated that consumption of antioxidant-rich foods and polyphenol treatment can enhance Anxiolytic and curative effects in the brain [26-32] and has also been reported to combat oxidative stress in rat model of Alzheimer's disease induced by colchicines such as vitamin C and vitamin E [33]. However, the scientific evidence concerning the effect of *M. oleifera* leaves extract on cognitive dysfunction induced by cholinergic function remains limited.

2. Materials and Methods

2.1. Sample Preparation and Extraction Procedure

2.1.1. Plant Materials

Fresh leaves of the plant, *Moringa oleifera* was harvested from plants grown in Enugu State, Nigeria. The leaves were

identified by a crop scientist of the Department of Agronomy and Ecological Management, Faculty of Agriculture and Natural resources Management, Enugu State University of Science and Technology, Agbani, Enugu, Nigeria on January 2020.

2.1.2. Preparation of *Moringa oleifera* Extract

The sample was dried in air for 7 days and after complete drying was crushed in the form of fine powder with a super Sony Japan electric blender. 200 g of dried plant extract was immersed in 2000 mls of water and was evaporated with a Soxhlet extractor to yield 21.2 g of gel extract, which was refrigerated until ready for use [34]. The experimental chemical Aluminium Chloride was procured from an approved pharmaceutical/chemical store in Enugu metropolis.

2.1.3. Animal Management and Grouping

The study was approved by the Ethical Committee of Enugu State University of Science and Technology, College of Medicine and carried out in accordance with the principles of laboratory animal care and standard experimental procedures.

Twenty four Wistar rats at the age of approximately three months each weighing 180-240g, were used for the study. The animals were kept in a plastic cage with iron nettings placed in a well ventilated standard housing conditions, twelve hours light and twelve hours darkness. The animals were acclimatized for two weeks before the commencement of the experiment. During this period, the animals were observed to ensure that they were disease free. They were fed with rat chows and given water ad libitum, and an ambient temperature range of 25-27 maintained at 50% humidity. The animals were weighed with an electronic weighing balance prior to the commencement and termination of the experiment.

2.2. Experimental Design

After an acclimatization, the rats were divided into five groups each consisting of four animals in accordance to their body weight and the experiment was run for twenty one days. Rats were administered their respective doses of *Moringa oleifera*, AlCl₃, and Simvastatin each for 21 days. The groups (A-F): A (positive control giving normal saline only for 21 days), B (*Moringa* extract for 21 days), C (negative control giving Aluminium Chloride for 21 days), D (Aluminium Chloride and *Moringa oleifera* extract co-treatment for 21 days), and standard group E (Aluminium Chloride and Simvastatin for 21 days).

Each group consisted of four rats. Alzheimer's disease was induced (in groups C, D and E 'test' rats) by oral gavage administration of AlCl₃ (200 mg/kg body weight), freshly dissolved in 0.9% saline. The positive control rats were injected with only 0.9% saline orally.

The brain analysis was carried out to check effect of the aqueous extracts of *Moringa oleifera* leaves on the Hippocampus.

The histological examination was also carried out to

determine the extent of degeneration, regeneration, ameliorative and curative effect of the *Moringa oleifera*

extract, the deposition of beta amyloid, and its curative and attenuation in comparison to the positive control.

Table 1. Table showing grouping of experimental animals.

| Groups | No of rats per cage | Inducing agent/ extract | Dosage | Duration |
|----------------------------------|---------------------|---|--|----------|
| Group A (positive control group) | 4 | Normal saline | Control | 21 days |
| Group B (negative control) | 4 | MOE extract | 400 mg/kg | 21 days |
| Group C | 4 | AlCl ₃ | 200 mg/kg body weight | 21 days |
| Group D | 4 | AlCl ₃ +MOE extract 3 simultaneously | AlCl ₃ =200 mg/kg, MOE=400mg/kg | 21days |
| Group E | 4 | AlCl ₃ + Simvastatin | AlCl ₃ =200 mg/kg, Simvastatin=40 mg/kg | 21 days |

2.3. Animal Sacrifice, Tissue Collection and Processing

Before sacrificing the wistar rats, the final weight of the test animals were collected. The wistar rats were anaesthetized using chloroform. The cranium of each of the animals was opened using brain Forceps, the hippocampus was then separated from the cerebrum. The tissues were then fixed in 10% formal saline and processed for histological observation using routine Haematoxylin and Eosin, and Congo red staining techniques.

2.4. Tissue Preparation for Microscopy

The tissue were subsequently trimmed, dehydrated in ascending grades of alcohol (70%, 80%, 90% and absolute alcohol), cleared in three (3) changes of xylene and embedded in molten paraffin wax. Sections of 5µm thickness with a rotary microtome were made, floated in water bath (45oc) and incubated at 60 oc to dry. The 5µm thick sections were subsequently stained in using hematoxylin and Eosin stains. The prepared slides was examined with a Motic™ compound light microscope using x4, x10 and x40 objective lenses. The photomicrographs was taken using a Motic™ 2.0 megapixels microscope camera at x100; x160 & x400 magnifications [35].

3. Results

3.1. Hippocampus (H and E)

Representative photomicrographs of the hippocampus showing the panoramic view of the CA3 region (Cornu Ammonis). The CA3 is the region with large densely packed pyramidal cells (black arrow) in the concavity of the dentate gyrus. Deducible histology presentation includes; cellular delineation, cellular density, staining intensity and histomorphology cellularity. Two copies of images are made available one of which is labelled and the other is plain.

3.2. Hippocampus (Congo Red)

Representative photomicrographs of the hippocampus showing deposition of amyloid β plaques (yellow arrow) in the CA3 region at a high magnification (X400). Slides presents with varying staining intensity and cellular density. Amyloid deposit is stained red and nuclei are stained blue. Two copies of images are made available one of which is

labelled and the other is plain.

4. Discussion

Poor neuronal connectivity, neuronal loss in the overall brain matter with specific loss of cerebellar and hippocampal neurons, decreased dendritic spine density and remodeling could lead to impaired performance on a hippocampal-dependent learning and memory task, context discrimination [36] and memory disturbances which are clinical signs in AD, even in the preclinical stages [37]. In this study (Figures 2, 3 and 5) the photomicrographs showed shrunken pyramidal cells which is an evidence of neurodegenerative disease [38] post chronic administration of the Moringa Leaves extract in the wistar rat.

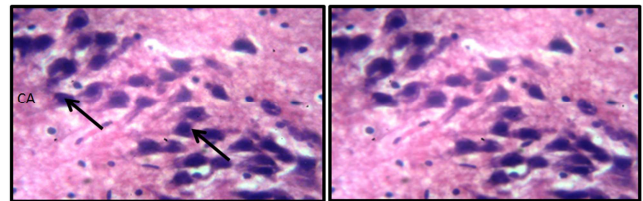


Figure 1. (Normal control): Representative photomicrograph of the CA3 region of the hippocampus of experimental animal. The Haematoxylin and Eosin photomicrograph shows normal histomorphology and staining intensity of the densely and tightly packed pyramidal cells of the Cornu Ammonis (CA3) region (H and E x400).

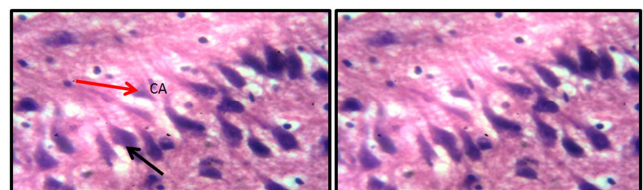


Figure 2. (Moringa group): Representative photomicrograph of the CA3 region of the hippocampus of experimental animal. The Haematoxylin and Eosin photomicrograph shows normal histomorphology and staining intensity but the pyramidal cells of the Cornu Ammonis 3 region are not densely and tightly packed. Few shrunken cells are present (red arrow) (H and E x400).

In this study, (Figure 3) histopathological evaluation of hippocampal sections of AlCl₃ treated group of rats confirmed neurodegenerative changes in CA3. This is consistent with previous study by [39] where AlCl₃ induction group showed massive depletion of cellular body, acidic cytoplasm, nuclear vacuolation and more intercellular space. However, the experimental group (Moringa extract + AlCl₃) (Figure 4) showed fewer shrunken cells than in Figure 3.

This shows that the administration of *Moringa* extract was able to attenuate the pathological changes of pyramidal cells in the CA3 region, in the Co-treatment.

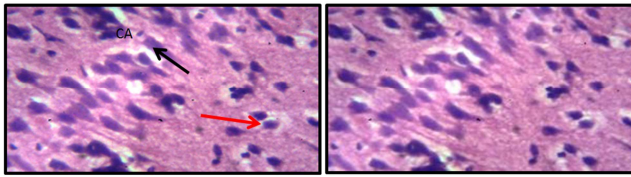


Figure 3. ($AlCl_3$ group): Representative photomicrograph of the CA3 region of the hippocampus of experimental animal. The Haematoxylin and Eosin photomicrograph shows normal histomorphology and staining intensity but the pyramidal cells of the Cornu Ammonis 3 region are not densely and tightly packed. Few shrunken cells are present (red arrow) (H and E x400)

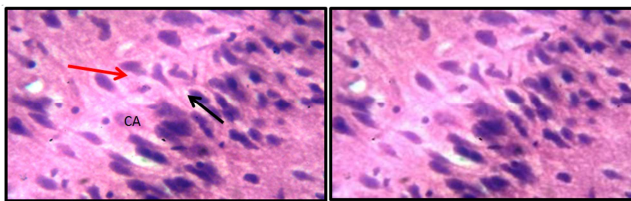


Figure 4. (*Moringa* and $AlCl_3$ group): Representative photomicrograph of the CA3 region of the hippocampus of experimental animal. The Haematoxylin and Eosin photomicrograph shows normal histomorphology and staining intensity of the densely and tightly packed pyramidal cells of the Cornu Ammonis 3 region. Few shrunken cells are present (red arrow) (H and E x400).

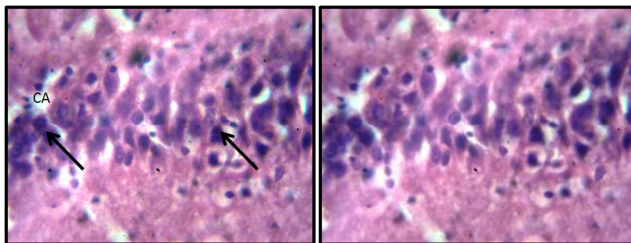


Figure 5. (*Moringa* extract and Simvastatin): Representative photomicrograph of the CA3 region of the hippocampus of experimental animal. The Haematoxylin and Eosin photomicrograph shows normal histomorphology and staining intensity of the densely and tightly packed shrunken pyramidal cells of the Cornu Ammonis 3 region. (H and E x400).

4.1. Hippocampus (Congo Red)

The histochemical changes in hippocampus CA3 region was examined with Congo red stain. Congo red stain demonstrated amyloid deposits in tissue sections. The normal saline treated groups (Figure 6) present normal histology and normal neuronal cells implying that there is no deposition of amyloid β plaque. The photomicrography of $AlCl_3$ treated wistar rats slide (Figure 8) showed high extracellular deposition of preformed senile plaques with hollow-spaced neuropils in pyramidal cells of hippocampus (CA3) suggesting Alzheimer's disease. This is in agreement with the previous study of [40] which identified the presence of beta amyloid plaque as hallmark pathology required for a diagnosis of Alzheimer's disease.

The *Moringa* extract treated group (Figure 7) in this study showed moderate deposition of amyloid β plaques suggesting

neurotoxicity in chronic consumption of overdose of *Moringa*. This implies that chronic consumption of *Moringa* at high dose has negative side effects on the body as also supported by the results from the H&E stain of the present study.

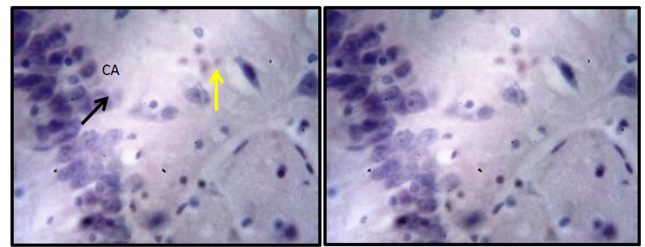


Figure 6. (Normal control): Representative photomicrograph of the CA3 region of the hippocampus of experimental animal showing pyramidal cells. This slide present pyramidal cells that are densely packed and properly delineated. Normal histology and normal neuronal cells. (Congo red X400).

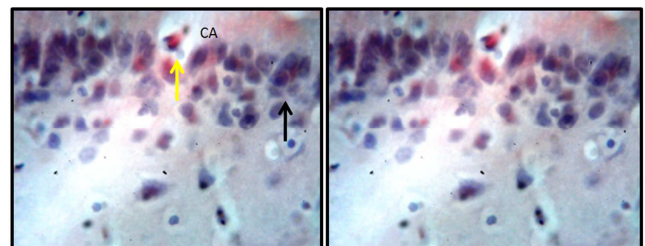


Figure 7. (*Moringa* group): Representative photomicrograph of the CA3 region of the hippocampus of experimental animal showing pyramidal cells. This slide present pyramidal cells that are densely packed and properly delineated. Moderate deposition of amyloid β plaques (yellow arrow) (Congo red X400).

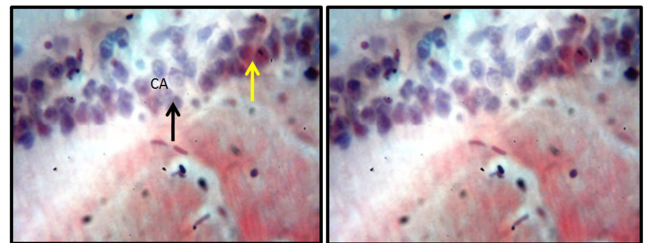


Figure 8. ($AlCl_3$ group): Representative photomicrograph of the CA3 region of the hippocampus of experimental animal showing pyramidal cells. This slide present pyramidal cells that are densely packed and properly delineated. High deposition of amyloid β plaques (yellow arrow) (Congo red X400).

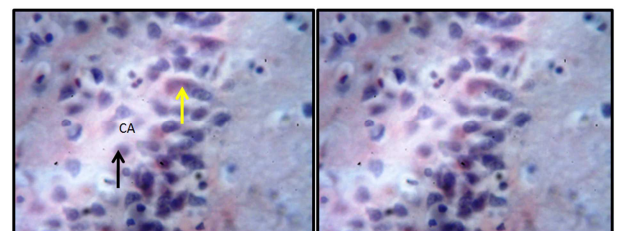


Figure 9. (*Moringa* and $AlCl_3$ group): Representative photomicrograph of the CA3 region of the hippocampus of experimental animal showing pyramidal cells. This slide present pyramidal cells that are densely packed and properly delineated. Moderate deposition of amyloid β plaques (yellow arrow). (Congo red X400).

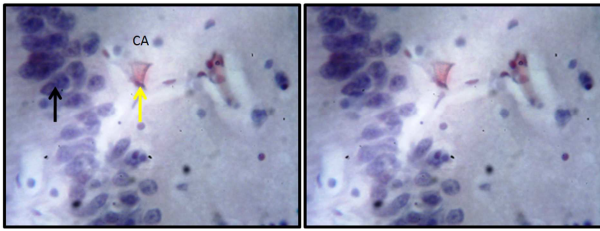


Figure 10. (*Moringa* extract and Simvastatin): Representative photomicrograph of the CA3 region of the hippocampus of experimental animal showing pyramidal cells. This slide present pyramidal cells that are densely packed and properly delineated. Moderate deposition of amyloid β plaques (yellow arrow) (Congo red X400).

Moringa extract and AlCl_3 co-treatment in this study (Figure 9), showed moderate deposition of amyloid β plaques, signifying that administration of *Moringa* extract attenuated the pathological changes induced by the AlCl_3 . The co-treatment of Simvastatin and AlCl_3 (Group E) showed moderate deposition of amyloid β plaques implying that the Simvastatin was able to ameliorate the pathological effects of AlCl_3 .

4.2. Comparison of Simvastatin with *Moringa* Leave Extract

Accumulative lines of evidence have demonstrated that consumption of antioxidant-rich foods has also been reported to combat oxidative stress in rat model of Alzheimer's disease induced by colchicines such as vitamin C and vitamin E [33]. H&E stain, (Figure 1 - Figure 5) result gotten from our study showed normal histomorphology and staining intensity but few shrunken pyramidal cells in both *moringa* extract and simvastatin. This implies that both remedies ameliorated neuronal degeneration.

From the result gotten from the Congo red stain, moderate deposition of amyloid β plaques in the photomicrographs of both *Moringa oleifera* extract group and simvastatin group was observed. This implies that both *Moringa* and Simvastatin attenuated the accumulation of amyloid β plaque in the nerve cell of the brain.

It can be deduced therefore that both *Moringa* Extract and Simvastatin ameliorated neuronal degeneration and as well attenuated the accumulation of amyloid β plaque hence *Moringa oleifera* extract can be used as an alternative drug to Simvastatin in the cure of Alzheimer's disease.

5. Conclusion

Herbal remedies have been on the increase especially due to side effects and high cost of some conventional medicines. The extract of *Moringa oleifera* despite its numerous benefits in treatment of various ailments also showed convincing evidence as a remedy for neurodegenerative diseases. The leave extract of *Moringa oleifera* application on the Alzheimer's disease-induced Wistar rat ameliorated the neurodegeneration and showed its protection against the disease. Thus advocate for the use of *Moringa* leave extract in the control and management of neurodegenerative diseases such as the Alzheimer's disease.

Further study should however be carried out to determine dose and duration dependent effect administration of *Moringa oleifera* extract on the ultrastructural changes in hippocampal histology and neuronal synaptogenesis.

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