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Research Article

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Moringa oleifera extract and neostigmine confer neuroprotection on aluminium chloride -induced Alzheimer's disease: Antioxidant and acetylcholinesterase inhibitory activities

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ABSTRACT

Alzheimer's disease (AD) is a neurological episode associated with the senescence process characterized by neuronal cell death in brain regions and loss of cognition. The study investigate the neuroprotective potentials of *Moringa oleifera* leaf extract and Neostigmine in a rat model of Alzheimer's disease induced by Aluminium chloride ($AlCl_3$). A total number of 20 rats weighing 180-240 kg were used for this study and they were grouped into 5 with 4 rats in each group (n=4) labelled A-E. Group A control, received 0.1 mL normal saline (placebo), Group B, received 100 mg/kg of Aluminium chloride, Group C was given Aluminium chloride 100 mg/kg and 200 mg/kg, Group D experimental animals received Aluminium chloride 100 mg/kg and were treated with 400 mg/kg of the extract orally for 21 days, while Group E received the same dose of $Alcl_3$ and 2 mg/kg Neostigmine given intraperitoneal (IP). Thereafter, the rats were sacrificed, and the hippocampi were harvested for histology and histochemical analysis. Our findings revealed visible protective changes in the group treated with the *M. oleifera* and Neostigmine as the pyramidal cells of CA3 and neurofibrillary tangles which were prominent in the positive control group were resolved. At the end of this study, it was proven that the toxicity induced by Aluminium chloride was reduced by *M. oleifera* leaf extract and Neostigmine. *M. oleifera* is a commonly consumed plant therefore the intake should be encouraged.

KEYWORDS: Neurofibrillary Tangle, Chromatolysis, Pyknosis, Nissls Granule, Oxidative Stress

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INTRODUCTION

Dementia is widely perceived as global cognitive impotence of the brain including the impairments of memory, attention, language, and problem-solving. The incidence is tied to the process of senescence where the elderly are the most affected people, with at least an estimated 35.6 million people recorded Worldwide (Prince *et al.*, 2013; Rizzi *et al.*, 2014; Valls-pedret *et al.*, 2015; Niu *et al.*, 2017). Dementia, particularly Alzheimer's, has immense health and social implications in developing nations. It is associated with an enormous economic burden on families and society due to the high outcome of the therapeutic strategy (Brookmeyer *et al.*, 2007).

One of the causal agents is an elevated oxidative stress status in the brain which results in hippocampal atrophy and the disruption of cholinergic neuronal activity (Mattson, 2004). Thus, insight into the modulatory functions of cholinergic neurons is a putative key to dementia treatment. However, most drugs in this class still exert adverse reactions requiring a prompt solution to better treatment outcomes (Doggrell & Evans, 2003). Consequently, research efforts are motivated in search of a protective agent(s) against Alzheimer's a subtype of dementia.

Preferences for plant-derived compounds over synthetic drugs have increased in recent years. Accumulative lines of evidence have demonstrated their consumption is safer and has potent bioactive agents that are rich in antioxidants and polyphenols which can enhance cognitive performance in elderly people (Valls-Pedret *et al.*, 2015; Akram & Nawaz, 2017). *M. oleifera*,

family *Moringaceae*, is a plant used as food and medicine in most parts of Nigeria especially Northern Nigeria. The leaves are rich sources of potassium, calcium, phosphorous, iron, vitamins A and D, essential amino acids, and antioxidants such as carotene, vitamin C, and flavonoids (Bennett *et al*., 2003.). In addition, alkaloids, tannins, phenolics, saponins, and steroids have been detected (Bamishaiye, 2011). The antioxidant activity of its leaves extract has been reported in a rat model of Alzheimer's disease induced by colchicines and compared to antioxidants such as vitamin C and vitamin E against oxidative stress (Ganguly & Guha, 2008; Ijeomah *et al*., 2012). Therefore, we investigated the neuroprotective effect of *M. oleifera* leaves extract in an animal model of Alzheimer's induced by aluminium chloride.

MATERIALS AND METHODS

Plant Preparation and Extraction

The leaves of *Moringa oleifera* were harvested from a cottage farm in Enugu metropolis, Enugu State Nigeria during the month of June 2019. It was authenticated by a curator in the Faculty of Agriculture, Department of Plant Science and Biotechnology, University of Nigeria Nsukka. The *M. oleifera* leaves were rinsed in distilled water and were air-dried at room temperature. The plant-derived aqueous extract tested in this study was prepared in our laboratory by mixing 100 g powdered leaves of *M. oleifer*a with 500 mL boiling water for 5 minutes. The mixture was then filtered twice through a 2 µm pore sterile filter paper into a sterile tube. The aqueous extract stock solution (100 mg/mL) was freshly prepared for each set of experiments and stored at 4°C for up to 5 days.

Animal Husbandry

Twenty (20) male Wistar rats weighing 180 g - 240 g were used for this study. The animals were procured from the animal husbandry unit at Ebonyi State University, Ebonyi State, and transferred to the animal experiment laboratory at the ESUT College of Medicine Parklane (ESUCOM), Enugu. Nigeria. Animals were housed in cages made with wire gauze under normal room temperature and maintained under a regular light/dark cycle. The rats were also fed twice daily and observed hygiene environment sanitation and acclimatized for two weeks prior to the experiment. The study design was approved by the Research and Ethics Committee of the Faculty of Basic Medical Sciences and the animals were cared for in accordance with institutional and international guidelines.

Experimental Design

Aluminium chloride and Neostigmine were procured from registered chemical and pharmaceutical stores in Enugu Metropolis. The experimental animals were grouped into five groups composed of 4 animals in each cage. The rats weighed between 180 g - 240 g. Group A control, received 0.1mL normal saline, Group B, received 100 mg/kg of Aluminium chloride only, Group C was given Aluminium chloride 100 mg/kg and 200 mg/kg, Group D experimental animals received Aluminium chloride 100 mg/kg and were treated with 400 mg/kg of the extract orally for 21 days, while Group E received the same dose of $Alcl_3$ and 2 mg/kg Neostigmine given intraperitoneal (IP).

Histochemical Studies

All the rats were sacrificed on the 22nd day of the experiment under ether anesthesia, rat brains were harvested and fixed in 10% formal saline for 48 hours. Thereafter, the hippocampus was manually processed and embedded in paraffin. 10um thick sections were obtained from blocks and stained variously for Alzheimer indicators using Nissls and Silver stain.

RESULTS AND DISCUSSION

This study has investigated the effect of *M. oleifera* leaves extract on the hippocampus. In Nissl stained sections, we have observed that $AlCl_3$-induced vacuolization, showing the cells that have undergone central chromatolysis present with faintly stained center and deeply stained periphery, depicting neuronal cell loss, atrophic neurons, shrunken cytoplasm, and damaged nuclei in pyramidal cells of the hippocampus (CA3) (Figure 1 & 2). However, the administration of *M. oleifera* attenuated these pathological changes in the group treated with the extract as well as the neostigmine standard drug. Showing the intact chromatogenic neural cells present with deeply stained cell bodies and axonal projections. In addition, compared to the control group, there were no obvious histopathological changes in the regions of rats treated with *M. oleifera* alone. Evidence from previous studies indicated *M. oleifera* leaf extract contains antioxidants such as vitamin C and E and is involved in enhancing memory and nootropics activity which is beneficial in combating stress associated with AD (Pakade *et al*., 2013). A few studies have also attributed the alteration of monoamines such as norepinephrine, dopamine, and serotonin in-memory process as the mechanism that might be responsible for the memory-enhancing activities of *M. oleifera* (Ganguly & Guha, 2008).

It is well known that amyloid (A) plaques deposition and formation of neurofibrillary tangles are key histopathological features of AD. Neurofibrillary tangles offer a plausible explanation for the pathophysiology and microanatomical changes of AD (Ansari & Khodagholi, 2013). In the present study, we stained neurofibrillary tangles with the Bielschowsky silver staining. The $AlCl_3$-induced rats showed impregnated soma of pyramidal neurons with impregnated apical and basal dendrites within the external pyramidal layers and impregnated soma of granule cells of dentate gyrus while treatment with *M. oleifera* showed little unimpregnated cell bodies with an unimpregnated axonal and dendritic projection of pyramidal cells, In addition, compared to the control group, there were no histopathological changes in the hippocampus treated with *M. oleifera* alone. In our previous study moringa, aqueous extracts ameliorated the reported histochemical and histopathological effects of $AlCl_3$ in treated rats using Congo red (Ozor *et al*., 2020).

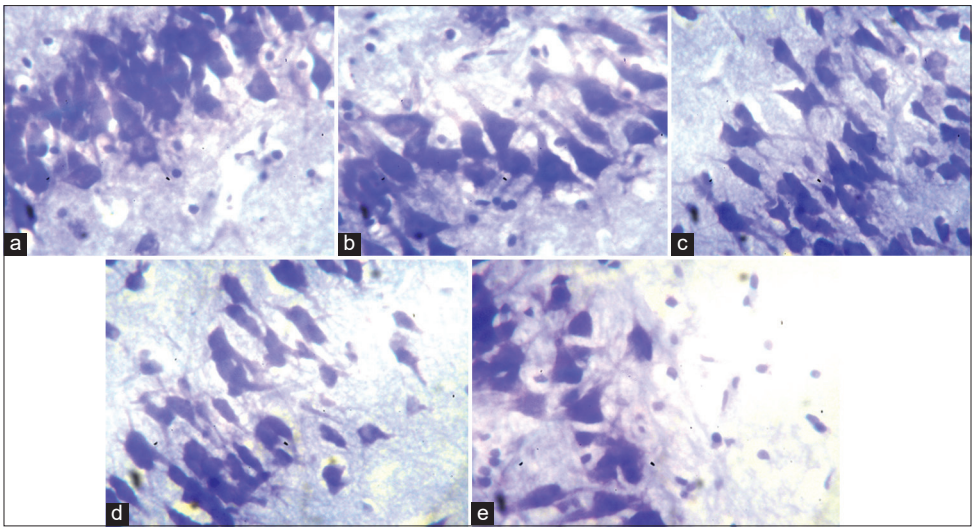

Figure 1: CA3 region of the hippocampus a) intensively stained Nissls substance and also well-defined and packed pyramidal cells. The pyramidal cells appear normal with large soma, with apical and basal dendrites. b) Stained Nissls substance but not well-defined and packed pyramidal cells. The pyramidal cells appear to have large soma with apical and basal dendrites. c) intensively stained Nissls substance but not well-defined and packed pyramidal cells. There appear to be mild chromatolytic changes. d) densely stained Nissls substance but not well defined and packed pyramidal cells. The pyramidal cells appear normal with large soma with apical and basal dendrites. e) deeply stained Nissls substance but Scanty pyramidal cell is observed (CFV. X400)

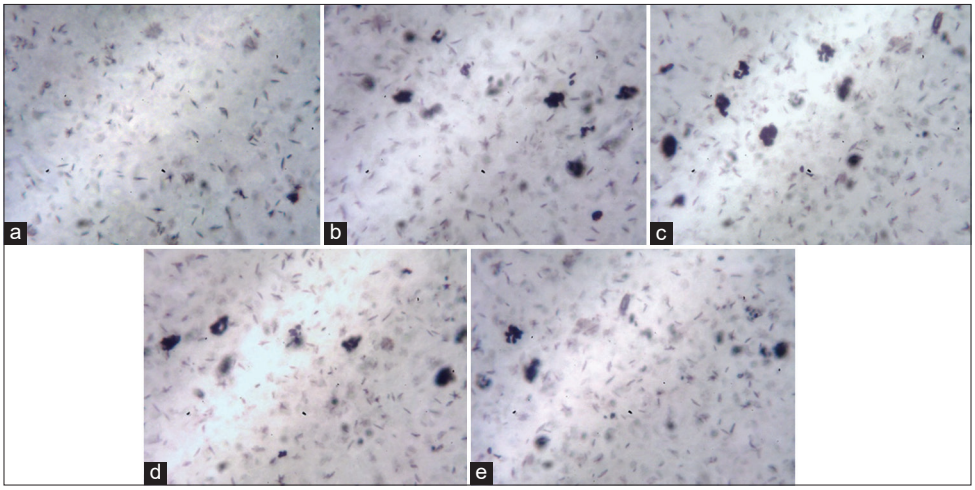

Figure 2: CA3 region of the hippocampus a) lacks neurofibrillary tangles, b) increase neurofibrillary tangles, c) reduced neurofibrillary tangles, d) reduced neurofibrillary tangles and e) reduced neurofibrillary tangles. (Bielschowsky Silver. X400)

This clearly implies the presence of neurofibrillary tangle which has been consistently reported in previous studies of AD. Particularly, Fu and Li (2011) show that neuronal changes in the Alzheimer's brain, in the early stage, are characterized by amyloidal deposits surrounding neurons and the formation of neurofibrillary tangles in the neuron. Neuroprotectivity from neurofibrillary tangles in neurons has been assessed for various herbs' potential to reverse the progressive course of the disease by resolving neurofibrillary tangles. In the present study, the extract was seen to have resolved the neurofibrillary tangles when compared with Neostigmine an inhibitor of acetyl-cholinesterase known to improve cognition in sufferers. These depicted similar outcomes although via different mechanisms. It is a normal finding to have cholinergic neuron functions decreased in Alzheimer's disease. But, upon administration of acetyl-cholinesterase inhibitor (AChEI) the degradation of acetylcholine is up-regulated, thereby increasing in resolving the cognitive decline and accompanying neuronal changes. This explains the possible mechanism by which Neostigmine has to attenuate the neuronal damage induced by aluminium chloride- hippocampal toxicity.

CONCLUSION

Histochemical findings indicate brain damage was induced by $AlCl_3$ but the observed protection afforded by *M. oleifera* and Neostigmine can be attributed to their antioxidant ability and acetyl-cholinesterase inhibitory activity respectively.

CONFLICTS OF INTEREST

The authors have declared that they have no competing interest

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