Asian Journal of Advances in Medical Science

3(4): 232-241, 2021

PROGRESSIVE CHANGES IN GAIT, KINEMATIC, HORIZONTAL AND VERTICAL ACTIVITIES IN RESPONSE TO OLEIC ACID AND HEAVY METAL EXPOSURE

O. N. ILOCHI[1*] AND A. N. CHUEMERE[1]

[1]Department of Human Physiology, Faculty of Basic Medical Sciences, University of Port Harcourt, Nigeria.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Received: 28 August 2021
Accepted: 03 November 2021
Published: 09 November 2021

Original Research Article

ABSTRACT

Oleic acid has a natural occurrence in most plants and is isolated for use in various industrial and medicinal purposes. The progressive changes in gait, kinematic, vertical and horizontal activities were studied in heavy metal exposure. Male wistar rats were randomly sampled in four groups of control as group *i* and oleic acid 1, 1.5 and 2ml co-treated with $AlCl_3$ at 50mg/kg as groups' *ii*, *iii* and *iv*, respectively. Oleic acid, especially at 1.5 and 2ml co-treatment with $AlCl_3$, significantly ($P<0.05$) enhanced gait, kinematic, vertical and horizontal activities compared to control. same co-treatment doses caused no significant change in calcium compared to control, but plasma level of muscle function markers changed significantly which may reveal a possible link between the dose of oleic acid treatment in heavy metal exposure and skeletal muscle activity. Oleic acid, at the studied dose may prevent or ameliorate the adverse neurologic changes caused by aluminum chloride.

Keywords: Oleic acid; heavy metal; AlCl3; gait; kinematic.

1. INTRODUCTION

Oleic acid (OA) is a 9Z-octadeconoic acid [1] with a natural occurrence [1,2]. Oleic acid is the most widely distributed and abundant fatty acid in nature [2]. It is used in the industrial preparation of food additives [3] [4], cosmetics and pharmaceutical solvents [5]. Oleic acid is a monounsaturated fatty acid and is the main component of olive oil [4]. Olive oil has beneficial effects in management of cardiovascular and gastrointestinal diseases [5,6,7]. Its effect on neurologic processes is not completely elucidated although they seem to be diverse depending on the division of the nervous system affected, age and extent of nerve injury [1,2]. Previous works have shown that oleic acid treatment leads to an accumulation of lipids in nervous system cells with no influence on cell viability [2]. Aluminum is a trivalent cation found in its ionic form in most kinds of animal and plant tissues [8]. It is the third most prevalent element and the most abundant metal in the earth's crust [9], representing approximately 8% of total mineral components [10]. Due to its reactive nature, aluminum can be found only combined with other elements like chloride and oxides [8]. Aluminum (Al) has the potential to be neurotoxic in humans and animals [9,10]. It is present in many psychopharmacologic agents and is used for water treatment and as a component in plant fertilizers [11]. Some modern vaccines contain aluminum [12]. The brain is a potential target for aluminum toxicity [11]. Aluminum can easily enter the blood-brain barrier via

**Corresponding author: Email: ilochiogadinma@gmail.com;*

its definitive high affinity to the receptors of transferrin [13]. In the brain, aluminum mostly accumulates in the motor and cognitive areas of frontal cortex and hippocampus, respectively [14]. Epidemiological investigations revealed that the accumulation of aluminum in the hippocampus in animals results in the anomalous Aβ accumulation, neuroinflammation and neuronal necrosis [15]. Various types of preceding animal investigations observed that the extended contact to the aluminum may lead to the neurobehavioral, neuropathological, and neurochemical alterations in brain that adversely affects the memory and learning capacities of the wistar rats [14]. There is paucity of scientific literatures concerning the progressive motor changes in response to oleic acid treatment before, during and/or after aluminum exposure. This necessitated the current study.

2. MATERIALS AND METHODS

2.1 Scope of Study

This study presented materials and methods relating primarily to neurosciences [12]. All observation tests were done using standard protocols.

2.2 Animal Collection

A total of 20 male wistar rats weighing 80-120 g were collected from the Research Animal House in Madonna University, Elele campus, Rivers State, Nigeria. They were allowed adaptation for one week in the animal house then weighed using a digital scale with accuracy of 0.001 gram, and randomly divided into 4 groups of 5 rats each. The study duration was 56 days.

2.3 Toxicity Study

The oral lethal dose 50 (LD_{50}) of oleic acid was determined using Lockes's method following standard protocols in correspondence to the Organization of Economic Co-operation and Development (OECD) regulations, code 432.

Table 1. Study design

Groups	Treatments
i	Water and feed *ad libitum*
ii	OA (1ml)+ $AlCl_3$ (50mg/kg)
iii	OA (1.5ml)+ $AlCl_3$ (50mg/kg)
iv	OA (2ml)+ $AlCl_3$ (50mg/kg)
***N*=5**	

Route of administration was oral. Treatment period for aluminum chloride was 22 days of equal interval while graded oleic acid treatment lasted throughout the study period.

2.4 Behavioral Test

2.4.1 Gait test

Gait test was done using a gait test apparatus. The parameters tested for include step length (SL), stride length (SDL), base of support (BOS), average speed (AS) and number of steps (BOS). The apparatus was cleaned after every test session.

2.4.2 Neuromuscular function tests

This test was done using the horizontal and vertical grids to determine the horizontal and vertical activities, respectively. Parameter tested for was hang time and descent time. Each test session lasted for a maximum test period.

2.5 Biochemical Tests

2.5.1 Brain tissue homogenization

This was done using standard procedures by Ilochi, et. al. [12]. The brain tissue was cut into slices of appropriate sizes for analysis (100 to 300mg) and placed into a microcentrifuge tube. The typical sample size was 100mg. The tissue was washed properly with 1 ml PBS. Glass beads (0.5mm) equal to tissue mass followed by 0.1 to 0.6ml of buffer was added. The microcentrifuge tubes were placed into blender at speed 6 at time 3 to homogenize.

2.5.2 Assay for creatine kinase

In the creatine kinase assay protocol, creatine kinase converts creatine into phosphocreatine and ADP. The phosphocreatine and ADP then reacts with creatine kinase enzyme mix to form an intermediate which reduces a colorless probe, to a colored product with strong absorbance at =450nm.

2.5.3 Assay for acetylcholinesterase

The principle was based on an improved Ellman's method. Thiocholine produced by acetylcholinesterase activity forms a yellow color with 5,5-dithiobis(2-nitrobenzoic acid). The intensity of the product color, measured at 412nm is proportionate to the enzyme activity in the sample.

2.5.4 Brain tissue electrolyte assay

Electrolytes are measured by a process known as Potentiometry. This method determines the potential difference that develops between the inner and outer phases of an ion selective electrode. The electrode is made of a selectively permeable material to the measured ion. The potential is measured by comparing it to the potential of reference electrode. Since the reference electrode has a constant potential, the voltage difference between the two electrodes is attributed to the concentration of ion in the sample.

2.6 Histochemistry

The craniums of each of the animals were removed after incisions were made across the anterior frontal and interparietal bones using brain Forceps, the cerebrum, hippocampus and cerebellum were isolated. The tissues were then fixed in 10% formal saline and processed for histological observation using routine Periodic Acid Schiff (PAS) staining techniques according to the methods of Bancroft and Gamble (1999). Photomicrography was done using a LEICA DM500 photomicrograph.

2.7 Statistical Analysis

In other to properly translate the outcome of this study, data collected from this study was analyzed using One-Way analysis of variance (ANOVA) and Post Hoc analysis with the aid of IBM®SPSS Version 20.0. The Percentage change (*%c*) was also presented in standard charts and tables, adopting methods used by Chuemere, et al., 2019 [12].

3. RESULTS

The results of this study include;

Table 2. Gait and kinematic function in oleic acid+$AlCl_3$ treatments

Groups	SL (cm)	SDL (cm)	BOS (cm)	NOS (Steps/cm)	AS (cm/s)
Control	6.18±0.06	9.75±0.34	7.63±0.04	0.14±0.01	13.36±0.21
Oleic acid (1ml)+$AlCl_3$	5.82±1.68*	9.68±1.57	7.58±2.26	0.15±0.01	10.26±0.32*
Oleic acid (1.5ml)+$AlCl_3$	6.56±2.73*	9.74±0.19	7.61±1.34	0.13±0.01	12.90±0.13*
Oleic acid (2ml)+$AlCl_3$	6.77±1.81*	9.83±3.67	7.89±1.95	0.12±0.01*	13.78±0.24
Total	**19.2**	**29.3**	**23.1**	**0.4**	**36.9**
Average	**6.4**	**9.8**	**7.7**	**0.13**	**12.3**

Table 1 shows gait and kinematic function in oleic acid+$AlCl_3$ treatments. There was a significant decrease ($P<0.05$) in SL and increase in NOS and AS in 1ml oleic acid co-treatment no significant change in all gait/kinematic function parameters except a significant increase in AS in 1.5ml co-treatment compared to control. There was a significant increase in SL and BOS in 2ml co-treatment.

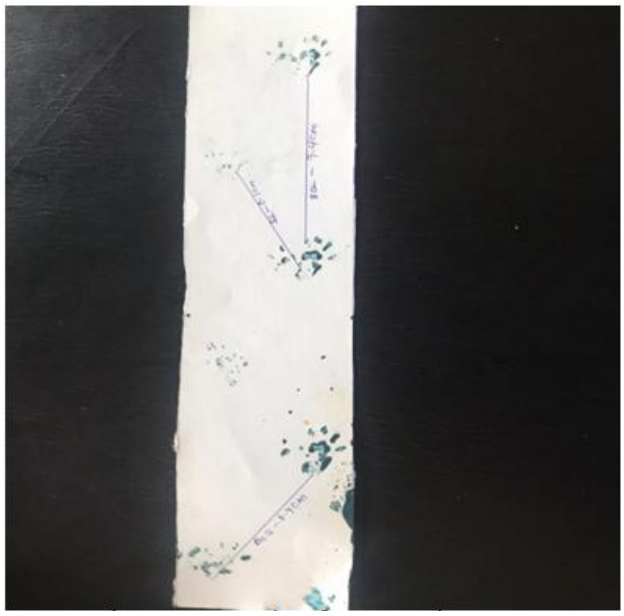

Fig. 1. Gait representation for control 1.5 ml+$AlCl_3$

Fig. 2. Gait representation for OA

Fig. 3. Gait representation for OA 1ml+$AlCl_3$

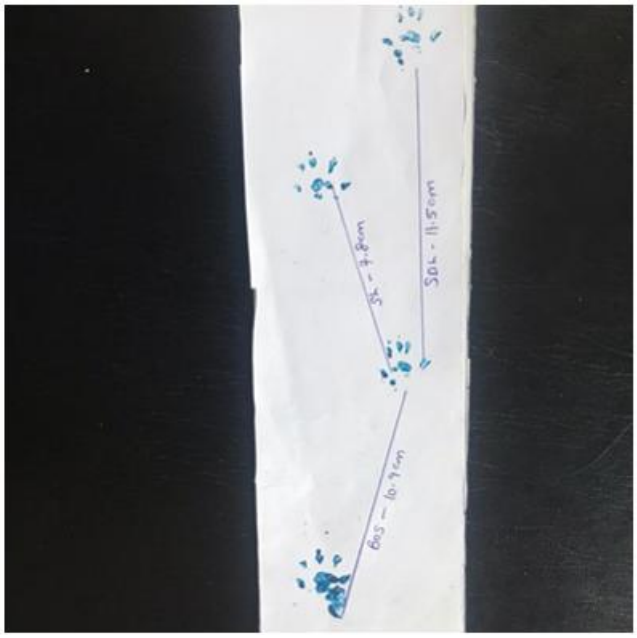

Fig. 4. Gait representation for OA 2ml+$AlCl_3$

Table 3. Neuromuscular strength and endurance in oleic acid+$AlCl_3$ using vertical grid test (VGT)

Groups	Decent Time (s)					
	0		28		55	
	T1	T2	T1	T2	T1	T2
Control	29.8±2.43	29.3±2.19	30.5±3.83	29.6±4.12	30.5±3.36	29.4±4.46
Oleic acid (1ml)+$AlCl_3$	30.3±2.73	30.8±3.36	37.6±6.48*	37.4±6.14*	33.3±8.35*	33.0±9.83*
Oleic acid (1.5ml)+$AlCl_3$	30.4±2.81	30.5±2.77	33.7±4.27*	32.2±1.16	28.4±9.17*	27.4±6.95*
Oleic acid (2ml)+$AlCl_3$	29.4±3.90	30.2±3.64	29.5±6.23	28.8±2.31*	26.1±5.18*	25.8±6.31*
Total	**90.1**	**91.5**	**100.8**	**98.4**	**87.8**	**86.2**
Average	**30**	**30.5**	**33.6**	**32.8**	**29.3**	**28.7**

Table 2 shows the neuromuscular strength and endurance in oleic acid+$AlCl_3$ treatments using VGT. Significant changes ($P<0.05$) were observed on day 28 and 55. There was a dose-dependent decrease in descent time in relation to the dose of oleic acid co-administered with aluminum chloride.

Table 4. Neuromuscular strength and endurance in oleic acid+$AlCl_3$ using horizontal grid test (HGT)

Groups	Hang Time (s)					
	0		28		55	
	T1	T2	T1	T2	T1	T2
Control	21.6±6.23	20.2±5.64	21.9±0.98	22.7±1.48	21.3±2.27	22.4±1.97
Oleic acid (1ml)+$AlCl_3$	20.4±2.23	21.4±1.07	12.2±6.45*	13.7±4.46*	14.4±5.31*	15.6±4.27*
Oleic acid (1.5ml)+$AlCl_3$	21.4±2.86	22.8±2.28	17.5±4.24*	18.8±3.61*	19.8±5.63*	20.2±5.81
Oleic acid (2ml)+$AlCl_3$	20.9±1.51	20.1±2.31	20.5±2.80	21.6±1.17	21.2±6.68	22.6±7.31
Total	**62.7**	**64.3**	**50.2**	**54.1**	**55.4**	**58.4**
Average	**20.9**	**21.4**	**16.7**	**18**	**18.5**	**19.5**

Table 3 shows the neuromuscular strength and endurance in oleic acid+$AlCl_3$ treatments using HGT tested on days 0, 28 and 55. There was no significant change ($P<0.05$) in horizontal activity or hang time in 2ml oleic acid co-treatment compared to control on days 28 and 55 but a significant decrease in hang time in days 28 and 55 for 1ml and 1.5 ml co-treatments.

Table 4 shows calcium in cerebral cortex in response to oleic acid+$AlCl_3$ treatments with no significant change ($P<0.05$) in any of the co-treatments of oleic acid and aluminium chloride compared to control.

From Table 5, creatine kinase and acetylcholinesterase in plasma in response to oleic acid+$AlCl_3$ treatments showed a significant decrease ($P<0.05$) in creatine kinase after 1.5 and 2ml oleic acid-aluminium chloride co-treatments and a significant increase in acetylcholinesterase for same doses of oleic acid-aluminium chloride treatment compared to control.

Table 5. Calcium in cerebral cortex in response to oleic acid+$AlCl_3$ treatments

Groups	Ca (mmol/L)	%c*
Control	2.27±1.32	-
Oleic acid (1ml)+$AlCl_3$	2.19±0.82	-3.5
Oleic acid (1.5ml)+$AlCl_3$	2.21±1.50	-2.6
Oleic acid (2ml)+$AlCl_3$	2.29±1.40	0.8
Total	**6.69**	
Average	**2.23**	

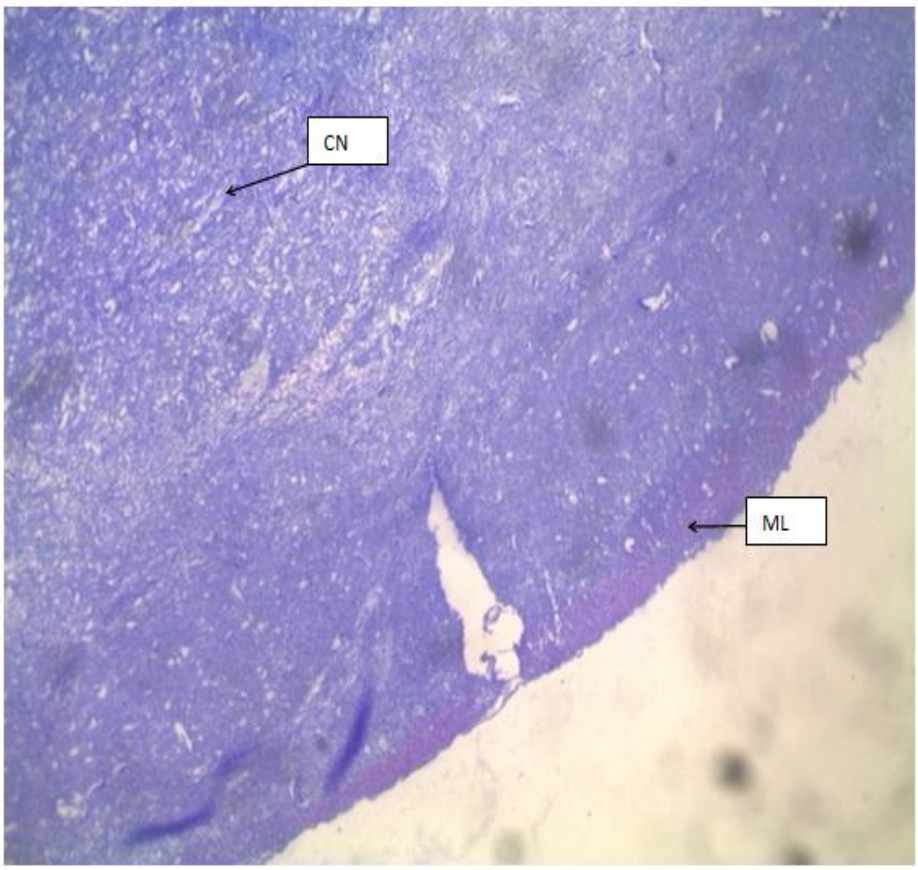

Plate 1. Cerebral cortex of control (Mag. x600) using PAS, shows normal histochemical make-up of the marginal layer and cortical neurons. ML= molecular layer; CN= cortical neurons

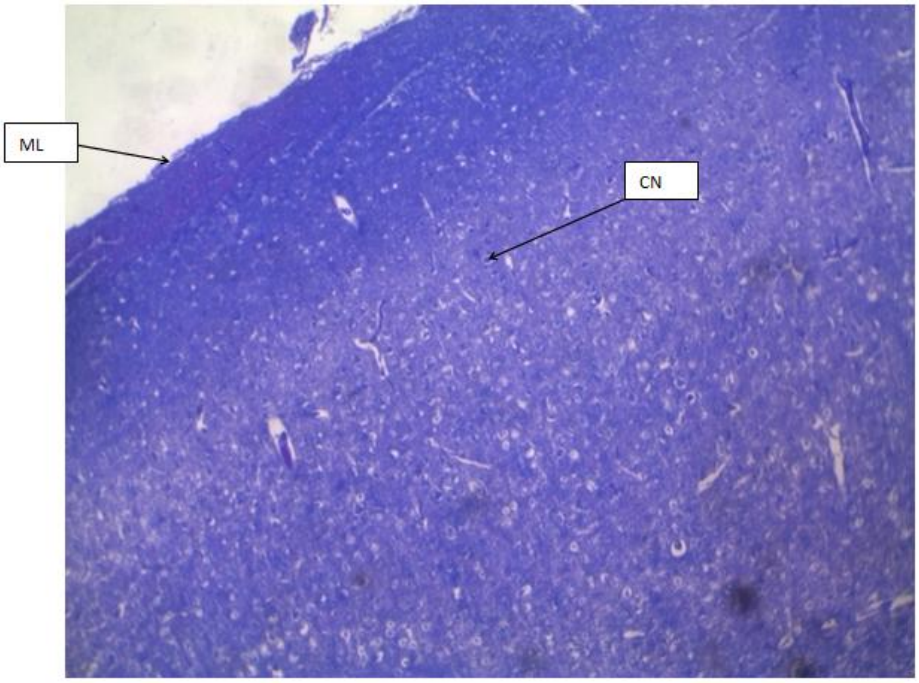

Plate 2. Cerebral cortex of oleic acid 1.5 ml + AlCl3 (Mag. X600) showing histochemical make up similar to control. ML= molecular layer; CN= cortical neurons

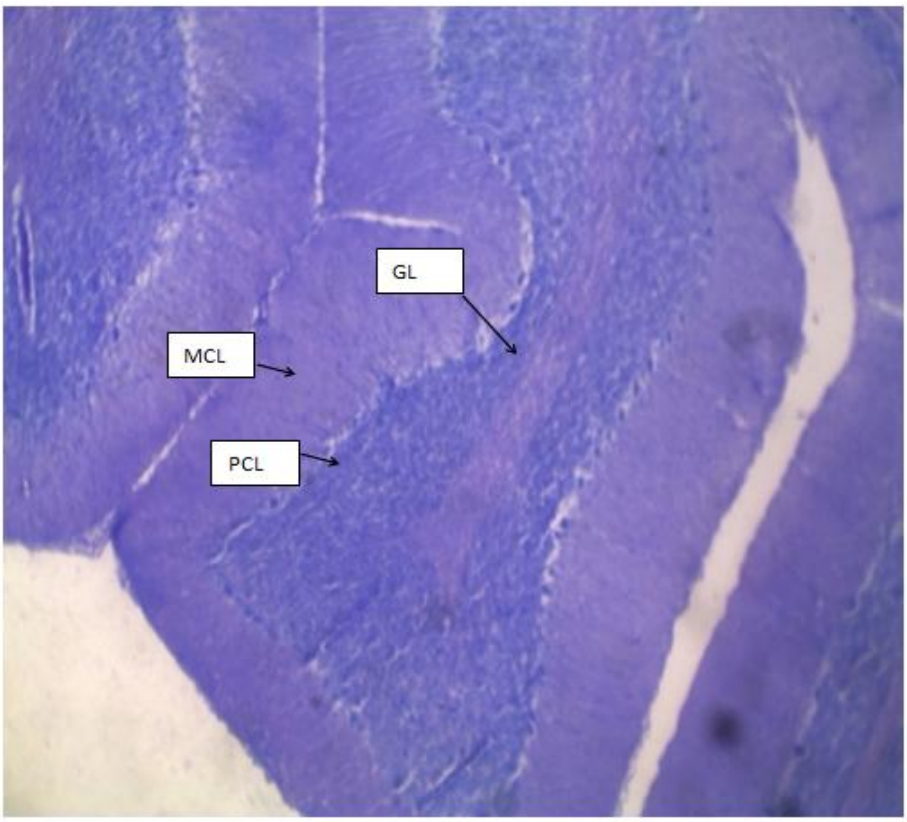

Plate 3. Cerebellar cortex of control (Mag. x600) using PAS, shows normal histochemical make-up of the molecular (ML), purkinje (PCL) and granule cell (GL) layers

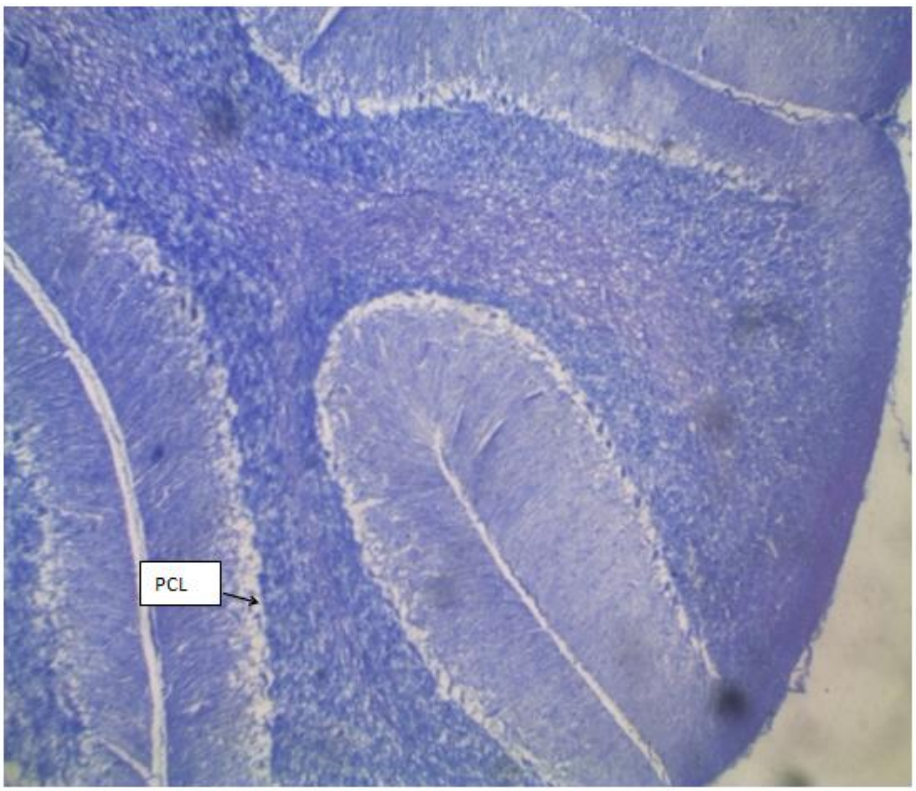

Plate 4. Cerebellar cortex of oleic acid 1.5ml+ $AlCl_3$ (Mag. X600) showing histochemical make up similar to control. PCL=purkinje cell layer

Table 6. Creatine kinase and acetylcholinesterase in plasma in response to oleic acid+$AlCl_3$ treatments

Groups	CK-MM (U/L)	%c*	AchE(U/Ml)	%c*
Control	53.12±0.31	-	32.30±2.13	-
Oleic acid (1ml)+$AlCl_3$	52.13±1.50	-1.9	34.93±0.12	8.1
Oleic acid (1.5ml)+$AlCl_3$	49.17±1.71*	-7.4	36.42±1.54*	12.8
Oleic acid (2ml)+$AlCl_3$	48.23±1.48*	-9.2	40.23±0.61*	24.6
Total	**149.53**		**111.58**	
Average	**49.84**		**37.19**	

*Key: *=values are statistically significant at 95% confidence interval (P<0.05) compared to control group; %c*=percentage change relative to control group*

4. DISCUSSION

The mechanism of neurotoxic substances is thought to be mainly due to pro-oxidative and subsequently cell biomolecular modifications [13]. Generally, nervous tissues are highly susceptible to attacks from free radicals due to their highly unsaturated lipid components [14,15]. Studies have shown that exposure to aluminum chloride results in the impairment of mitochondrial functions *in vivo* and *in vitro*, as well as destroying the antioxidant defense system by decreasing the antioxidant enzyme and non-enzyme status in heart, kidney [14], lungs and specific brain areas [16]. Pro-oxidative changes may interfere with calcium ion homeostasis which is essential for synaptic vesicular rupture and neurotransmission [15,16]. Alteration in calcium ion homeostasis is common is neurotoxicity-related conditions with exposure to heavy metals not an exception. Oleic acid affects cognitive function positively in ageing subjects [2], the mechanism behind its cognitive effect may be linked to the possibility that it may influence central nervous system (CNS) electrolyte balance. Cholinesterases play a crucial role in skeletal muscle activity [16]. Neuromuscular strength and endurance is linked to the rate of neuromuscular transmission across a neuromuscular junction. Cholinergic neurons are positive markers for the evolution of motor and motor-related disorders [12]. Neurologic diseases affecting acetylcholinesterase and impaired

cholinergic transmission are usually associated with motor and cognitive impairment. In addition to findings of the present study, cholinotoxic effects exerted by $AlCl_3$ were prevented by oleic acid co-treatments [12,16]. The level of acetylcholinesterase increased significantly in plasma in response to graded oleic acid treatment which may be because it stimulates the transcription and translation processes in the liver for the specific enzyme synthesis or it enhances the activity of the enzyme in the synaptic cleft. Oleic acid at especially 1.5ml and 2ml co-treatments caused a positive change in gait and kinematic response, vertical and horizontal activity because at these doses the adverse effect of aluminum chloride on the parameters was prevented, suppressed or reversed. Also, the ability for oleic acid to induce acetylcholinesterase upregulation when co-administered with aluminum chloride is a tendency it restores and/or prevents locomotor defects. The Interplay between alteration of acetylcholinesterase synthesis and increased tissue oxidative modification has been described [16], spotlighting a possible bi-functional mechanism of action for oleic acid in this present study. Oleic acid co-treatment with aluminum chloride also showed the tendency to stimulate general skeletal muscular activity by significantly reducing the plasma creatine kinase level possibly by a mop up process. Elevated creatine kinase has been reported in some clinical manifestations like skeletal muscle tissue necrosis [15,16] and some other myopathies [16,17,18].

5. CONCLUSION

Oleic acid, especially at 1.5ml and 2ml co-treatments with aluminium chloride, may prevent some neurotoxic manifestations peculiar to heavy metals exposure like impaired gait and kinematic, vertical and horizontal activities. Oleic acid at the reported doses may be neuroprotective.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The animal experimental procedures in this study were strictly in adherence to the regulations by the Experimental Research Ethics Committee of the University of Port Harcourt, Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Amtul Z, Westaway D, Cechetto DF, Rozmahel RF. "Oleic acid ameliorates amyloidosis in cellular and mouse models of Alzheimer's disease". Brain Pathology. 2011; 21:321–329.
2. Blondeau N. Alpha-linolenic acid: an omega-3 fatty acid with neuroprotective properties-ready for use in the stroke clinic? Biomedical Research Institute. 2015;519830. 5
3. Debbabi M. Protective Effects of α-tocopherol, γ-tocopherol and oleic acid, three compounds of olive oils, and no effect of trolox, on 7-ketocholesterol-induced mitochondrial and peroxisomal dysfunction in microglial BV-2 cells. Int. J. Mol. Science. 2016;17-1973.
4. Lewis MD, Bailes J. Neuroprotection for the warrior: dietary supplementation with omega-3 fatty acids. Mil. Med. 2011;176: 1120–1127.
5. Rioux FM, Innis SM. Oleic acid (18:1) in plasma, liver and brain myelin lipid of piglets fed from birth with formulas differing in 18:1 content. J. Nutr. 1992;122:1521–1528.
6. Velasco A, Tabernero A, Medina JM. Role of oleic acid as a neurotrophic factor is supported *in vivo* by the expression of GAP-43 subsequent to the activation of SREBP-1 and the up-regulation of stearoyl-CoA desaturase during postnatal development of the brain. Brain Research. 2003;977:103–111.
7. Arous C, Naimi M, Van Obberghen E. Oleate-mediated activation of phospholipase D and mammalian target of rapamycin (mTOR) regulates proliferation and rapamycin sensitivity of hepatocarcinoma cells. Diabetologia. 2011;54: 954–964.
8. Akinola OB, Biliaminu SA, Adediran RA. Characterization of prefrontal cortex microstructure and antioxidant status in a rat model of neurodegeneration induced by aluminium chloride and multiple lowdose streptozotocin. Metab Brain Dis. 2015;30: 1531–1536.
9. Bhattacharjee S, Zhao Y, Hill JM. Aluminum and its potential contribution to Alzheimer's disease (AD). Front. Aging Neurosci. 2014;6: 32-37.
10. Chakrabarty M, Bhat P, Kumari S. Cortico-hippocampal salvage in chronic aluminium induced neurodegeneration by Celastruspaniculatus seed oil: Neurobehavioural, biochemical, histological study. J Pharmacol Pharmacother. 2012;5:35-39.

11. Kumar V and Gill KD. Oxidative stress and mitochondrial dysfunction in aluminium neurotoxicity and its amelioration: a review. Neurotoxicology. 2014;41:154-66.
12. Ilochi Ogadinma, Arthur Nwafor Chuemere, Ekwem Ikechukwu, Bassey Samuel. Neuroprotective and Antitoxic Potential of Hydromethanolic Extract of *Allium cepa* in Experimental Rats. European Journal of Pharmaceutical and Medical Research. EJPMR. 2018;5(9):138-143.
ISSN 2394-3211 EJPMR.
13. Paul C. Akangbou, Arthur N. Chuemere, Ogadinma Ilochi. Impairment of cardiovascular function indices in male rats induced by aluminium-tainted water: Atherogenic indices and predictor ratio assessment. Discovery. 2018;54(275):442-446.
ISSN: 2278–5469.
EISSN:2278–5450.
14. Arthur N. Chuemere, Paul C. Akangbou, Ogadinma Ilochi. Evaluation and predictor ratio of toxicity of aluminium-tainted water impact male rats: Oxidative stress in heart and kidney. Science & Technology. 2018;4:183-188.
ISSN 2394–3750, EISSN 2394–3769.
15. Ilochi ON, Chuemere AN, Olorunfemi OJ, Amah-Tariah FS. Evaluation of pharmacognostic, nutraceutic and phytotherapeutic constituents of unripe *Musa sapientum* hydromethanolic extracts. Journal of Phytopharmacology. 2021;10(3):156-161.
16. Ilochi Ogadinma, Daniel Yaro Onoja, Chuemere Arthur Nwafor. Neurodynamic Influence of Hydrocortisone on Rhinencephalic and Telencephalic Brain Areas. International Neuropsychiatric Disease Journal. 2019;13(2): 1-7. Article no.INDJ.52924 ISSN: 2321-7235, NLM ID: 101632319..
17. Yao HR, Liu J, Plumeri D, Cao YB, He T, Lin L, et al. Lipotoxicity in HepG2 cells triggered by free fatty acids. Am. J. Transl. Res. 2011;3: 284–291.
18. Sales-Campos H, Souza PR, Peghini BC, Da Silva JS, Cardoso CR. An overview of the modulatory effects of oleic acid in health and disease. Mini. Rev. Med. Chem. 2013;13:201–210.